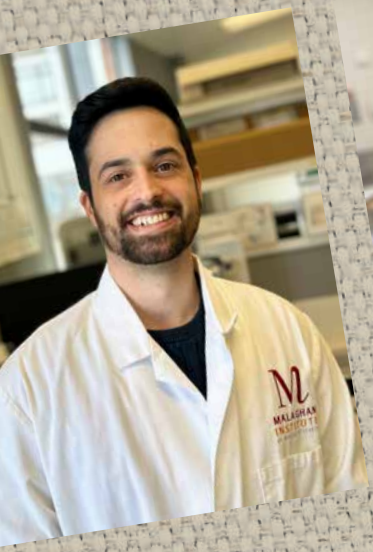
2025

RESEARCH REVIEW



Research For Life

WELLINGTON MEDICAL
RESEARCH FOUNDATION

RESEARCH REVIEW 2025

COVER PHOTO:

Grant recipients:
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Kayden Borchowsky

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Research For Life
The Wellington Medical
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RESEARCH PROJECT GRANTS 2025

TRAVEL GRANT REPORTS

Editorial

BY PROFESSOR REBECCA GRAINGER

Each year when I sit down to write this editorial, I am again struck by the range of research that Research For Life supports across Wellington's academic institutes.

As Chair of the Research Committee, I read the proposals for all these endeavours when they arrive as grant applications, and it is deeply satisfying to later review the research reports and see those bold ideas come to life. I also think about the talented young people developing biomedical research skills, many hoping for careers in science. Some two decades ago I was fortunate to receive a Research For Life grant for my doctoral studies, and now, as a clinician-scientist, it is a personal delight to see the next generation of Wellington researchers pursuing similar paths.

Each research report is unique, but what stands out this year are the cutting-edge tools and new collaborations emerging in Wellington. Research For Life continues to play a key role in enabling students, emerging researchers, and their teams to undertake this work.

Biomedical research has always been driven by technology and critical thinking. The tools in use today were barely imaginable when I was a doctoral student. We now have single-cell sequencing that can read the genetic "voice" of each immune cell, and organoids that better mimic human tumours. These technologies are revealing biological phenomena once overlooked. One such example is the ground-breaking work at the Malaghan Institute on Chimeric Antigen Receptor T (CAR-T) cells — a form of cancer immunotherapy that modifies a patient's own immune cells to fight cancer. In this review, we see a collaboration exploring how to protect CAR-T cells from chemotherapy so both therapies can be used together.

Our grant recipients are also investigating areas once dismissed as biological "waste" — such as neutrophil extracellular traps (NETs) and extracellular vesicles — now known to transmit important biological messages. Others are exploring how opioid receptor agonists, typically used for pain, might also calm mast cells and reduce food allergy symptoms. These are not abstract advances; they are being used right now by the students and early-career investigators we fund. Each project contributes to future improvements in medical care, ensuring Wellington and New Zealand continue to advance health outcomes.

Another striking feature is how interconnected Wellington's biomedical scientists are. Few work in isolation — most collaborate across institutions and disciplines, from surgery and cardiology to endocrinology and neuroscience. Even the most lab-based discoveries often involve shared methods and cross-mentoring.

As someone who works across both clinical and academic settings, I see daily how collaboration multiplies impact. Research For Life grants may be modest in dollar value, but they often spark connections and new opportunities. A consumables budget for one PhD student can generate data that supports a national HRC application or a link with an overseas lab. The effect is like dropping a pebble in a pond — the ripples extend far beyond the initial splash.

What excites me most is this combination of advanced tools and teamwork. No single lab can master every technology, but together, Wellington researchers are maximising infrastructure, sharing expertise, and training one another's students. This culture of openness and generosity is one of our community's greatest strengths.

Travel grants extend this collaboration further. Each time a student or postdoctoral scholar attends an international conference, they return with new techniques, insights, and partnerships that strengthen our local research ecosystem. While some move overseas, they often bring those skills and connections back to Wellington. We now see former grant recipients mentoring the next generation.

To all who support Research For Life — thank you. I hope this report excites you about the work being done by our Wellington researchers and their teams. To the researchers — thank you for your ideas, persistence, and energy. And to my colleagues on the Research Committee — thank you for the care and expertise you bring to every application.

Research For Life exists to give new ideas their first chance and new researchers the support to grow. In the hands of Wellington's talented biomedical scientists, those ideas are becoming knowledge and innovation that will improve health for our communities. That is something we can all be proud of.

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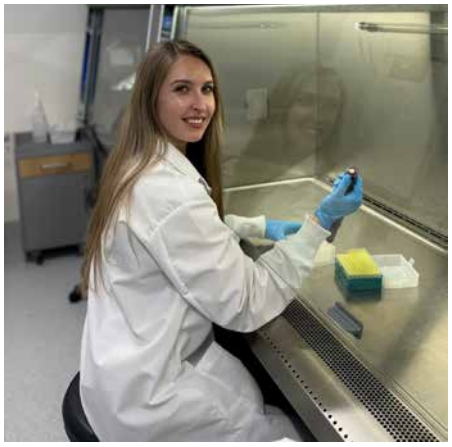
RESEARCH REPORTS

What the Post Doctoral Fellowship meant to me



The Research For Life Postdoctoral Fellowship was the first fellowship I was fortunate to receive after completing my PhD, and it has provided a solid foundation for my research career.

I completed my doctorate in 2019, just prior to the upheavals of the COVID-19 pandemic. Amidst lockdowns and international travel restrictions, it was an uncertain time to be an emerging postdoctoral researcher. Traditionally, the well-trodden path has been to seek postdoctoral opportunities overseas, but such options were suddenly less feasible for me, and the pool of opportunities within New Zealand was limited. I was grateful that my Victoria University of Wellington PhD supervisor, Professor David Ackerley, was able to offer me a Postdoctoral Research Fellow role supported by NIH and Marsden grants he held with collaborators at Johns Hopkins University and the Malaghan Institute of Medical Research. This position enabled me to continue and expand upon my PhD research, while also benefiting greatly from Professor Ackerley's mentoring and support.

While I thoroughly enjoyed contributing to the NIH and Marsden projects, after three years of postdoctoral work I felt increasingly ready to take on greater responsibility and ownership of my own research direction. The Research For Life Postdoctoral Fellowship, awarded to me in 2023, provided the perfect opportunity to do so. It gave me both the security and freedom to establish myself as a semi-independent researcher within the School of Biological Sciences at Victoria University of Wellington. With my salary supported for two years, I was able to focus fully on progressing my research, generating the preliminary data and momentum that directly underpinned my success in securing further competitive funding – including a Health Research Council Emerging Researcher grant in 2024 and a Marsden Fund Fast-Start grant in 2025. The work I undertook during the fellowship was also recognised through research awards such as the Illumina™ Emerging Researcher Award (2023) and the VUW Ke Ti Pae Early Career Research Excellence Award (2024).

The fellowship also marked the beginning of my supervisory career. Over its course, I had the privilege of guiding two Master's students and one PhD student through to completion, in my role as secondary/co-supervisor alongside Professor Ackerley. The experiences I gained from this supervision not only deepened my leadership skills but also reinforced my commitment to mentoring the next generation of scientists. At the same time, my fellowship research strengthened relationships with collaborators across institutes, broadening my professional networks and fostering new avenues of inquiry that continue to shape my work today. Beyond the laboratory, Research For Life has supported my attendance at international conferences during both my PhD and fellowship years. These opportunities were invaluable for professional development – enhancing my profile, building international networks, and allowing me to share my work on a global stage. The fellowship gave me the confidence to step into the wider academic community, developing skills in project management, grant writing, and cross-disciplinary collaboration that will be essential for my long-term research career.

In every sense, this fellowship provided the launchpad for the trajectory I am now on. I am deeply grateful to Research For Life for investing in early-career researchers and for the trust and support they placed in me at this formative stage. Its impact extends well beyond the research outcomes achieved during its tenure – it truly laid the foundation for the independent research career that I continue to build today.

Enhancing mucosal immunity by determining the immunological basis of pulmonary tertiary lymphoid structure formation using micro-dissection through photoconversion.

K MOLONEY-GEANY[1], A SCHMIDT[2], K HILLIGAN[1]

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KEY FINDINGS

The formation of ectopic lymphoid aggregates in perivascular lung tissue, commonly referred to as tertiary lymphoid structures (TLS), occur in response to infection or inflammation. One hinderance to studying TLS biology is the limited ability to isolate single cells specifically from TLS as spatial information is lost during tissue processing. Here, we aim to leverage a novel method, known as photoconversion of areas to dissect micro-environments (PADME), to fluorescently mark cells within TLS prior to tissue dissociation, thus enabling downstream study of cellular interactions specifically within TLS.

OBJECTIVES

i. Develop and optimise the identification and photoconversion of tertiary lymphoid structures using fixed lung tissue.

ii. Optimise the disassociation of lung tissue and efficient recovery of photoconverted cells by fluorescence-activated cell sorting.

iii. Apply single-cell RNA sequencing to photoconverted cells enabling a deeper understanding of the initiation and maintenance of tertiary lymphoid structures.

METHODS

i. C57BL/6 mice were infected with influenza A virus X31. On days 0-, 3-, 7-, and 18 days post infection, left lung lobes were fixed using DSP dissolved in methanol. Lung tissue was then snap frozen, followed sectioning at 160µm thick. Lung sections were then incubated with B220 to identify TLS and PA-JF-646 for photoconversion. Lung sections were then visualised under a confocal microscope and regions of B220+ cells were exposed to UV light, uncaging PA-JF-646.

ii. Photoconverted lung tissue sections were digested and filtered through a 40µm mesh. Digested cells were then washed and prepared for fluorescent-activated cell sorting. Cells were isolated based on PA-JF-646 staining.

iii. To ensure RNA quality was sufficient for single-cell RNA sequencing, whole RNA was extracted across multiple steps of the processes. Extracted RNA was analysed using a Tapestation to obtain RNA length and integrity.

RESULTS

To demonstrate the ability to visualise TLS cells, fixed lung tissue sections were stained with the B cell marker B220. This highlights the B cell follicle at the centre of TLS (Figure 1A). To ensure all cells involved in TLS formation were photoconverted, regions adjacent to the B220+ cells was exposed to UV light resulting in PA-JF-646 uncaging (Figure 1A). To facilitate isolation of photoconverted cells, we used FACS to separate PA-JF-646+ cells from cells which were not exposed to UV light (Figure 1B). Finally, to ensure that RNA

was adequately preserved for single-cell RNA sequencing, RNA was extracted from cells after photoconversion and tissue digestion. a RIN score of 7 confirmed that these cells would be applicable for sequencing.

CONCLUSION

All steps involved in facilitating PADME have been optimised. The final step is to perform single-cell RNA sequencing. To gain a greater understanding of what occurs within a TLS, we will sequence both photoconverted and non-photoconverted cells to allow a direct comparison within each lung sample processed.

All reagents have been obtained, and sequencing will commence in early October once the final influenza A infection time course has been completed. We anticipate the conclusion of this research by early November.

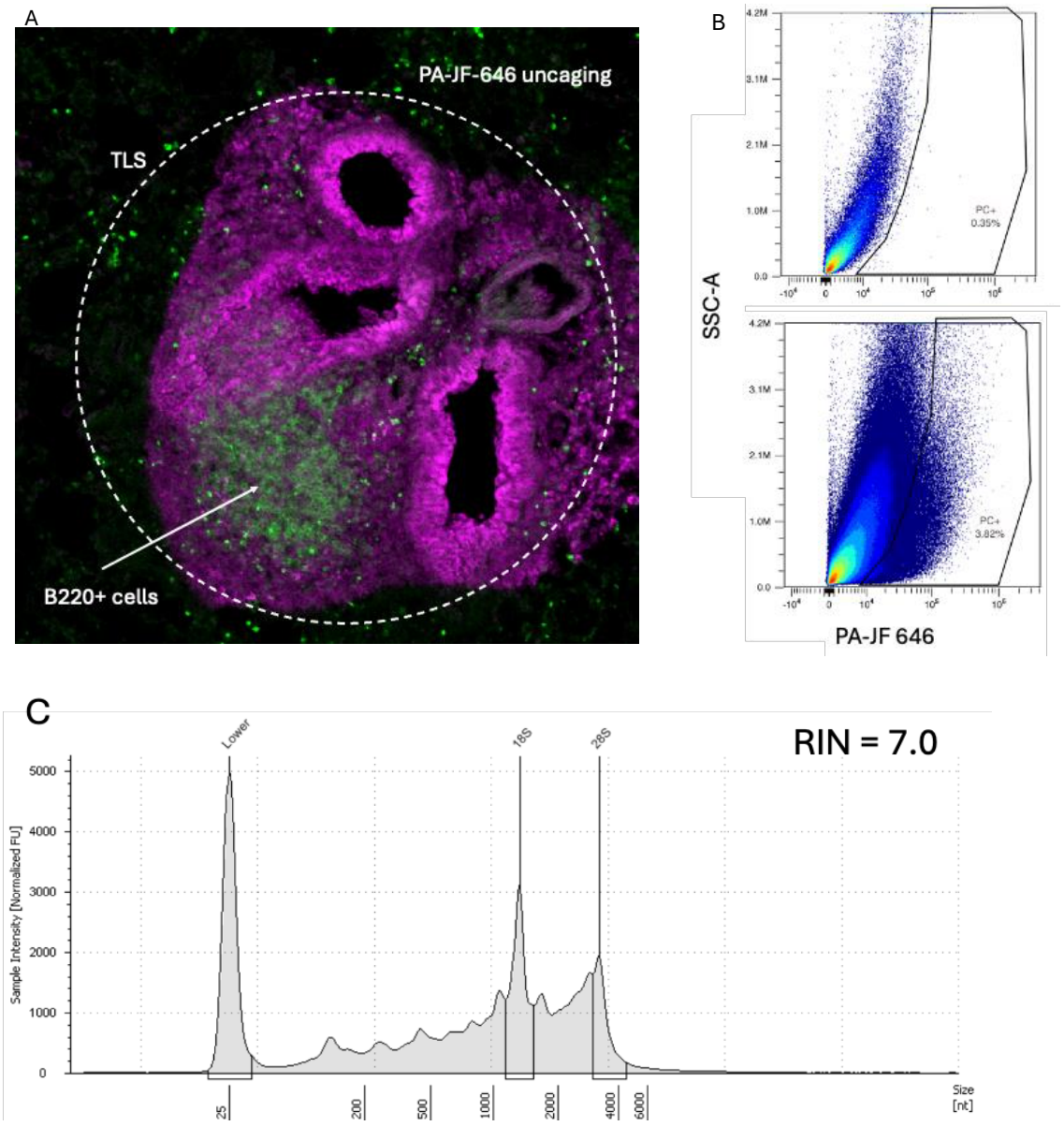

FIGURE 1: Optimising PADME to isolate TLS-resident cells. **(A)** immunofluorescent image of fixed lung tissue incubated with B220 (green) and PA-JF-646 (magenta). B220 highlights B cells residing in the TLS. The adjacent regions were exposed to UV light resulting in PA-JF-646 uncaging. **(B)** FACS plots detailing the ability to separate out PA-JF-646 cells (PC+). The top plot displays a control sample which was stained with PA-JF-646 but was not exposed to UV light. **(C)** a fragment analyser plot demonstrating the RNA integrity after tissue digestion following photoconversion. RIN = RNA integrity number.

Malaghan Institute of Medical Research
Te Herenga Waka
Victoria University of Wellington

A calming influence from your MAITs: Unveiling MAIT cell mechanisms safeguarding lung tissue during influenza

H BOSWELL, I HERMANS, L CONNOR

KEY FINDINGS

Our work aims to harness the activity of Mucosal Associated Invariant T (MAIT) cells to shift the lung immune landscape towards an anti-inflammatory state, aiming to protect against influenza infection. We performed single-cell RNA sequencing of lung immune cells and developed a new protocol to prepare single-cell suspension of lung tissue enriched with MAIT cells. Single cell RNA sequencing analysis has revealed that MAIT cells alter their phenotype following treatment of 5-OP-RU, providing new information on the mechanism of how MAIT cells can reduce the negative effects of influenza infection.

OBJECTIVE/S OR AIM

This project aimed to develop a method to isolate MAIT and other immune cells that protect the lung from damage during influenza. This method was used to better understand the different activated states of MAIT cells following enrichment and influenza infection.

METHODS

MAIT cells represent a very small percentage of a lung immune compartment. These numbers can be increased by performing intranasal administration of the MAIT cell activating ligand, 5-OP-RU. To assess how enhancement of MAIT cells can alter the lung immune landscape, two 'vaccine groups' were produced. Mice were given intranasal treatment of 5-OP-RU or a vehicle control three times, two weeks apart. These mice were culled at day 49 and the lungs harvested and were labelled "vehicle uninfected" and "5-OP-RU uninfected". To assess how MAIT cell enhancement can alter the course of an influenza infection, two 'influenza groups' were made. In the influenza groups mice were treated with the vaccination schedule and given influenza two weeks after the final 5-OP-RU or vehicle treatment. These mice were culled at day three post influenza infection, labelled "vehicle infected" and "5-OP-RU infected", to look at the changes in MAIT cells which have the potential to aid viral clearance and lung pathology. Lungs from three mice per experimental condition were collected for single-cell RNA sequencing.

Isolated lung immune cells were obtained for single-cell RNA sequencing by cell sorting. The lung tissue was processed to obtain single cell suspensions. Cells were tagged with fluorescent antibodies to detect live cells, immune cells, and MAIT cells. Cells were run through an influx cell sorter to obtain live MAIT and immune cell populations. The cells were mixed at a ratio of 1:10 MAIT to immune cells. Once mixed, the cells were tagged with specific oligonucleotide antibodies to identify each treatment group, mixed, and run on the BD Rhapsody. The BD Rhapsody works in a well and magnetic bead-based selection of RNA. Cells were gently flowed over a cartridge containing microwells, a magnetic bead capable of catching RNA was then added to each well. The cells were then lysed, RNA bound to the bead, which were magnetically removed from the wells. The RNA was converted to stable cDNA and sequenced at the Ramaciotti Centre for Genomics, UNSW Sydney. The data was analysed using the Seurat package in R to identify the mRNA expressed in each cell which was used to infer the cells phenotype and function.

RESULTS OR FINDINGS

We developed a cell sorting method to isolate and enrich MAIT cells from the lung, addressing their rarity in the murine lung which makes them difficult to detect with single-cell RNA sequencing. Using an influx cell sorter (Fig

2), we stained cells with fluorescent antibody markers for MAIT cells (TCRß and MR1 tetramer), total immune cells (CD45.2) as well as markers for high abundant cell types such as monocytes and B cells (Ly6G and CD19). Populations that were doublets, dead, or low quality were separated out from MAIT cells or non-MAIT immune cells based on their fluorescence and size (Fig 2). MAIT cells and non-MAIT immune cells were isolated in the 'MAIT cell sort gate' and 'Live immune cell sort gate' and separately stained with cell tags to allow for identification during RNA sequencing analysis. These cells were then mixed at a 1:10 ratio to enrich for MAIT cells while retaining the lung immune cell environment. After pooling, RNA extraction, and collection, we obtained 36,097 cells from the vaccine groups and 41,945 from the influenza groups for analysis.

Single cell RNA sequencing is a powerful tool to investigate what genes individual cells expressed in response to 5-OP-RU treatment. The MAIT cells were identified using the oligonucleotide antibodies and specifically analysed. The MAIT cells with similar phenotypes and functions were grouped together into 'clusters' based on which genes were expressed in each cell, which is visualised in a UMAP graph where one dot represented one cells (Fig 2A). These clusters were largely dependent on the treatment received, where cluster 3 is found primarily in the vehicle treated MAIT cells prior to infection and represented the 'baseline' MAIT cell phenotype (Fig 2B&C). After treatment with 5-OP-RU we see a shift in the gene expression of the MAIT cells, with less cells in cluster 3 and the generation of two new clusters (cluster 0 and cluster 4), which represented the population of MAIT cells that were activated by 5-OP-RU. The gene expression of these three clusters is significantly different, where 843 genes were found to be specific to the baseline MAIT cells, and the two 5-OP-RU activated MAIT cell clusters had 305 and 92 genes upregulated respectively (Fig 3). This indicated that 5-OP-RU enrichment of MAIT cells in the lung produced unique MAIT cell populations, which may then impact the influenza infection.

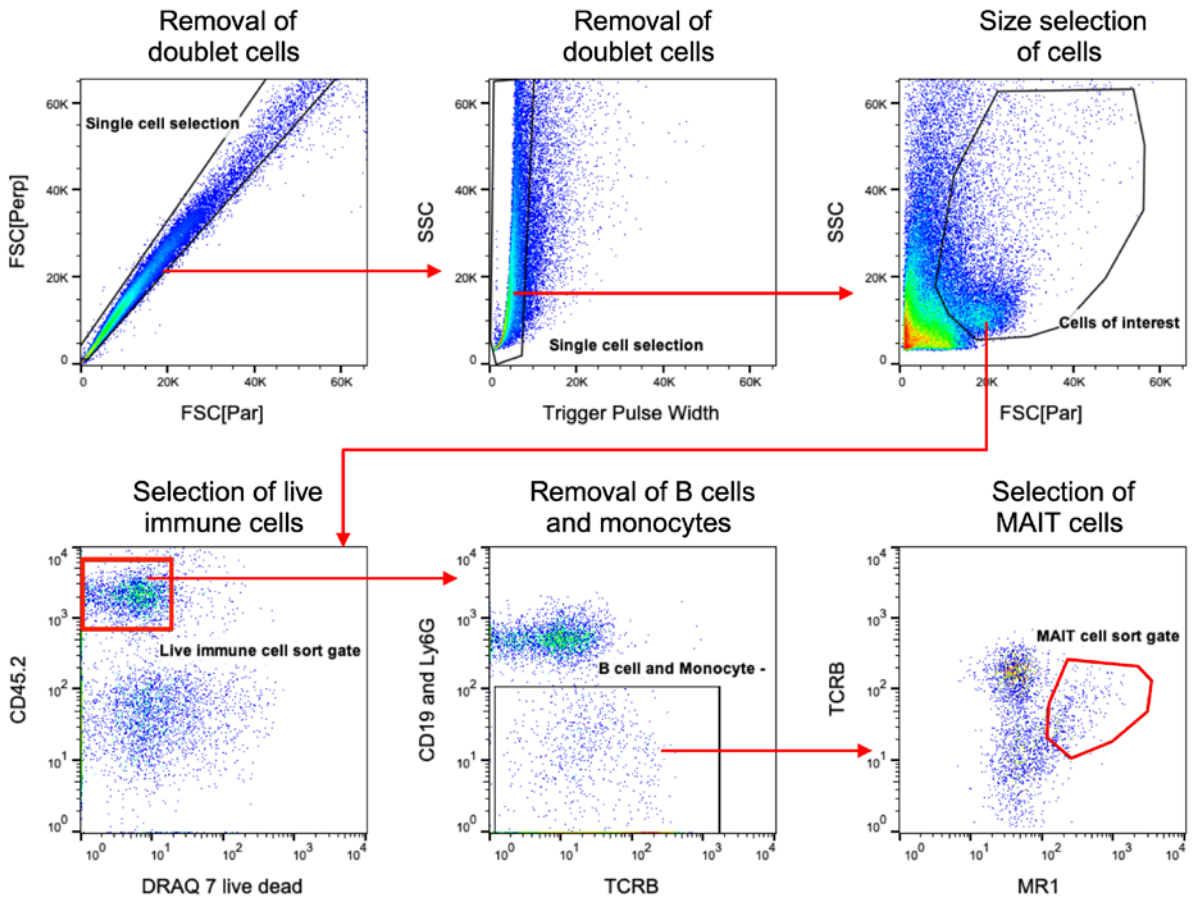

FIGURE 1: Example of 5-OP-RU and immune cell purification by the influx cell sorter.

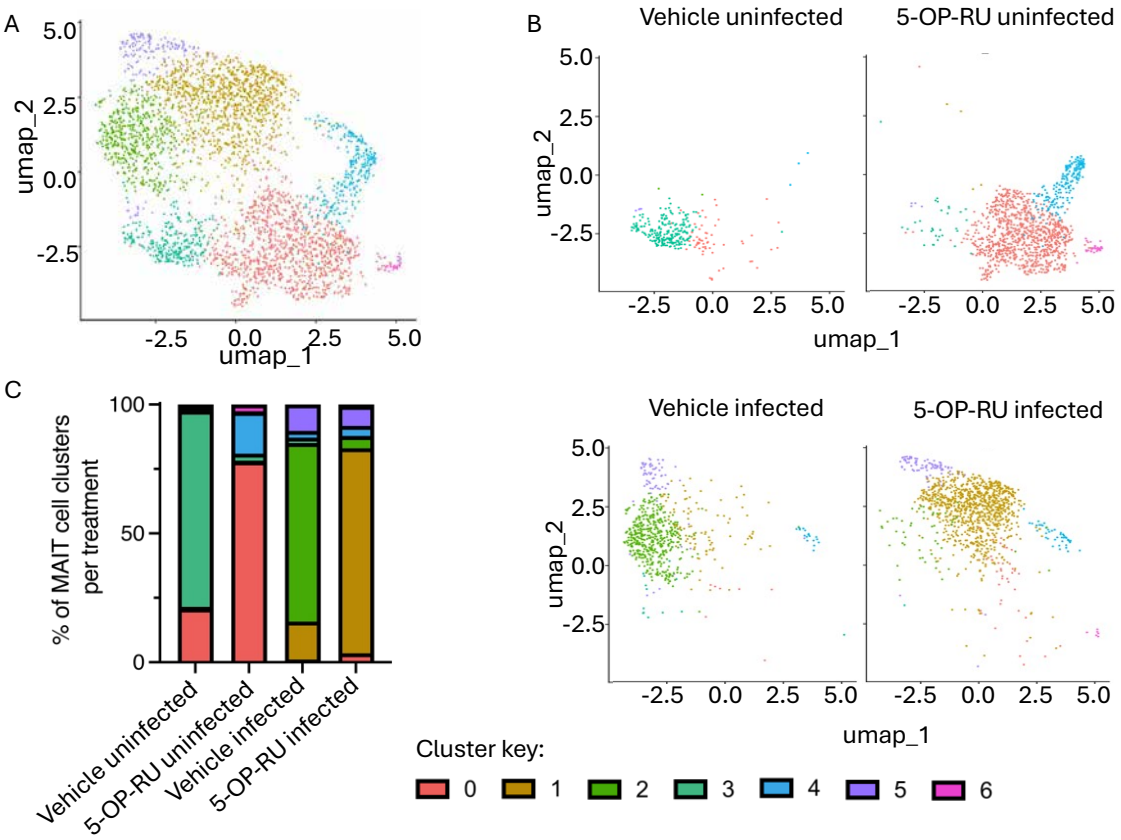

FIGURE 2: Single cell RNA sequencing revealed unique MAIT cell populations dependent on 5-OP-RU treatment and influenza infection. UMAP of seven clusters of MAIT cells for all treatment groups **(A)** or for each treatment group **(B)**. **(C)** The percentage of MAIT cells in each cluster for each treatment group.

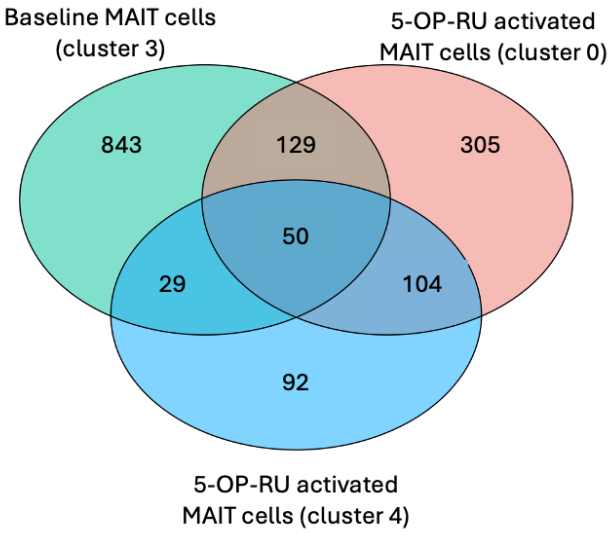

FIGURE 3: Venn Diagram of upregulated genes in the clusters associated with either vehicle MAIT cells or 5-OP-RU activated MAIT cells prior to influenza infection.

CONCLUSION

We have successfully developed a novel method to prepare single-cell suspensions of lung cells enriched with MAIT cells. High quality sequence data from mice treated with MAIT ligand 5-OP-RU and infected with Influenza has been generated and shows us that treatment with 5-OP-RU can induce unique MAIT cell phenotypes in the lung. This data will help us understand how enriching MAIT cells can aid in protection during influenza infection.

Te Herenga Waka
Victoria University of Wellington

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

A SHARROCK, R WEINKOVE, M TERCEL, D ACKERLEY

KEY FINDINGS

My research focused on discovering new enzymes that can protect healthy cells from the toxic effects of a next-generation chemotherapy drug. To achieve this, I combined computational database mining, functional metagenomic screening, and a bacterial testing pipeline to identify entirely new classes of protective enzymes. Using protein engineering strategies, including directed evolution and the addition of intracellular trafficking tags, I investigated whether their baseline protective activities could be enhancwed. Two lead candidates emerged that offer low-to-moderate protection to human cells in vitro; when co-expressed they complement one another to confer substantial protection. In ongoing work, these enzymes and other lead candidates are being evaluated in human immune cells, with a longer-term goal of incorporating them into engineered CAR T-cells. Our over-arching goal is to enable these therapeutic cells to be administered alongside chemotherapy drugs without being destroyed in the process, to develop combined chemotherapy and immunotherapy cancer treatments as safer and more-effective options for patients.

AIM

The primary aim of this investigation was to identify and evolve/engineer optimised enzyme candidates that can be expressed in CAR T-cells, to provide protection against the cytotoxicity of a promising next-generation chemotherapy drug.

METHODS

To discover protective enzymes, a 'metagenomic library' – a cloned collection 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 (published in Cell Chemical Biology, 2023). Our strategy 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), selecting for bacteria that had acquired DNA fragments enabling their survival. The DNA from surviving cells was extracted and analysed to identify protein-encoding genes responsible for drug resistance. In parallel, database mining was used to identify resistance genes from bacteria that produce toxic compounds structurally similar to our target drug, reasoning that these may encode different kinds of protective enzymes. Candidate genes from both strategies were expressed and tested in isolation in E. coli growth assays to confirm their protective function. The most promising candidates were then further tested by integrating them into the genome of a human cell line, using cell survival assays to assess how well they functioned in the human cellular environment.

To improve activities, error-prone PCR was used to generate random mutations in the genes that encoded lead enzymes. These libraries were screened using growth assays in both solid and liquid media to identify improved versions with enhanced protective activity. Additional tests were performed to explore whether use of genetic fusion tags (nuclear localisation signals, NLS) directing key enzymes to specific locations inside the cell could improve protection. Confocal microscopy was used to check whether target enzymes were successfully trafficked to the nucleus, and cell viability assays were carried out to assess whether this improved protection against the drug.

Following identification and engineering of top candidates from the first metagenomic library, a second library was screened using similar methods.

RESULTS

Through database mining, function-based metagenomic screening, and a bacterial testing pipeline, I uncovered over 50 novel enzymes and other proteins able to protect cells from our target chemotherapy drug. Many of these share very little sequence similarity with any previously known proteins, highlighting their novelty. Following screening of an initial metagenomic library, proteins from three particularly promising classes were tested in human HEK-293 cells. Of these, two ('Alkrep', a putative DNA repair protein and 'MG', a protein of unknown function) demonstrated successful expression and function in the human cell environment, providing significant protection against the drug.

To enhance protective activity, I constructed error-prone PCR libraries of the Alkrep and MG genes, in each case generating 0.5 to 4 million variants. Initial screening efforts identified clones that conferred improved protection through elevated expression levels, but not through enhanced enzymatic activity. In ongoing work, screening conditions are being refined to select for functional improvements rather than expression-driven effects. Given the importance of intracellular localisation for protein function, I modified the MG and Alkrep enzymes, with nuclear localisation signals (NLS) to investigate their activities in different cellular compartments. Excitingly, in HEK-293 cells, Alkrep showed improved protective capacity when directed to the nucleus - consistent with its putative DNA repair role - while MG was more effective in the cytoplasm (Fig 1A). Confocal microscopy confirmed the localisation of each construct (Fig 1B). Furthermore, expressing both MG and Alkrep together in HEK-293 cells significantly increased resistance to DNA-alkylating drug toxicity compared to either protein alone, highlighting the potential for additive or even synergistic protection (Fig 1C).

Screening of a second metagenomic library was recently completed, leading to identification of a new set of candidate enzymes, eight of which are now being advanced to testing in human cells.

CONCLUSION

Through bioinformatic analysis and metagenomic screening, this project has identified a diverse set of novel proteins capable of protecting cells against our target chemotherapy drug. Two – MG and Alkrep – have been studied in detail and shown to protect human HEK-293 cells from drug toxicity. Intracellular localisation plays a large role in determining the protective activity of each of these proteins, and co-expression provides greater protection than either enzyme alone, suggesting a synergistic effect. Work is ongoing to further assess these and other lead enzymes, via collaborative efforts focused on testing their performance in human immune cell models. These efforts aim to lay the groundwork for engineering chemo-resistant immune cells, opening new possibilities for combining chemotherapy with immune cell-based therapies.

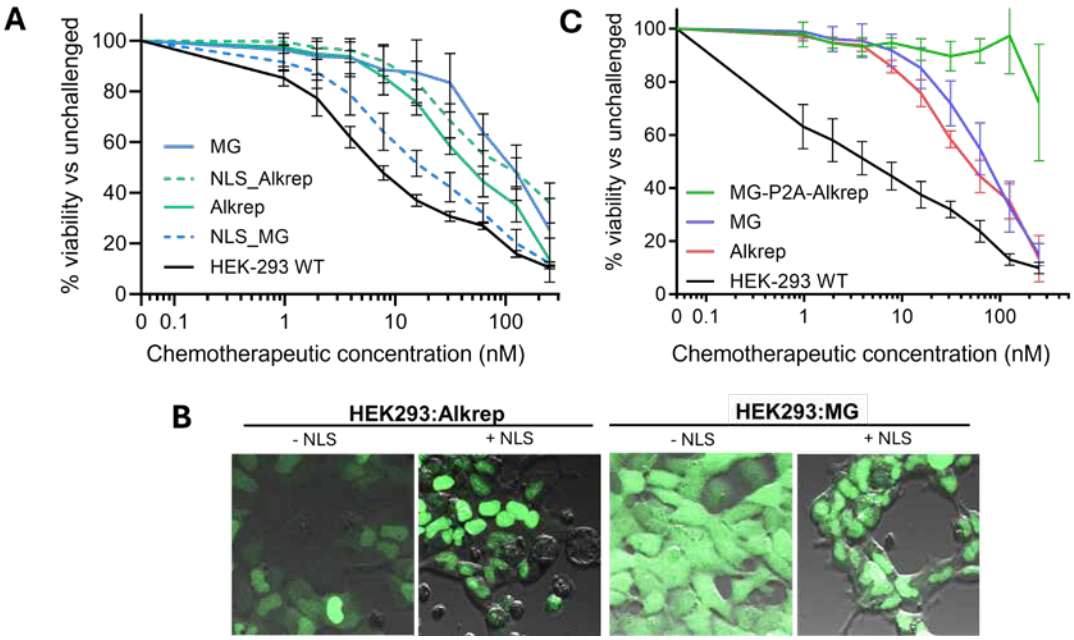

FIGURE 1: (A) Drug sensitivity of HEK-293 cell lines expressing protective proteins with or without a fused NLS. **(B)** Confocal fluorescence microscope images of HEK-293 cells expressing GFP-tagged proteins with or without a fused NLS. **(C)** Drug sensitivity of HEK-293 cell lines expressing one, or both,

Te Herenga Waka
Victoria University of Wellington

Investigating the effects of kappa opioid receptor agonists on CD4+ T cell subsets

B WALDRAM, A LA FLAMME

KEY FINDINGS

This project investigated the effect of activating the kappa opioid receptor (KOR) on CD4+ T helper cell differentiation into effector subsets such as Th1, Th2, Th17 and Treg. We found that activating the KOR with nalfurafine reduced inflammatory Th17 cells and shifted the balance of CD4+ T cell subsets in the periphery toward a more regulatory state. This, in turn, may contribute to downstream changes in the central nervous system (CNS) previously observed with nalfurafine treatment by our lab group, such as increased remyelination and reduced infiltration of CD4+ T cells. Additionally, we observed a reduction in the expression of Th17-associated genes after nalfurafine treatment during a mouse model of multiple sclerosis, highlighting the immunomodulatory potential of this treatment.

OBJECTIVE/S OR AIM

Multiple sclerosis (MS) is a chronic autoimmune disease affecting the CNS. CD4+ T helper cells are key drivers of inflammation in MS, where they migrate into the CNS and attack the myelin sheath, the protective layer surrounding the nerves. CD4+ T cells can be divided into several functional subsets. Inflammatory subsets Th1 and Th17 are key contributors to CNS damage, while anti-inflammatory Tregs support immune resolution.

Kappa opioid receptor agonists such as nalfurafine have been shown to promote remyelination in the experimental autoimmune encephalomyelitis (EAE) model of MS.

Nalfurafine treatment was also found to reduce the infiltration of CD4+ T cells into the CNS and reduce cytokine production by these cells, highlighting the potential of nalfurafine to modulate immune responses. However, the direct effects of KOR activation on CD4+ T helper cell differentiation were not clear.

The overall aim of this project was to further characterise the influence of nalfurafine treatment on CD4+ T cell subsets and determine any downstream immune-modifying effects. This aim had two main objectives

Objective 1: Explore the effect of nalfurafine treatment in vitro and in vivo on peripheral CD4+ T cell subsets.

Objective 2: Investigate the influence of nalfurafine treatment on CD4+ T cell-associated gene expression in the context of EAE.

RESULTS

Objective 1: Explore the effect of nalfurafine treatment in vitro and in vivo on peripheral CD4+ T cell subsets.

Splenic T cells were activated with T cell activation beads and treated with nalfurafine, U50,488 (the prototypic KOR agonist) or vehicle alone. Nalfurafine but not U50,488 treatment induced a modest reduction in the expression of RORγT, the marker for Th17 cells, on CD4+ T cells (Figure 1A).

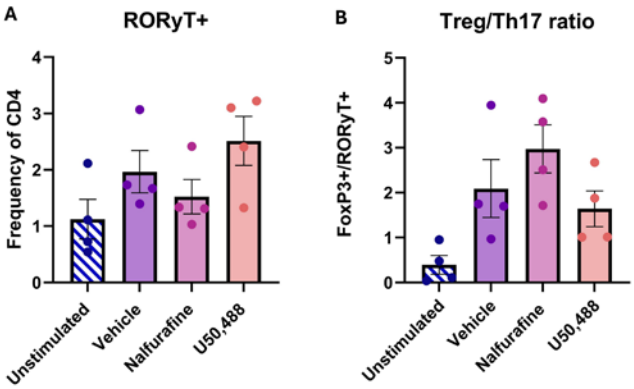

FIGURE 1: Splenocytes were stimulated with anti-CD3/CD28 beads and treated with either vehicle control, 50nM nalfurafine.

There is considerable transcriptional overlap between Th17 and Treg cells, with TGFβ playing a key role in the induction of both subsets. A dysregulation in the balance of these two subsets is known to be a key factor in the development of both autoimmune diseases and cancer. The ratio of FoxP3+ Tregs to RORγT+ Th17 cells was increased by nalfurafine treatment, indicating a shift towards a more regulatory environment (Figure 1B).

Next, we explored the impact of nalfurafine treatment on CD4+ T cell subsets in peripheral tissue using a modified version of the EAE mouse model of MS. By focusing on peripheral immune processes, this approach allowed us to narrow down the localisation of nalfurafine's effects and whether treatment affected the differentiation and expansion of CD4+ T cells before they enter the brain and spinal cord. Flow cytometry analysis demonstrated that nalfurafine treatment reduced the frequency and number of RORγT+ CD4+ T cells in the spleen and blood of mice immunised with a myelin peptide, indicating a downregulation of Th17 responses (Figure 2)

Objective 2: Investigate the influence of nalfurafine treatment on CD4+ T cell-associated gene expression in the context of EAE

To investigate changes in the CD4+ T helper cell compartment during EAE, we analysed the transcriptome of spinal cords by RNA sequencing (RNAseq). We assessed this data for changes in gene expression associated with CD4+ T cell subsets, including their differentiation and production of cytokines. Overall, genes associated with CD4+ T cell subsets were reduced in nalfurafine compared to vehicle-treated animals (Figure 3A). As objective 1 determined that nalfurafine treatment may reduce Th17 cells, we also investigated changes in gene expression associated with this subset. Nalfurafine treatment downregulated the expression of the Th17 inflammatory cytokine IL-17A. (Figure 3B). The production of inflammatory cytokines by Th17 cells is known to contribute to the progression of EAE and MS by disrupting tight junctions and altering adhesion molecules. This impairs the blood-brain barrier, facilitating infiltration of immune cells into the CNS. Nalfurafine treatment downregulated the expression of adhesion molecule genes ICAM-1, VCAM-1 and P-selectin (data not shown), which could contribute to the reduction in CD4+ T cell infiltration seen in earlier studies.

The expression of migration-associated markers such as LFA-1 on T cells was not significantly altered by nalfurafine in EAE. This suggests that nalfurafine is unlikely to have a direct effect on T cell homing to the CNS (Figure 4) despite previous data indicating a reduction CD4+ infiltration into the brain and spinal cord following nalfurafine treatment. Instead, nalfurafine may dampen the overall inflammatory potential of CD4+ T cells in the periphery which in turn could contribute to reduced T cell infiltration into the CNS. This hypothesis is supported by the reduction in cytokine production, particularly IL-17A and IL-6, by Th17 cells, which are critical in driving blood brain barrier disruption and immune cell infiltration into the CNS.

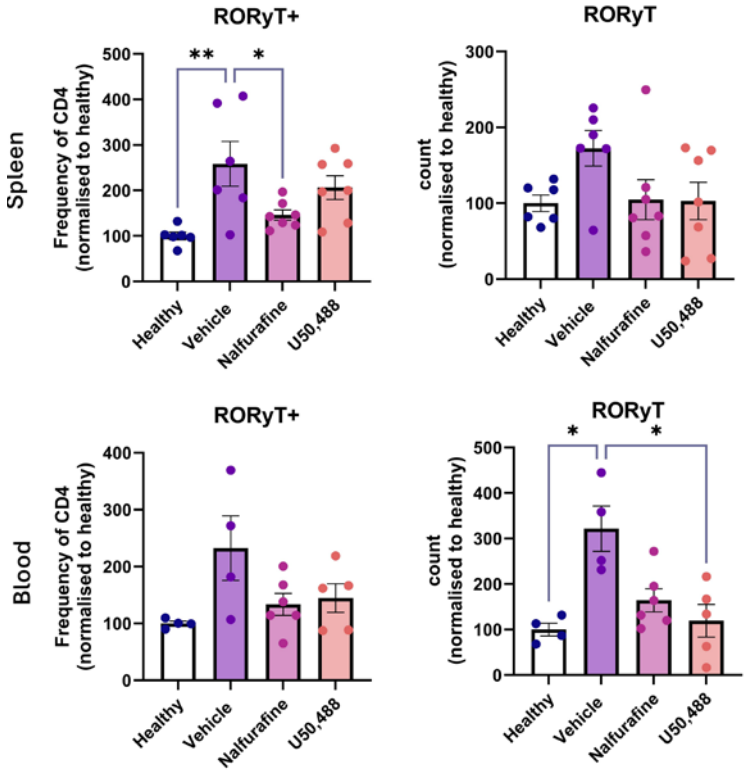

FIGURE 2: Frequency and count of cells in the spleen (top) and blood (bottom) following 7 days KOR treatment. Data represents 3 individual experiments. n = 4-8. Data are mean ± SEM.

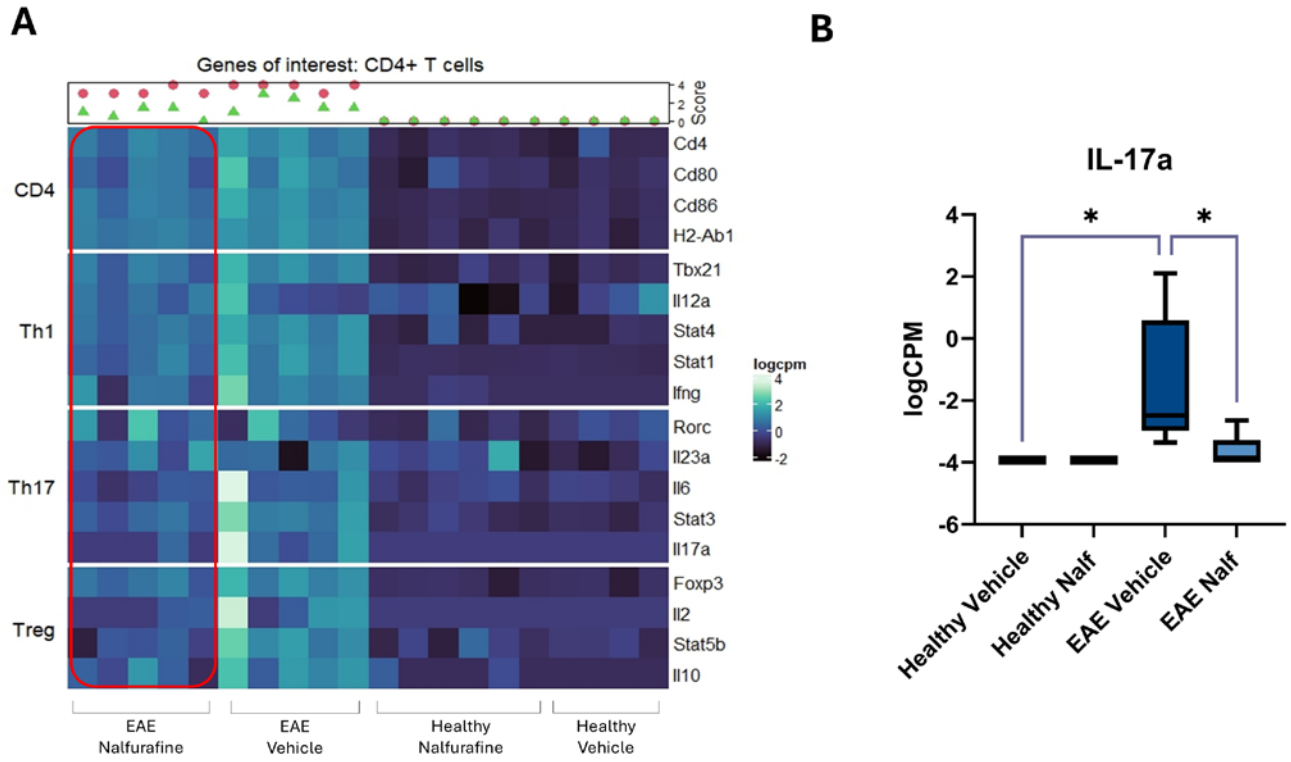

FIGURE 3: (A) Heatmap showing CD4 associated genes. Top row represents disease scores at peak disease (red) and end of experiment (green). **(B)** Expression level (logCPM) of IL-17A across treatment groups. n = 4-6.

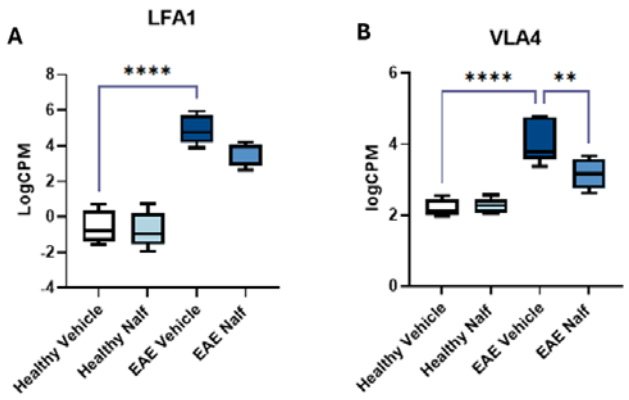

FIGURE 4: Expression level (logCPM) of migration associated genes. A) LFA-1. B) VLA4. n = 4-6.

CONCLUSION

These results support the hypothesis that KOR agonists have immunomodulatory potential that can influence the CD4+ T cell compartment. Our data suggests an ability of nalfurafine treatment to downregulate the expansion of inflammatory CD4+ T cell subsets in the periphery, particularly Th17 cells. In turn, this downregulation could contribute to the reduction in disease progression and CD4+ T cell infiltration into the CNS observed in studies using the EAE model. Additionally, nalfurafine treatment during the EAE mouse model of multiple sclerosis reduced the expression of Th17-associated genes, further highlighting the immunomodulatory potential of this treatment.

Te Herenga Waka
Victoria University of Wellington

The effects of *in vivo* anti-platelet therapy on neutrophils.

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

KEY FINDINGS

In this study, we examined the effects of *in vivo* dual anti-platelet therapy (DAPT) – as the mainstay therapeutic management of acute myocardial infarction (AMI) – on neutrophil function and phenotype. We demonstrated that neutrophil superoxide production was dampened following loading doses of DAPT. We also demonstrated that neutrophils exhibited phenotypic changes following *in vitro* stimulation and loading and maintenance doses of DAPT, however other functional pathways were not altered. These findings suggest that *in vivo* DAPT indeed elicits off-target effects on neutrophils, providing a foundation for future studies investigating the implications of DAPT on the neutrophil response to AMI.

OBJECTIVES

The overall aim of this project was to characterise the neutrophil response to *in vivo* anti-platelet treatment. The specific objectives of this project were:

Objective 1: Determine the effects of short-term DAPT (aspirin and ticagrelor) on key neutrophil functions including activation, degranulation, phagocytosis, and production of reactive oxygen species (ROS) and soluble inflammatory mediators.

Objective 2: Determine the long-term effects of DAPT (aspirin and ticagrelor) on key neutrophil functions as described in Objective 1.

METHODS

We recruited eight healthy subjects to examine the effects of *in vivo* DAPT on neutrophils (HDEC 2024 EXP 19948). All study participants received DAPT for seven days. DAPT was defined as a combination of aspirin and ticagrelor. Study participants received loading doses of DAPT on Day 1 (300 mg aspirin and 180 mg ticagrelor). For the remainder of the study period, participants received daily maintenance doses of DAPT (100 mg aspirin once daily and 90 mg ticagrelor twice daily). We chose to examine neutrophil phenotype and function after 24-hours and 7-days of DAPT to investigate whether short- and longer-term DAPT provoked different effects on neutrophils.

Peripheral venous blood was collected via venepuncture into sodium citrate or EDTA collection tubes for monitoring platelet and neutrophil activity, respectively. Blood samples were collected before loading doses of DAPT on Day 1, 24-hours after DAPT loading doses, and after seven days of DAPT. Platelet-rich (PRP) and platelet-poor (PPP) plasma was isolated from sodium citrate tubes for monitoring of platelet activity using a variation of light transmission aggregometry (LTA).

Neutrophils were isolated from EDTA tubes by negative magnetic separation and stimulated with lipopolysaccharide (LPS), N-Formylmethionyl-leucyl-phenylalanine (fMLP), or pHrodo Green Bioparticles, or supplemented with cell culture media for 1 hour at 37°C/5% CO2. To monitor neutrophil ROS production, isolated neutrophils were incubated in the presence of the colorimetric agent water salt tetrazolium (WST)-1 alongside fMLP for 20-minutes at 37°C. Superoxide anion production was assessed at 450nm every 30 seconds for 20 minutes, whereby an increase in absorbance over this time was indicative of reduction of WST-1 to formazan by superoxide anions.

Neutrophil phagocytic capacity was assessed by flow cytometry using pHrodo Green bioparticles. To assess the specificity of this assay for examining phagocytosis, neutrophils were incubated with the phagocytosis inhibitor, latrunculin A, alongside pHrodo Green bioparticles for this time.

Neutrophil release of interleukin (IL)-1β, IL-6, IL-8, IL-10, and IL-12p70 were assessed by cytometric bead array (CBA) in the culture supernatant of unstimulated stimulated neutrophils, however median fluorescent intensities (MFIs) were only obtained for IL-8.

Neutrophil phenotype was examined by flow cytometry using an 18-colour panel we have previously developed. Isolated neutrophils were stained with this 18-colour panel post-stimulation, and neutrophil phenotype was analysed in two different ways to determine the effects of DAPT: 1) by assessing changes in the ability of neutrophils to activate in response to stimulation with fMLP and LPS, and how this changed with DAPT on board, and 2) by assessing changes in the phenotype of unstimulated neutrophils pre- and post-DAPT.

RESULTS

LTA indicated effective platelet inhibition in response to DAPT (Figure 1). After 24-hours of DAPT, neutrophils exhibited dampened superoxide anion production compared to baseline (mean difference 19.55±20.15%, $p = 0.03$; Figure 2A), however superoxide anion production was similar to baseline after seven days of DAPT, and between 24-hours and seven-days of DAPT. We did not detect a significant change in neutrophil phagocytic capacity after 24-hours or seven days of DAPT (Figure 2B). We did not detect a significant difference in neutrophil release of IL-8 in fMLP- or LPS-supernatants after 24-hours or seven days of DAPT (Figure 2C).

Unstimulated neutrophils exhibited increased expression of CD11c and CD177 after 24 hours and CD62L after 7 days of DAPT (Figure 3A). We did not detect a significant difference in the proportion of $CD63^+$ neutrophils in unstimulated neutrophils after 24 hours or 7 days of DAPT, as these proportions were highly variable. Neutrophils also exhibited altered expression of activation markers in response to *in vitro* stimulation after DAPT (Figure 3B-C). Compared to baseline, fMLP- and LPS-stimulated neutrophils exhibited reduced expression of CD16 after 7 days, but not 24 hours of DAPT (both $p = 0.02$). fMLP-stimulated neutrophils also exhibited reduced CD11b expression ($p = 0.04$) alongside increased expression of CXCR1 ($p = 0.02$) after 7 days of DAPT (Figure 3B). Further, the proportion of $CD63^+$ neutrophils was higher in response to stimulation with fMLP after 24 hours and 7 days of DAPT, compared to baseline (both $p = 0.04$). The proportion of $CD63^+$ neutrophils was also higher in response to LPS stimulation after DAPT, however this difference was only statistically significant after 7 days (Figure 3C, $p = 0.04$). LPS-stimulated neutrophils also exhibited decreased CD177 expression after 7 days of DAPT ($p = 0.04$).

CONCLUSION

We demonstrated that following platelet inhibition with *in vivo* DAPT, neutrophils exhibited dampened ROS production after 24-hours, but not 7-days, and similar phagocytic capacity and IL-8 production at all timepoints. Additionally, we demonstrated that the distributions of neutrophil populations were not altered with *in vivo* DAPT. However, *in vivo* DAPT did alter the expression of some canonical and non-canonical activation markers, both in the absence and presence of an *in vitro* stimulant. These findings suggest that the therapeutic management of AMI patients with DAPT may inadvertently dampen some, but not all, neutrophil functions, and that DAPT does not affect neutrophil differentiation or maturation.

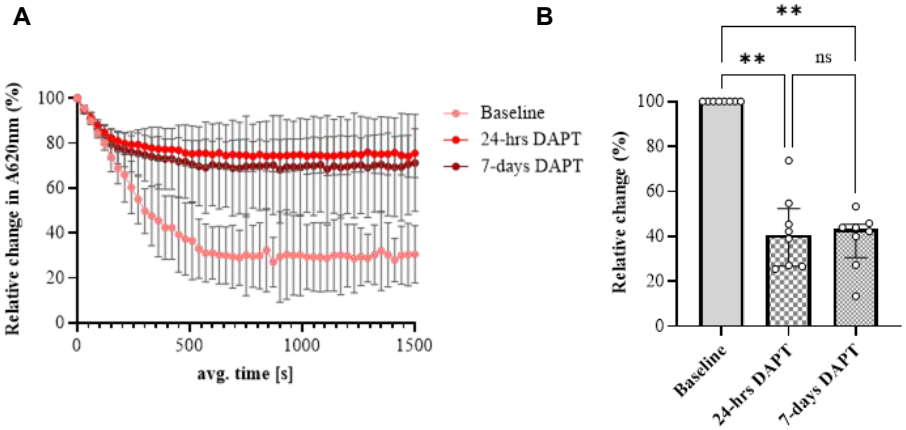

FIGURE 1: Platelet aggregation before and after DAPT. PRP and PPP were isolated from whole blood by density centrifugation and stimulated for 25-minutes with 20.00 µM ADP. Maximum aggregation was reported as the change in A620nm of PRP from baseline (0 minutes) to 25 minutes, relative to the difference in absorbance between PRP and PPP at 25 minutes and expressed as a percentage of the initial baseline difference. **(A)** A620nm readings were taken every 30-seconds to monitor platelet aggregation during this time. Mean ± SD of the eight study participants. **(B)** Platelet aggregation in response to ADP was significantly reduced after 24-hours and 7-days of DAPT. Median ± IQR of the eight study participants. All experiments were performed in triplicate. **p < 0.01 with Friedman test with Dunn's multiple comparisons.

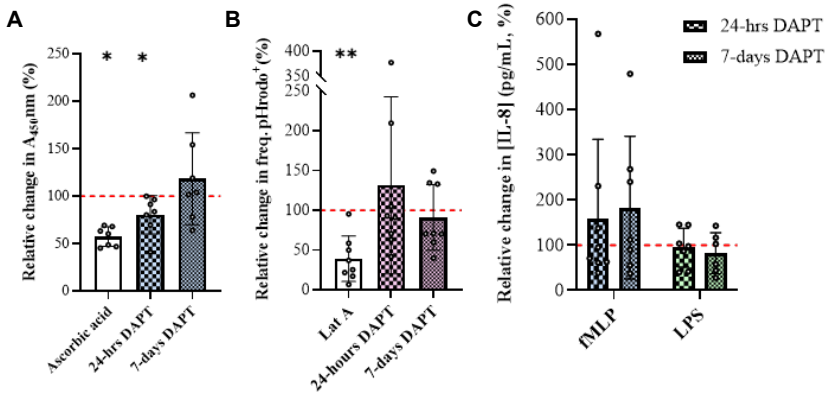

FIGURE 2: The effect of in vivo DAPT on neutrophil effector functions in vitro. **(A)** Magnetically isolated neutrophils were stimulated for 20-minutes with fMLP alongside the colorimetric agent WST-1. A450nm was read at the end of this time and is as proportional to superoxide anion production. Neutrophils exhibited dampened superoxide anion production after 24-hours, but not 7-days of DAPT compared to baseline (red-dotted line at y = 100). **(B)** Isolated neutrophils were stimulated for 1-hour with 781.1 nM pHrodo bioparticles and the proportion of pHrodo+ neutrophils (CD66b+CD15+) was examined by flow cytometry. The proportion of pHrodo+ neutrophils did not differ significantly to baseline after DAPT treatment. **(C)** Neutrophil IL-8 production was quantified by CBA in stored supernatants from neutrophils stimulated for 1-hour with fMLP and LPS. IL-8 production was highly variable, and did not differ after DAPT treatment. Values are expressed as relative change compared to baseline (red-dotted line at y = 100). Mean ± SD of the eight study participants, except for the superoxide anion assay (A) which was mean ± SD of seven study participants. *p < 0.05 **p < 0.01 with Wilcoxon matched-pairs signed rank test.

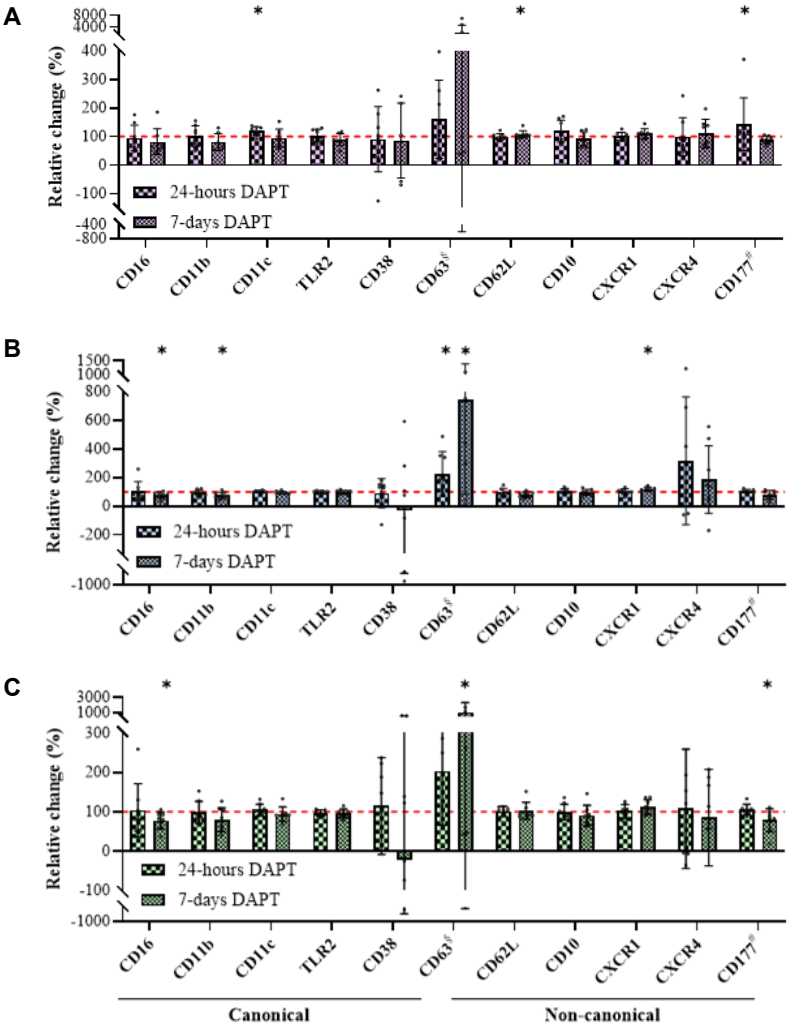

FIGURE 3: The effect of in vivo DAPT on cell-surface activation marker expression in vitro. Magnetically isolated neutrophils were cultured with CCM **(A)**, fMLP **(B)**, or LPS **(C)** for 1-hour and stained with our 18-colour panel to identify neutrophil subsets and monitor activation before and after DAPT. Subsets were identified utilising the FlowSOM-guided manual gating strategy from Chapter 6. Values are expressed as the relative change in MFI (on the CD66b+CD15+ neutrophil population) compared to baseline (red-dotted line at y = 100). Mean ± SD of the eight study participants. #change in MFI of CD177+ population. *p < 0.05 with Wilcoxon matched-pairs signed rank test.

Te Herenga Waka
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Characterising the immunomodulatory properties and mechanism of action of a novel Btk and Bmx inhibitor in B cells and microglia.

E-R GRANT, J MCCONE, R KEYZERS, J HARVEY, A LA FLAMME

KEY FINDINGS

While B cells exposed to pro-inflammatory stimuli were not influenced by co-treatment with novel Btk and Bmx inhibitor (–)-TAN-2483B, inflammatory microglial functions were mitigated in a manner distinct from potent Btk inhibitors ibrutinib and fenebrutinib. These results indicate that dual inhibition of Btk and Bmx proteins found in microglia, compared to sole Btk inhibition in B cells, lead to the unique immunomodulatory activity of (–)-TAN-2483B.

OBJECTIVES

Multiple sclerosis (MS) is a chronic inflammatory autoimmune disease of the central nervous system (CNS), characterised by periods of demyelination that lead to neurodegeneration. Immune cells infiltrate into the CNS, and in combination with microglia, these cells work together to induce neuronal demyelination. While a few disease-modifying therapies target the peripheral immune response, none impact the CNS-compartmentalised inflammation in progressive MS. Bruton's tyrosine kinase (Btk) is involved in the signalling pathways of many immune cells including B cells, microglia, and macrophages. Btk has been implicated in the pathogenesis of chronic diseases such as MS, making it an attractive target for MS therapy.

(–)-TAN-2483B is a fungal metabolite and novel Bmx (bone marrow tyrosine kinase on chromosome X) and Btk inhibitor (McCone et al., 2020) , which has shown distinct bioactivity from pre-existing Btk inhibitors, by modulating macrophage phenotypes away from pro-inflammatory states. The aim of this project was to investigate the immunomodulatory activity of the novel inhibitor (–)-TAN-2483B in B cells and microglia, this was achieved in 2 specific objectives:

Objective 1: *Investigate immunomodulation of B cells upon in vitro stimulation and treatment with Btk inhibitors*

Objective 2: *Characterise the immunomodulatory potential of ex vivo Btk inhibitor treatment of microglia isolated from a pre-clinical model of multiple sclerosis.*

RESULTS

Immunomodulation of B cells upon in vitro stimulation and treatment with Btk inhibitors. Owing to the high expression of Btk in B cells, our next avenue of research was to investigate the dual Btk and Bmx inhibitor (–)-TAN-2483B in B cells. After determining a non-cytotoxic dose of (–)-TAN-2483B in splenocytes, along with potent Btk inhibitors ibrutinib and fenebrutinib, an investigation into different aspects of B cell function, such as cytokine and antibody secretion, expression of activation markers, and cellular proliferation was conducted. While ibrutinib and fenebrutinib strongly inhibited B cell proliferation (**Figure 1a**) and expression of co-stimulatory markers such as CD86 (**Figure 1b**), (–)-TAN-2483B did not influence B cell function in the assays conducted, summarised by **Figure 1c.**

Modulation of microglia isolated from a pre-clinical model of MS upon ex vivo treatment with Btk inhibitors. Microglia (MG) were isolated from mice induced with an animal model of MS called experimental autoimmune encephalomyelitis (EAE) and healthy controls, and were subject to Btk inhibitor treatment *ex vivo*. Unlike in B cell assays, (–)-TAN-2483B was shown to modulate pro-inflammatory microglia responses, by reducing chemokine and cytokine secretion (**Figure 2a-d**), lowering iNOS and CD80 expression whilst enhancing Arg-1 (**Figure 2e-h**), suggesting an ability of (–)-TAN-2483B to re-polarise microglia phenotype to one associated with anti-inflammatory functions.

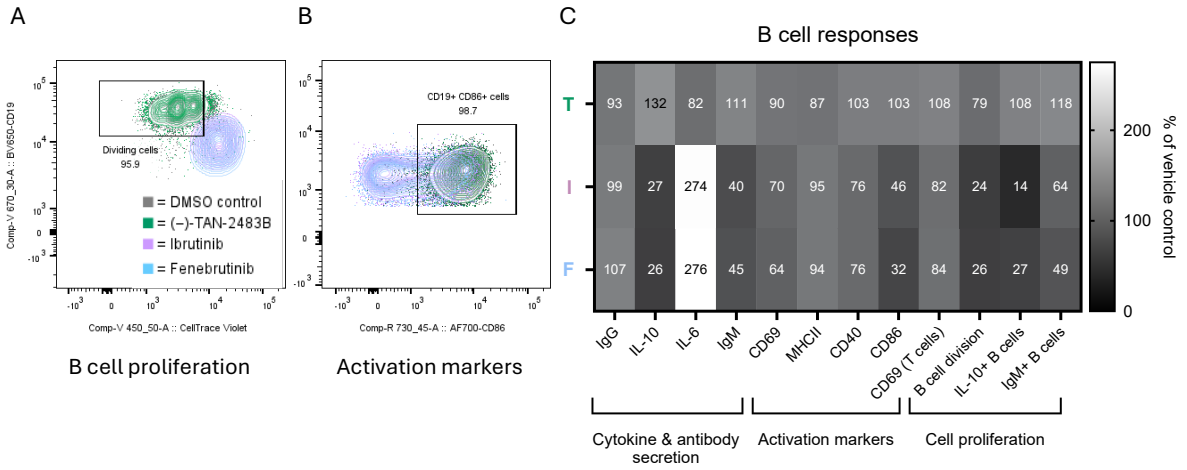

FIGURE 1: CD19+ B cells were stimulated with anti-IgM and co-incubated with (-)-TAN-2483B, ibrutinib, fenebrutinib or equivalent vehicle control for **(A)** 96-hours to assess cellular proliferation, **(B)** 24-hours for activation marker expression, or **(C)** 48-hours to detect cytokine and antibody secretion.

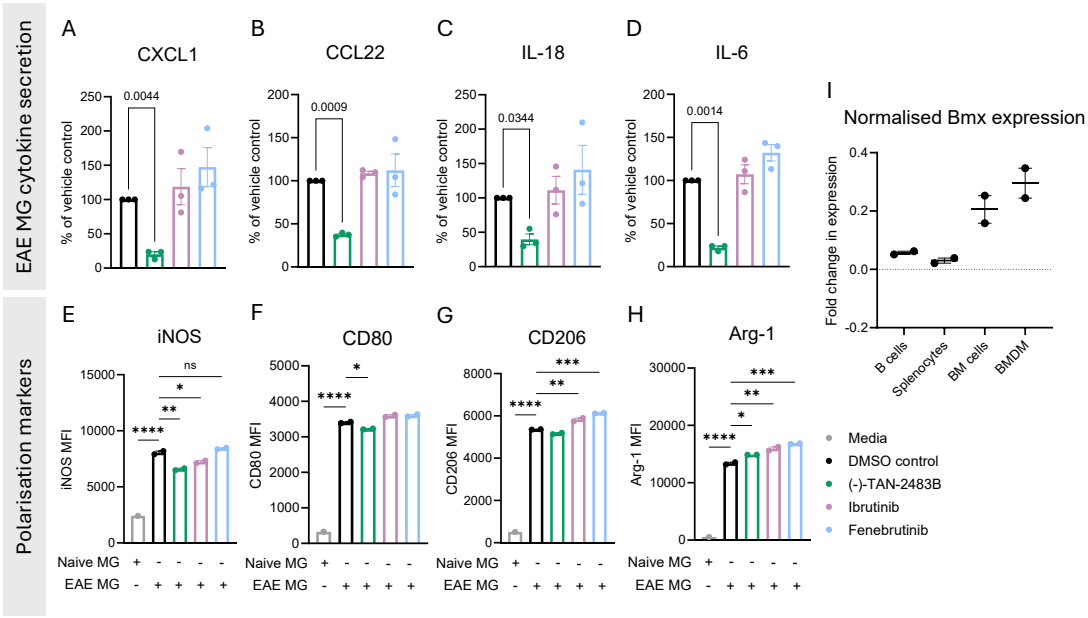

FIGURE 2: P2RY12+ microglia (MG) were magnetically isolated from brains and spinal cords of naive and EAE mice and treated with (-)-TAN-2483B, ibrutinib, fenebrutinib or equivalent vehicle control for 48 hours. **(A-D)** Cytokines and chemokines accumulated in supernatants were assessed cytometric bead array, and pro- **(E-F)** and anti-inflammatory markers **(G-H)** were assessed by flow cytometry. Cell lysates from B cells, splenocytes, bone marrow (BM) cells and bone marrow derived macrophages (BMDM) were used to detect total Bmx protein levels by western blotting, normalised to B-actin loading control **(I).**

It was hypothesised that the effectiveness of (–)-TAN-2483B in macrophages and microglia was due to the expression of both Btk and Bmx proteins in these myeloid cells, where the presence of both these proteins was confirmed by western blot (**Figure 2i**).

CONCLUSION

In this project, we explored how (–)-TAN-2483B modulates B cell responses, assessing B cell activation, proliferation, and protein phosphorylation. In addition, utilising a pre-clinical model of MS, we demonstrated the capacity of this compound to mitigate pro-inflammatory microglial functions. As macrophages and microglia have fundamental roles in the pathogenesis of MS, this research demonstrates the immunomodulatory potential of our dual Btk and Bmx inhibitor (–)-TAN-2483B in this context.

SCHOOL OF BIOLOGICAL SCIENCES
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Forewarned is forearmed: Investigating and mitigating resistance to the clinical antibiotic candidate niclosamide

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DAVID F. ACKERLEY

KEY FINDINGS

This research sought to evaluate four different classes of enzyme (a methyl-transferase, an α/β hydrolase and two unrelated nitroreductases) that previous work had shown can confer low-level resistance to the clinically-advanced antibiotic candidate niclosamide. We showed that the nitroreductases pose the greatest risk, not only because they are ubiquitous enzymes in bacteria, but because they can readily evolve to confer total or near-total niclosamide resistance. However, we also found that as these enzymes evolve high-level niclosamide resistance, they consistently sensitise their host cells to nitro-prodrug antibiotics (the PnbA nitroreductases strongly, the AzoR nitroreductases to a lesser extent), offering prospects for combination therapies to mitigate the emergence of niclosamide resistance.

OBJECTIVES AND METHODS

Directed evolution and selection

We tested whether our most-active methyltransferase and α/β hydrolase, as well as the most-active enzyme from two predominant nitroreductase families (PnbA and AzoR), could readily be evolved to confer high-level niclosamide resistance. Each resistance element was subjected to recursive rounds of random (error-prone PCR) mutagenesis to generate many millions of gene variants that were then expressed in model *E. coli* bacteria to select the most resistant strains.

Collateral sensitivity testing

A panel of nitroaromatic prodrugs comprising metronidazole, tinidazole, ronidazole, RB6145, azomycin, nifurpirinol, and nitrofurantoin was assembled for collateral sensitivity screening, to identify antibiotics to which the niclosamide-resistant bacteria had become hypersensitive.

RESULTS

Directed evolution

Previously, we had been unable to recover improved variants of our top methyltransferase enzyme. Here, we employed an alternative batch evolution approach involving multiple top variants. This likewise failed to recover improved variants. Overall, it appears there is no easy route for methylating enzymes to evolve to confer clinical levels of niclosamide resistance.

Conversely, we had previously made the worrying observation that a single mutation (C95F) in PnbA family nitroreductases was sufficient to yield high-level (albeit not total) niclosamide resistance. An additional round of evolution was performed for our top PnbA enzyme, yielding further improved variants. All contained the C95F mutation recovered in variants from the first round and a diversity of additional mutations that provided further increases in protection, up to the maximum achievable concentrations of niclosamide in solution (i.e., total resistance).

We discovered that AzoR nitroreductases could be evolved to near-complete niclosamide resistance, but that this was a lengthier process than for the PnbA enzymes, and did not quite attain full resistance. The α/β hydrolase proved even slower to evolve than the AzoR variants, but over several rounds of evolution gradually achieved near-complete resistance (**Figure 1**).

Collateral sensitivity

Collateral sensitivity analysis focused on the top variants of the nitro-reducing enzymes AzoR and PnbA4, on the basis

that a panel of clinical nitro-prodrug compounds could be rationally constructed for these. Here the rationale was that as mutations accrue to improve nitro-reduction (and thereby detoxification) of niclosamide, cells might inevitably become more sensitive to certain prodrugs, the toxicity of which is *activated* by nitro-reduction. Powerful collateral sensitivities were consistently observed for PnbA4, with the key C95F mutation playing a key role in underpinning sensitivity to all prodrugs tested. For AzoR, a similar pattern of collateral sensitivities was observed, but the extent of these was less than observed for PnbA4. Overall, it appears likely that clinical nitro-prodrugs could have utility in preventing or mitigating the emergence of niclosamide resistance driven by evolved PnbA enzymes, but that if AzoR-mediated resistance emerges, this might prove more difficult to combat. We additionally tested a veterinary analogue of niclosamide that lacks a nitro substituent, oxyclozanide, against all evolved enzymes and showed that it retained full activity against the nitroreductase-expressing strains, but that strains expressing the evolved α/β hydrolase variants had generally become more resistant to oxyclozanide (targeting the same amide bond as in niclosamide).

CONCLUSIONS

In contrast with methyltransferases, for which various directed evolution efforts failed to yield variants better able to defend *E. coli* host cells against niclosamide, a single mutation (C95F) in diverse PnbA family nitroreductase enzymes was sufficient to confer high-level niclosamide resistance. We showed that additional mutations could accrue to provide essentially complete niclosamide resistance, i.e. at levels beyond the limits of drug solubility. The leading AzoR family variant from our metagenomic screening was slower to gain resistance-conferring mutations, but after 3 rounds of evolution, combinations of 5+ mutations were sufficient to confer near-complete niclosamide resistance. The unique α/β hydrolase enzyme we recovered was even slower to evolve, but did eventually provide high-level niclosamide resistance after four rounds of evolution (resistant variants bearing 7+ mutations). Overall, based on the ubiquity of the diverse nitroreductase enzymes recovered from our metagenomic screening relative to the single α/β hydrolase, and the rapidity with which the former could evolve to provide complete or near-complete niclosamide resistance, we concluded that nitroreductases pose the greatest threat to clinical progression or utility of niclosamide as an antibacterial drug.

We next investigated collateral sensitivities, demonstrating that the mutations giving rise to high-level niclosamide resistant PnbA4 variants also confer hypersensitivity to clinical nitro-prodrug antibiotics including metronidazole, nitrofurazone and nitrofurantoin – an important finding to guide future stewardship measures. For example, these alternative antibiotics could be co-administered alongside niclosamide therapy to prevent resistance arising, or else used to treat evolved cases of resistance post-emergence. Unfortunately, the evolved AzoR family variants conferred substantially less sensitivity to either metronidazole, nitrofurantoin or nitrofurazone, or other antibiotics we tested that are in current human clinical or veterinary use. Thus, AzoR enzymes may ultimately pose a greater risk to the clinical longevity of niclosamide than PnbA nitroreductases. We also made the promising finding that a veterinary analogue of niclosamide that lacks a nitro substituent, oxyclozanide, retains full activity against cells expressing any of the evolved reductase variants. Oxyclozanide may therefore offer a future solution against the emergence of nitroreductase-driven niclosamide resistance.

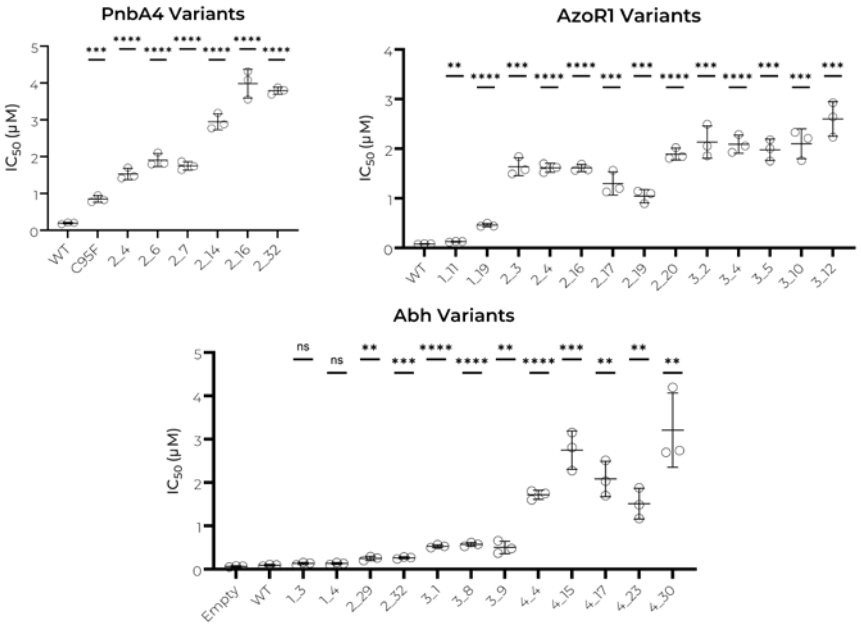

FIGURE 1: Evolutionary trajectories of lead PnbA, AzoR and α/β hydrolase (Abh) variants. *E. coli* cells producing evolved enzyme variants were challenged with 0-10 μM niclosamide over four hours. Growth was monitored by OD_{600} pre- and post-incubation. Percentage growth was normalised to an unchallenged replicate and used to calculate the IC_{50} value. Data are the means of three biological replicates, two technical repeats each. Error bars represent ± 1 std. dev. The first number of each variant identifier indicates the round of directed evolution from which it arose.

Te Herenga Waka
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Investigate The Effect of Kappa Opioid Agonism in Food Allergies Model

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KEY FINDINGS

This project demonstrated that mast cells, which drive immediate allergic reactions, can be regulated through the kappa-opioid receptor (KOR) pathway. In laboratory experiments, the KOR agonists Nalfurafine and U50,488H suppressed mast cell activation in a concentration-dependent manner, reducing the release of allergy-related mediators. In a mouse model of food allergy, treatment with these agonists lessened clinical symptoms, with mice showing milder reactions such as occurrence of diarrhoea and weight loss compared to untreated controls. These findings provide evidence that KOR signalling can calm mast cells and reduce the severity of food allergy responses, highlighting a promising pathway for future therapies.

OBJECTIVES

The project aimed to investigate whether activating the KOR could regulate mast cell activity and reduce food allergy symptoms. Specifically, I tested Nalfurafine and U50,488H to:

1. Assess their ability to inhibit mast cell activation *in vitro*.
2. Evaluate their potential to reduce allergic symptoms in a food allergy mouse model.

METHODS

Mast cells were generated from mouse bone marrow under culture conditions supported by IL-3 cytokines, which promote their development into functional cells. These mast cells were treated with Nalfurafine or U50,488H and then stimulated with PMA and ionomycin, chemical triggers that activate mast cells. Their response was measured by the surface marker CD107α, which indicates degranulation, the process by which mast cells release histamine and other inflammatory chemicals. Cell viability was assessed using Zombie NIR™ staining.

To test the effects *in vivo*, I used a mouse model of food allergy that I previously optimised. Mice were sensitised to ovalbumin (OVA), an egg-white protein, together with the adjuvant alum. Upon challenge with OVA, the mice developed allergic features such as diarrhoea and weight loss. After each challenge, mice were monitored for 60 minutes for any signs of food allergy. Symptoms were scored on a 0–4 scale:

0 = no symptoms, well-formed stool

1 = soft, sticky, well-formed stool

2 = soft, sticky, poorly formed stool

3 = liquid diarrhoea

4 = more than two episodes of liquid diarrhoea within 40–60 minutes post-challenge

This model was then used to evaluate whether treatment with KOR agonists could reduce the severity of food allergy symptoms.

RESULTS

C57BL/6 bone marrow–derived mast cells were seeded into wells and pre-treated with Nalfurafine (0.5–200 ng/mL), U50,488H (1–4 μM), or DMSO (control) for two hours, followed by stimulation with PMA (100 ng/mL) and ionomycin (500 ng/mL) for another two hours. Clemastine fumarate (1 μg/mL) was used as a positive control (dashed line). Activated cells were assessed by CD107α expression, and cell viability was determined using Zombie NIR™ by flow cytometry. (A,C) Activated mast cells were normalised to untreated controls and are shown as mean ± SEM. (B,D) Live

cells were normalised to untreated controls, with each point representing an independent culture (N = 3–20).

As shown in **Figure 1**, mast cells treated with the KOR agonists Nalfurafine and U50,488H displayed reduced activation after stimulation. Both drugs inhibited mast cell activation in a concentration-dependent manner (**Figure 1A,C**), with the strongest inhibition observed at 5 nM for Nalfurafine and 3 μM for U50,488H. Nalfurafine demonstrated the strongest effect, achieving ~60% inhibition at 5 nM, requiring nearly 600-fold lower concentration than U50,488H. At higher or lower concentrations, the inhibitory effect was diminished, producing an inverted bell-shaped concentration–response pattern, indicating that the drugs lose effectiveness beyond an optimal range. Importantly, neither Nalfurafine nor U50,488H altered mast cell viability (**Figure 1B,D**), as live cell frequencies were comparable to untreated controls. These findings suggest both compounds are effective at reducing mast cell activation while remaining safe and non-toxic to the cells.

(A) Schematic of the prophylactic treatment schedule. Male BALB/cByJ mice (8–12 weeks old) were sensitised and challenged with 25 mg/mL OVA. KOR agonist treatments were administered intragastrically 40 minutes before each challenge. (B) Mice were monitored for 60 minutes post-challenge for disease symptoms, scored on a 0–4 scale. (C) The area under the curve (AUC) of disease scores was calculated for each mouse. (D) Body weight was measured before each challenge, and data are shown as the percentage change relative to the first challenge (day 28). (E) The AUC for percentage weight change was calculated for each group. Statistical analysis of disease scores and body weight was performed using mixed-effects two-way repeated measures ANOVA, with significance indicated as *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001 by Sidak's test. AUC comparisons for disease scores and body weight relative to naïve controls were performed by one-way ANOVA (***p < 0.001, ****p < 0.0001). Results are combined from three independent experiments (n = 9–13 per group). Data are presented as mean ± SEM.

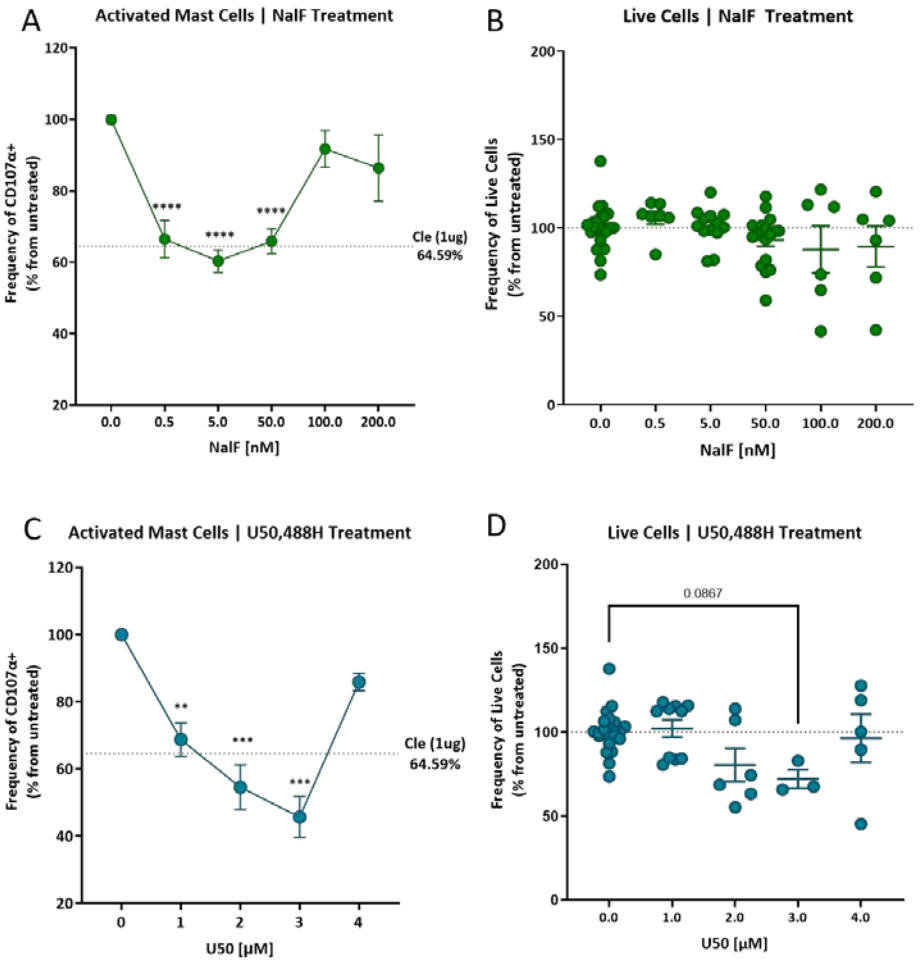

FIGURE 1: Nalfurafine and U50,488H reduced mast cell activation in a concentration-dependent pattern.

As shown in **Figure 2**, prophylactic treatment with the KOR agonists Nalfurafine and U50,488H reduced food allergy symptoms in OVA-sensitised mice, but Nalfurafine was more effective. Mice receiving Nalfurafine had significantly lower disease scores during the 60-minute monitoring period (**Figure 2.B**) and reduced cumulative disease severity, as reflected by lower AUC values (**Figure 2.C**), compared to U50,488H-treated mice. Nalfurafine also prevented body weight loss more consistently across repeated challenges (**Figure 2.D**), again reflected in significantly lower AUC values for weight change (**Figure 2.E**). Together, these results indicate that Nalfurafine provides stronger protection against food allergy symptoms than U50,488H, supporting its potential as a promising therapeutic candidate.

CONCLUSION

This project shows that KOR agonists can regulate mast cells and reduce allergic reactions, advancing understanding of immune regulation. These findings provide early evidence to guide future therapies and may ultimately improve safety, treatment options, and quality of life for individuals and families affected by food allergies.

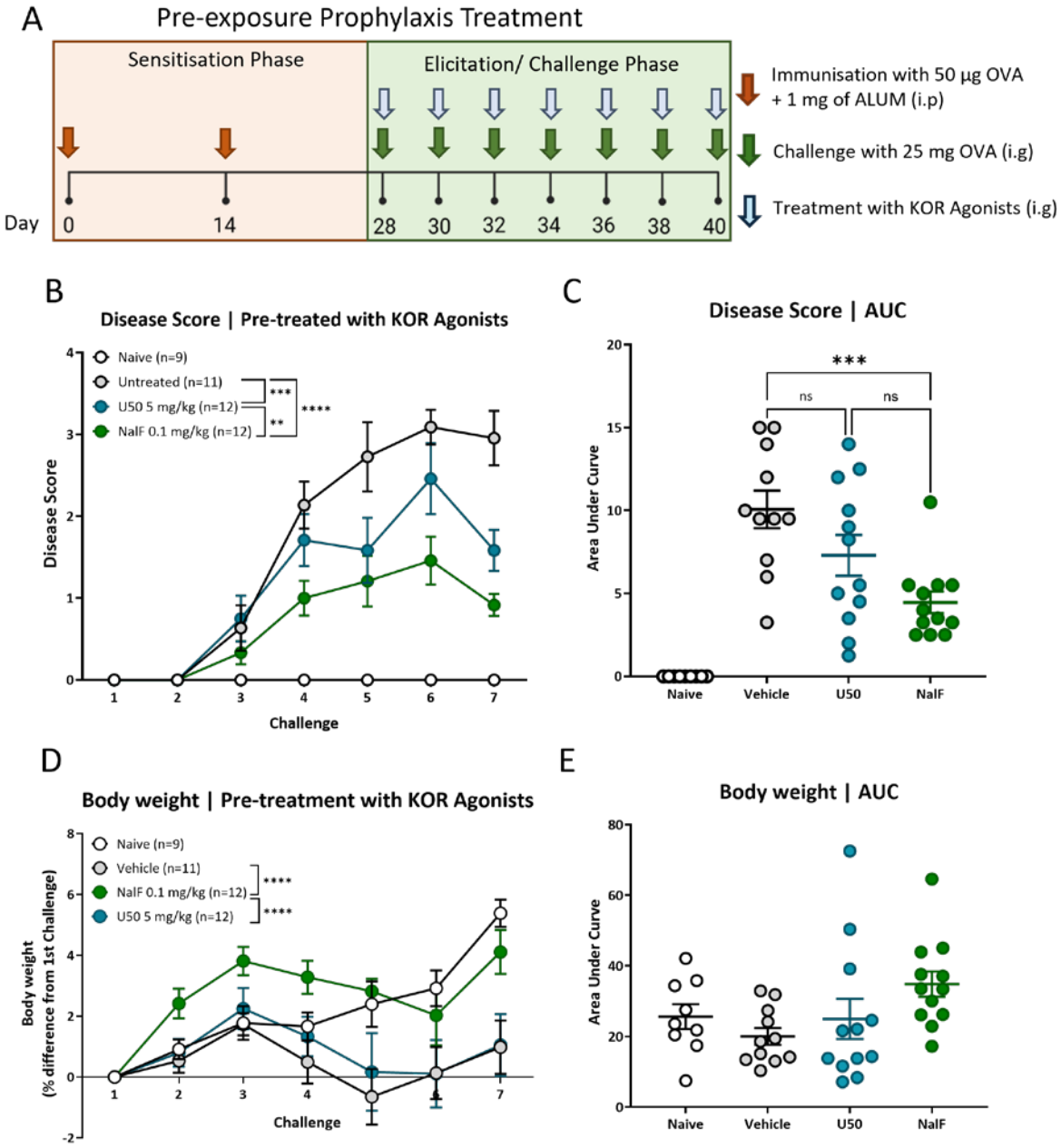

FIGURE 2: Nalfurafine more effectively reduces food allergy symptoms than U50,488H.

Te Herenga Waka
Victoria University of Wellington

Investigating remyelination as a novel treatment avenue for Alzheimer's disease

R VAN DE WETERING, B KIVELL

KEY FINDINGS

Our research indicates that loss of myelin, the protective coating that facilitates nerve conduction, directly causes an increase in amyloid plaques, a hallmark pathological feature of Alzheimer's disease. Future work will further explore this link and evaluate whether pharmacotherapies that restore myelin function might serve to delay the progression of Alzheimer's disease.

OBJECTIVES

Old age, more so than any genetic or environmental variable, is the primary risk factor for Alzheimer's disease (AD), yet the mechanisms linking brain aging to AD pathology remain unclear. A recent hypothesis proposes that premature demyelination in the aging brain directly contributes to disease progression [1]. Demyelination naturally occurs with aging, but it is exacerbated in AD patients and animal models of AD [2–5]. These observations are correlational, however, so it is not known whether demyelination is a secondary consequence of AD-induced neurodegeneration or if demyelination plays a more causative role. Therefore, this research aimed to investigate whether experimentally induced demyelination directly causes an increase in AD-like pathology by:

- Combining a cuprizone-induced demyelination model with the 5xFAD genetic mouse model of AD.
- Assessing whether demyelination increases amyloid plaque formation.
- Defining the region- and time-dependent nature of this effect.
- Evaluating the mediating role of microglia.

METHODS

To induce demyelination, male and female 12-week-old 5xFAD mice were fed a diet containing 0.2% cuprizone for 8 weeks. To control for potential confounding effects of disrupted copper homeostasis, which has limited previous investigations [1], some mice also received dietary supplementation with 0.1% $CuSO_4$. Cuprizone binds to copper with a stoichiometry of 2 cuprizone:1 copper; therefore, this ratio should any net change in copper levels [6]. After 8 or 12 weeks, mice were anaesthetised, perfused, and brains processed for immunohistochemistry. Coronal sections were labelled for myelin (MBP), microglia (IBA1), and amyloid-beta (6E10) and imaged using an Olympus FC3000 confocal microscope.

RESULTS

A summary of the major results is shown in Figure 1. Compared to controls that did not receive cuprizone, 8 weeks of cuprizone administration caused widespread demyelination with partial recovery by the 12-week time point. Demyelination was observed in both grey (cortex, hippocampus) and white (alveus) matter regions. In the alveus, demyelination was also accompanied by significant microgliosis (increase in % area IBA1). Interestingly, dietary copper supplementation had little impact on cuprizone-induced demyelination or microgliosis, supporting the idea that cuprizone does not cause demyelination through a homeostatic copper deficit, but rather through the formation of a toxic copper-cuprizone complex [6].

Importantly, cuprizone-induced demyelination significantly increased amyloid plaque burden, but this effect was limited to the alveus, a major white matter tract of the hippocampus, and was most pronounced at the 12-week time point. The basis for this region and time-dependent effect is unclear; however, it is worth noting that the alveus was the only demyelinated white matter region examined that also exhibited substantial plaque formation under normal conditions. Copper supplementation had no effect on plaque formation, indicating that this effect is not copper-dependent, as was previously thought [1].

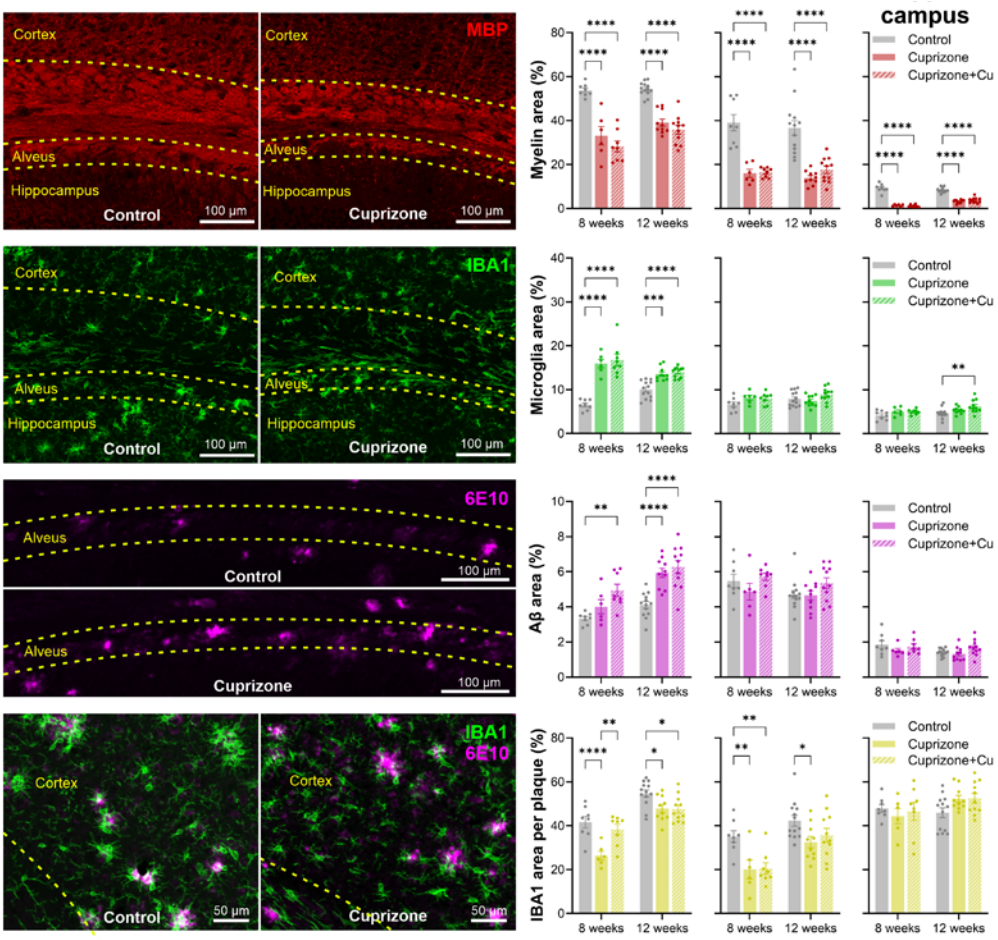

FIGURE 1: Effect of cuprizone administration on myelin (MBP), microglia (IBA1), amyloid β (6E10) and plaque-associated microglia (IBA1 on 6E10) in the alveus, hippocampus, and cortex of 5xFAD mice, as determined by immunohistochemistry and confocal microscopy. Data are presented as mean ±SEM. n = 6-13/group/time-point. ****p<.0001, ***p<.001, **p<.01, *p<.05, two-way ANOVA, Tukey post-tests.

To investigate a potential mechanism mediating the link between demyelination and amyloidosis, we then determined if there was also a corresponding shift in microglial activity. It is thought that a 'distraction' of microglia towards the processing of myelin debris at the expense of amyloid plaques might drive demyelination-induced amyloidosis. Our findings provide mixed support for this idea. We found that demyelination significantly reduced the plaque-associated microglia in the hippocampus alveus, but this effect was the greatest in the cortex, an area that did not show any increases in amyloid plaques.

CONCLUSION

It has been proposed that premature demyelination in the aging forebrain leads to neuroinflammation and microgliosis, which in turn, interferes with the ability of microglia to clear amyloid β deposits, leading to an increase in plaque formation [1] . To investigate this idea, we experimentally induced demyelination in mice genetically predisposed to develop amyloid plaques. Our findings show that demyelination plays a causal role in driving AD-like pathology by increasing amyloid plaque formation, but this effect may be both region- and time-dependent. Future analyses will determine the impact of demyelination in other brain regions before exploring the viability of therapeutic intervention with remyelinating pharmaceuticals as a novel strategy to prevent amyloid plaque aggregation.

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Te Herenga Waka
Victoria University of Wellington

Structural and functional characterisation the FAM171 family of neuronal proteins

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KEY FINDINGS

Protein structure determines protein function, thus, characterising a proteins three-dimensional structure allows us to understand at a molecular level how a protein interacts with other proteins and ligands within the human body. The FAM171 neuronal receptors (A1, A2, and B) are an understudied family of three proteins with no published functional or structural data despite their genetic implications in multiple types of cancer, and neurodegenerative diseases. In this ongoing project, we have previously identified an interaction network within the FAM171 family, where A1 and A2 exhibit selfinteraction, and both also interact with B. Despite their high sequence similarity, we have also identified distinct structural features for each protein using complementary biophysical techniques. A1 forms a stable trimeric structure while A2 and B exist in monomer-trimer equilibria. Recently, we have solved high resolution structures for A1 and A2, revealing potential molecular mechanisms underlying their function in the body in health and disease.

OBJECTIVES

1. Determination of the binding characteristics of the FAM171 family of proteins by the biophysical analysis of these interactions using ITC and ELISA.
2. Determination of the three-dimensional structures of each protein and protein complex. Specifically,
   a. Low-resolution, in-solution structures (SAXS).
   b. High-resolution structures (Cryo-EM).

METHODS

a. Large scale expression of FAM171 proteins required generation of stable cell lines using HEK293 GnTI- cells. Protein was secreted into the cell culture media and purified using Protein A affinity resin, followed by size exclusion chromatography (SEC).

b. Isothermal titration calorimetry (ITC) and ELISA assays were utilised to measure protein interactions in solution.

c. Small angle x-ray scattering (SAXS) was also used for investigating protein behaviour in solution. These experiments were carried out on the SAXS/WAXS and BioSAXS beamlines at the Australian Synchrotron. SEC-SAXS with protein at a concentration of 5–10 mg/mL with a co-flow setup was used for data collection.

d. Determination of high-resolution protein structure utilised cryogenic electron microscopy (cryo-EM) techniques at the Ian Holmes Imaging Centre, Bio 21 Institute, University of Melbourne. Purified FAM171 proteins were first screened for data collection suitability by negative stain EM at 10 ug/mL. FAM171 protein at 1–10 mg/mL was then used for full data collection on a Titan Krios G4 EM at 105,000x magnification, followed by data processing in CryoSPARC, and structure solution using Coot and Phenix.

RESULTS

Truncated FAM171 protein constructs were designed, successfully improving expression and purification without altering their behaviour in solution (**Figure 1 A,B**). ITC

experiments utilised these truncated constructs to further validate FAM171 selfinteraction, as well as verifying the nanomolar affinity of each interaction previously determined using an optimised ELISA affinity assay (**Figure 1 C**).

SAXS experiments were employed to determine FAM171 behaviour in solution. Each protein was found to form trimers at high concentrations, consistent with previous SEC and analytical ultracentrifugation experiments. Follow up experiments on the new BioSAXS beamline at the Australian Synchrotron confirmed these results, and also identified the formation of a much larger, non-aggregated FAM171A1 species at high concentrations, consistent with a 60-mer (see below).

Finally, cryo-EM was able to provide the first high resolution structures of FAM171A1 and FAM171A2 at resolutions of 2.30 and 2.78 Å, respectively (**Figure 1 D,E**), both of which were in agreement with the in solution SAXS data. The extracellular domain of FAM171A1 was identified to form an icosahedron consisting of 20 identical trimers (60-mer), while FAM171A2 was found to form a similar trimeric structure. The full length FAM171A1 cell surface receptor is unlikely to form this spherical structure *in vivo* due to the physical constraints of a cell. However, the interactions identified between trimers are possible and may allow for distinct cell signalling upon clustering of FAM171A1 trimers at the cell surface. Conversely, FAM171A2 was only found as a trimer at high resolution, as well as a monomer in solution in prior experiments. Comparison of the trimer interfaces of FAM171A1 and FAM171A2 identified key interactions that may justify the stability of the FAM171A1 trimer compared to the monomer-trimer equilibrium of FAM171A2 (**Figure 1 F**). In particular, FAM171A1 contains two additional hydrogen bonds and a salt bridge that likely stabilise its trimeric structure.

CONCLUSION

We have made good progress in resolving the structure and function of the FAM171 family of proteins, with high resolution structures of FAM171A1 and FAM171A2 providing insights into their different behaviours in solution. A manuscript describing this work has been prepared and will be uploaded shortly to bioRxiv to make it publicly available prior to submission to a peer-reviewed journal.

Meanwhile, investigations of the FAM171 proteins is ongoing in the Comoletti lab, focussing on resolving a high resolution structure for the FAM171A2-FAM171B complex. Additionally, cell-based assays are being optimised by collaborators Joris de Wit (VIB Leuven, Belgium) and Thomas Biederer (Yale University, CT, USA). This new collaboration will focus on better understanding the nature of FAM171 interactions on the cell surface. Combined, we hope the results of the current and future work will further our knowledge of the FAM171 receptors in the brain and provide insights into their roles in human diseases.

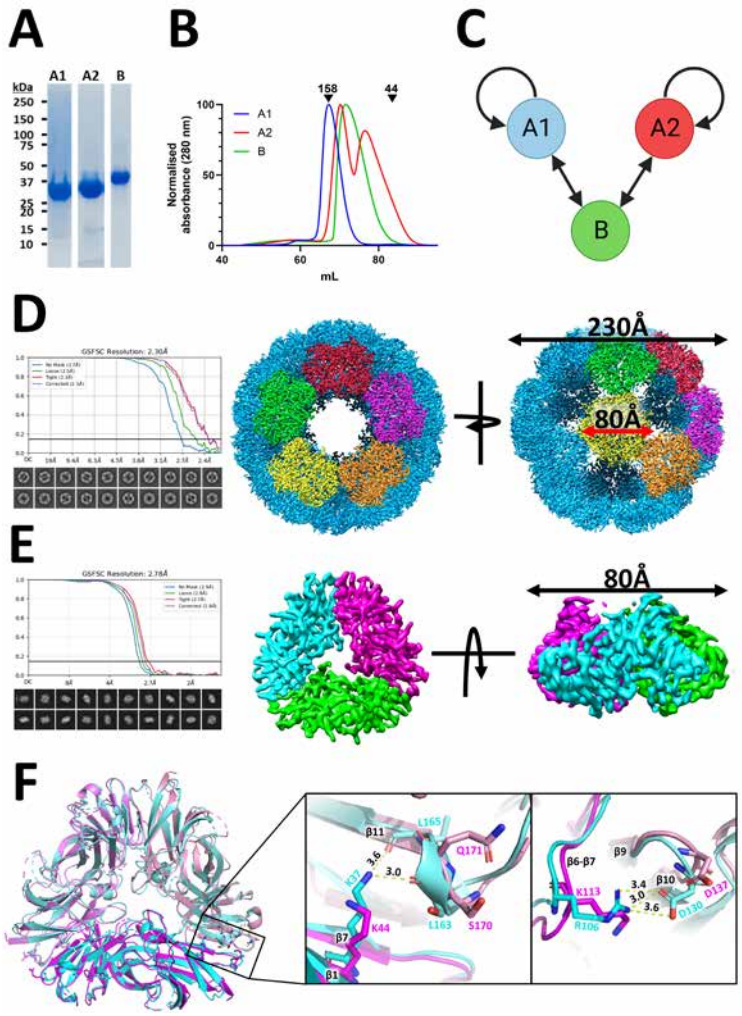

FIGURE 1: (A) Coomassie-stained SDS-PAGE of purified FAM171 extracellular domains. **(B)** Superdex SEC trace of purified FAM171 extracellular domains. Protein standards indicated with arrows (kDa). **(C)** FAM171 interaction network. **(D)** (Left) FAM171A1 cryo-EM FSC curve and 20 representative 2D classes. (Right) 3D model of the FAM171A1 60-mer showing five trimers in different colours. **(E)** (Left) FAM171A2 cryo- EM FSC curve and 20 representative 2D classes. (Right) 3D model of the FAM171A2 trimer showing each monomer in different colours. **(F)** Overlayed structures of FAM171A1 (shades of cyan), FAM171A2 (shades of magenta) (left) and key trimer stabilising interactions (right). H-bond and salt bridges distances highlighted by yellow dashed lines.

University of Otago, Wellington

Safely improving outcomes for babies after birth planned by caesarean section in Wellington – the C*STEROID trial

INTERIM REPORT

ORMANDY, J

Te Whatu Ora Capital & Coast

KEY FINDINGS

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 parents.

Administering corticosteroids before birth to mothers giving birth prior to 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.

AIMS

To assess whether antenatal administration of corticosteroids prior to planned Caesarean will reduce neonatal respiratory morbidity and whether it can cause neonatal hypoglycaemia.

METHODS

Study Design: Multi-centre, triple-blind, placebo-controlled, parallel, phase III trials conducted across hospitals in Aotearoa and Australia.

Study Setting and Population: C*STEROID invites women without diabetes undergoing planned pre-labour caesarean section at 25 hospitals to participate.

Inclusion Criteria: Planned CS at 35^{+0} - 39^{+6} weeks gestation; >24 hours and <7 days until planned birth; Singleton or twin pregnancy.

Exclusion Criteria: Major fetal or chromosomal abnormalities, prior corticosteroid use for fetal lung maturity, prior trial participation, diabetes (pre-existing or gestational).

Intervention and randomisation: Women are randomised 1:1 into one of two groups; Corticosteroid Group - two doses of 11.4 mg betamethasone (Celestone Chronodose®) or Placebo Group - two doses of visually matching placebo, both given by intramuscular injection 24 hours apart.

Neonatal monitoring: Neonatal blood glucose concentrations will be measured after first-feed at 1-2 hours of age, then pre-feed 3-4 hourly until 12 hours of age. If hypoglycaemia occurs treatment following the national Oral Dextrose Gel guideline will be recommended and testing continued until at least 12 hours after last low glucose.

Primary Outcome:

- Co-primary outcomes to accurately assess benefit and harm — neonatal respiratory distress requiring >60 minutes of support (benefit), and hypoglycaemia <2.6 mmol/L (harm).

Secondary Outcomes:

Neonatal: incidence of hypoglycaemia and treatment, neonatal unit admission/duration, respiratory distress severity, need for/duration of respiratory support, infection

Maternal: Infection post-randomisation, reported side effects, breastfeeding rates, maternal wellbeing at baseline and 6 weeks postpartum; duration of hospital stay;

Long-term follow-up: Future studies are planned to assess childhood neurodevelopment, growth, and respiratory, cardiovascular, and metabolic outcomes - at 6–7 years

RESULTS

There are now 25 hospitals across Australasia participating in C*STEROID and Wellington Hospital is one of eight New Zealand hospitals participating. The trial started in October 2020 and Wellington joined in October 2021. The trial aims to recruit 2500 women and in August 2025 hit the '1500 babies' milestone. Wellington hospital has recruited 53 women and 54 babies.

CONCLUSION

Recruitment is ongoing and the trial it is anticipated that the trial will continue until at least the end of 2027. The grant provided by Research For Life supported our first 50 participants.

Wellington Hospital continues to recruit for C*STEROID and our site target is 3 recruits per month.

University of Otago, Wellington

NETendo: Neutrophil Extracellular Traps in endometriosis

K BORCHOWSKY, E PATERSON, C JONES, K HALLY, C HENRY

KEY FINDINGS

The proportion of neutrophils with appendant neutrophil extracellular traps (NET burden) was significantly greater in those with non-endometriosis pelvic symptoms compared to those with endometriosis and to healthy controls. There was no significant difference in NET burden between endometriosis patients and healthy controls.

AIMS

This study aimed to compare NET burden in neutrophils from the venous blood of endometriosis patients to those having undergone surgery with no endometriosis found, and to healthy control using a novel flow cytometry panel.

METHODS

Consenting participants undergoing surgery for suspected endometriosis were recruited from Wellington and Kenepuru hospitals on the day of surgery. Blood was collected into ethylenediaminetetraacetic acid (EDTA) tubes and serum separation tubes (SST) prior to commencing anaesthesia. Participants were grouped into the 'endometriosis' group or the 'symptomatic control' group based on surgical and histopathological findings. Furthermore, blood was collected from healthy volunteers with no endometriosis-type symptoms forming the asymptomatic control group.

Processing began as soon as possible after sample collection, and always within four hours. 2mL of EDTA-anticoagulated blood was used to isolate neutrophils through negative magnetic bead separation. Neutrophils, at a concentration of 4×10^6 cells/mL, were stained with optimised antibodies titres in accordance with a seven-colour flow cytometry panel designed to identify NET appendant neutrophils (Jones et al., 2024). Markers used included neutrophil markers (CD15 and CD66b), putative markers of NETosis (extracellular DNA, myeloperoxidase (MPO), neutrophil elastase (NE) and citrullinated histones) and a viability marker (Zombie NIR). Immediately after staining, samples were acquired on a three laser (violet-blue-red) Cytek Aurora flow cytometer.

Gating strategy: The gating strategy can be found in Figure 1. Granulocytes were identified by their forward scatter (FSC) and side scatter (SSC) profiles. After doublet exclusion, neutrophils were identified through positive surface expression of the two neutrophil markers. Cells which were Zombie NIR^{bright} were deemed necrotic and excluded. Live, NET appendant neutrophils were identified through their positive surface expression of putative markers of NETosis, namely extracellular DNA, MPO, NE and citrullinated histones. NET burden was defined as the proportion of neutrophils which were NET positive. A one-way analysis of variance (ANOVA) was performed. Post-hoc Tukey tests were subsequently performed to confirm which groups had significantly different mean NET burdens.

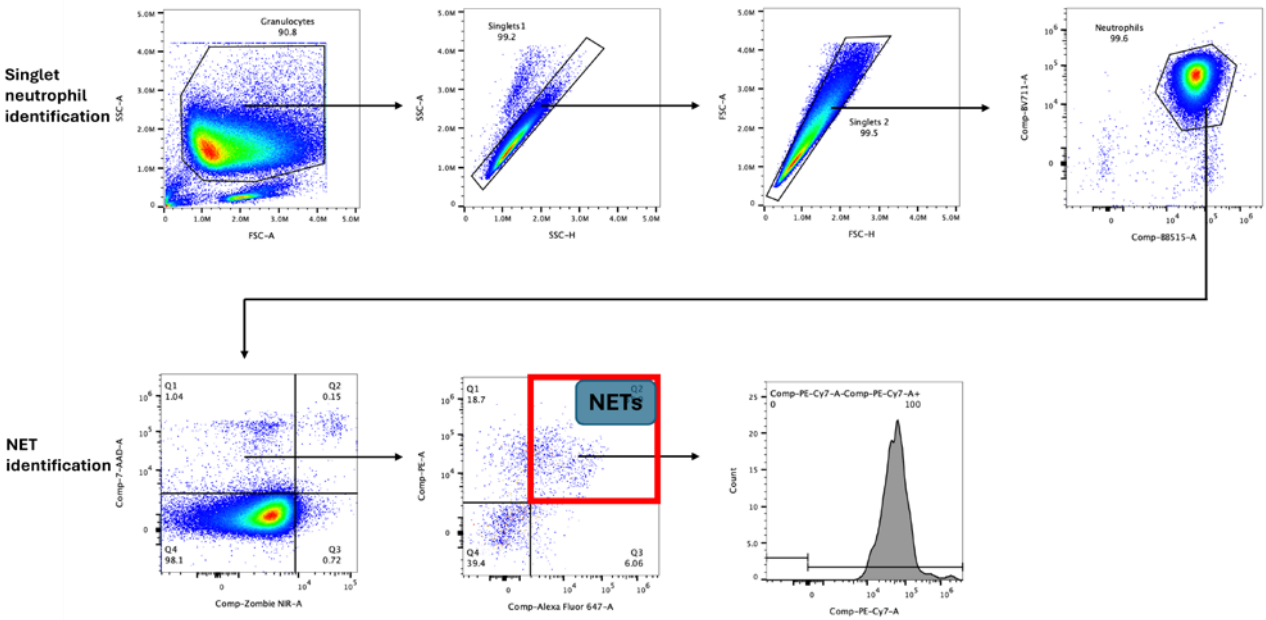

FIGURE 1: Neutrophil extracellular trap gating strategy. Singlet neutrophils were first identified as being $FSC^{high}SSC^{high}CD15^{+}CD66b^{+}$ **(A-D)**. Necrotic neutrophils were excluded through excluding Zombie NIR^{bright} cells (E). NET appendant neutrophils were identified through their expression of extracellular DNA, MPO and NE **(E-F)**. Expression of citrullinated histones was also examined, however, in all cases, close to all NETs expressed this marker **(G)**.

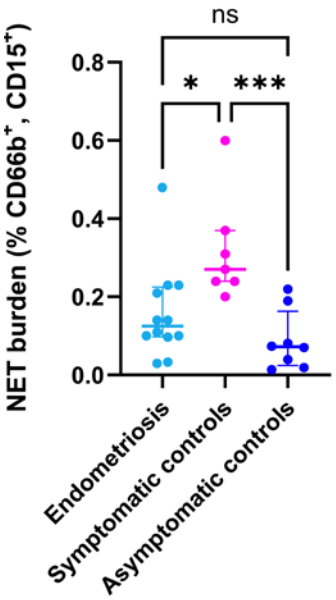

FIGURE 2: Comparison of NET burdens between endometriosis participants (n=12), symptomatic controls (n=7) and asymptomatic controls (n=8). Data is displayed as median ± interquartile range. *p<0.05, *** p<0.001, as assessed by one-way analysis of variance (ANOVA). ns – no significance.

RESULTS

NET burden did not significantly differ in endometriosis patients (n=12) compared to asymptomatic controls (n=8; 0.13%, IQR=0.13 vs 0.07%, IQR=0.14 p=0.41) and was significantly lower compared to symptomatic controls (n=8; 0.13%, IQR=0.13 vs 0.27%, IQR=0.13, p=0.04; Figure 2).

CONCLUSION

Venous NET burden is not elevated in endometriosis cases compared asymptomatic controls, suggesting NET burden is not a biomarker of endometriosis. However, NET burden was significantly higher in people displaying a similar symptom profile without endometriotic disease, presenting an interesting avenue for future research.

University of Otago, Wellington

Novel flow cytometry techniques to detect extracellular vesicles for diagnosing endometriosis

E S J PATERSON, J E GIRLING, C E HENRY

KEY FINDINGS

This project developed techniques to use flow cytometry to examine single, fluorescently labelled extracellular vesicles. The methods developed were applied to assay plasma and cervicovaginal fluid EVs in people with endometriosis and without endometriosis, with no differences between the groups identified.

OBJECTIVES

Endometriosis is a common, painful condition defined by the presence of tissue resembling the endometrium (the lining of the uterus) growing outside the uterus. Diagnosing endometriosis non-invasively remains challenging, meaning in Aotearoa New Zealand most people are diagnosed with endometriosis via laparoscopic surgery. Furthermore, with a non-specific symptom profile, around one third of surgeries for suspected endometriosis find no evidence of disease (Paterson et al., 2023). Thus, non-invasive diagnostic biomarkers are critically required to shorten the time to diagnosis and predict which patients will benefit from surgical treatment.

This project focused on extracellular vesicles (EV) as biomarkers of endometriosis. EVs are membrane delimited particles released by cells that are involved in intercellular communication and cellular homeostasis. EVs have been implicated in the pathogenesis of endometriosis in a multitude of studies, yet only a few studies have investigated EVs in the context of diagnostic biomarkers.

The overall aim of this project was to develop methodologies to measure EVs using flow cytometry to investigate potential biomarkers of endometriosis. Specifically: Objective 1: Demonstrate ability to detect single, fluorescently labelled vesicles using flow cytometry. Objective 2: Evaluate cervicovaginal fluid and plasma EVs as diagnostic biomarkers of endometriosis.

METHODS

Blood samples and cervicovaginal fluid sampled with a low vaginal swab were collected from consenting people having surgery for suspected endometriosis at Wellington and Kenepuru Hospitals. Based on surgical and histological reports, patients were grouped into either the endometriosis group or no endometriosis group.

EVs were isolated from 500µL of plasma or cervicovaginal fluid using size exclusion chromatography, enriching for EVs sized 70-1000nm. EVs were stained overnight at 4°C. The plasma EVs were stained with antibodies targeting markers of endometrial stromal cells, anti-CD10-BB515, anti-CD90-BV711 and anti-CD140b-PE. Cervicovaginal fluid EVs were stained with antibodies targeting immunoregulatory proteins, anti-CD55-APC, anti-CD59-PE, anti-CCR2-APC and anti-CD82-AF647. The next day, EVs were fluorescently labelled with Aco-430, a membrane dye used to identify the EV population. Samples were then analysed on a three-laser Cytek Aurora flow cytometer equipped with the enhanced small particle detection module. Each experimental day, 8-Peak rainbow beads and polystyrene beads sized 70-400nm were acquired and resulting data was used to calibrate FCS files from arbitrary units to standardised units using FCMpass software. Aco-430^{+} events were deemed EVs. Samples were subjected to detergent lysis and serial dilution. Antigen positive EVs were presented as a proportion of the total EV population. Median fluorescence intensity (MFI) statistics were acquired for each population, as a measure of antigen density. To compare the endometriosis and no endometriosis groups, Mann-Whitney tests with Holm-Šídák correction for multiple comparisons were performed.

RESULTS

Data calibration into standardised units determined that the lower limit of detection of the cytometer was 100nm. An upper limit of 1000nm was set based on calibrated SSC-H. Detergent lysis of EVs showed a significant reduction in EV events by 88.6±4.1% after lysis (p=0.011, n=3), demonstrating particles detected were true EV events rather than other non-membranous particles such as protein complexes (Figure 1A). Samples had a linear relationship between sample dilution factor and the number of EV events detected, with a consistent Aco-430 MFI (Figure 1B).

For plasma EVs analysis, there were 15 participants per group. There were no differences in the proportions of $CD10^+$, $CD90^+$, $CD10^+CD90^+$ or $CD140b^+$ EVs or MFIs (Figure 2A) between the endometriosis and no endometriosis groups. This held true when analysing small EVs (EVs < 200nm) and large EVs (EVs > 200nm) separately (data not shown). Similarly, in the cervicovaginal fluid EV analysis there were no differences between the endometriosis and no endometriosis groups in the proportion of positive EVs or MFIs of $CCR2^+$, $CD55^+$, $CD59^+$ or $CD82^+$ total EVs, small EVs or large EVs (Figure 2B).

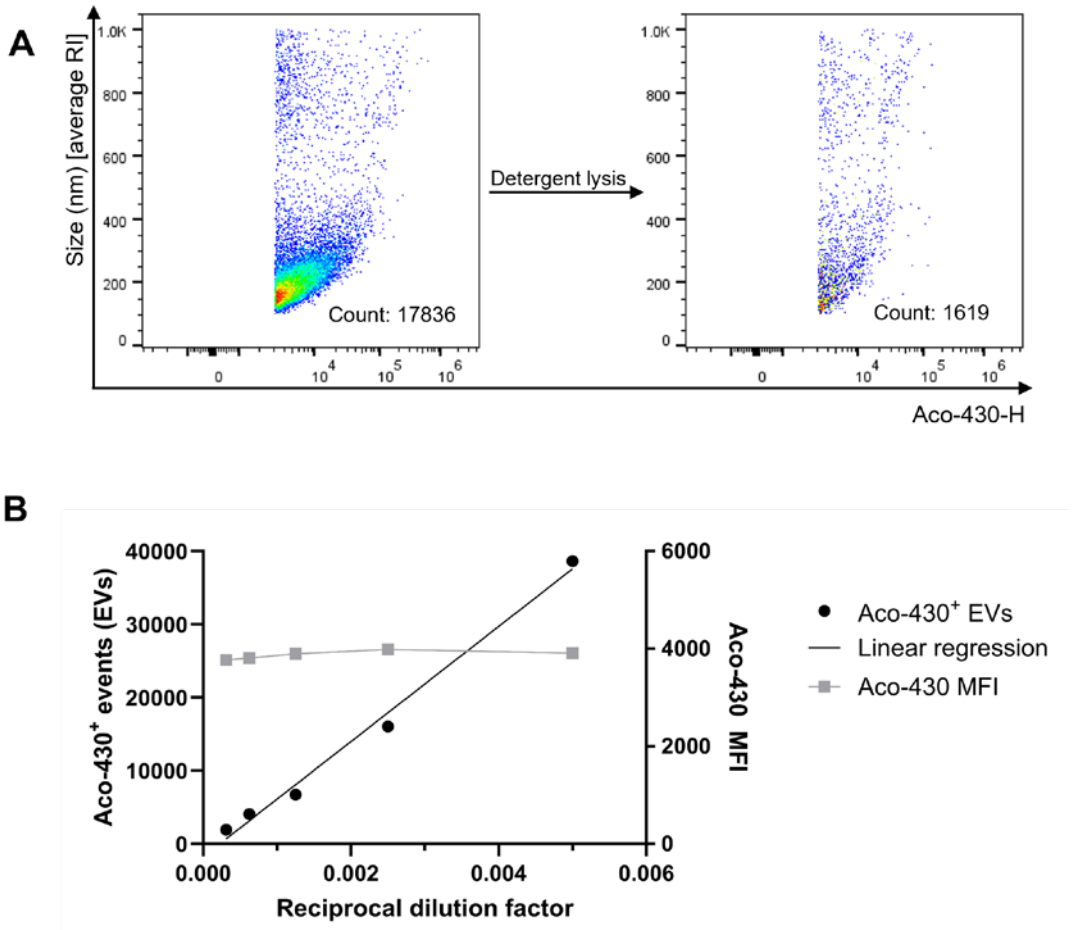

FIGURE 1: Single vesicle detection. **(A)** Representative plot showing significant reduction in EVs (Aco-430+ events) after detergent lysis. **(B)** Representative graph showing a linear reduction in EV count with sample dilution and a constant Aco-430 median fluorescence intensity (MFI).

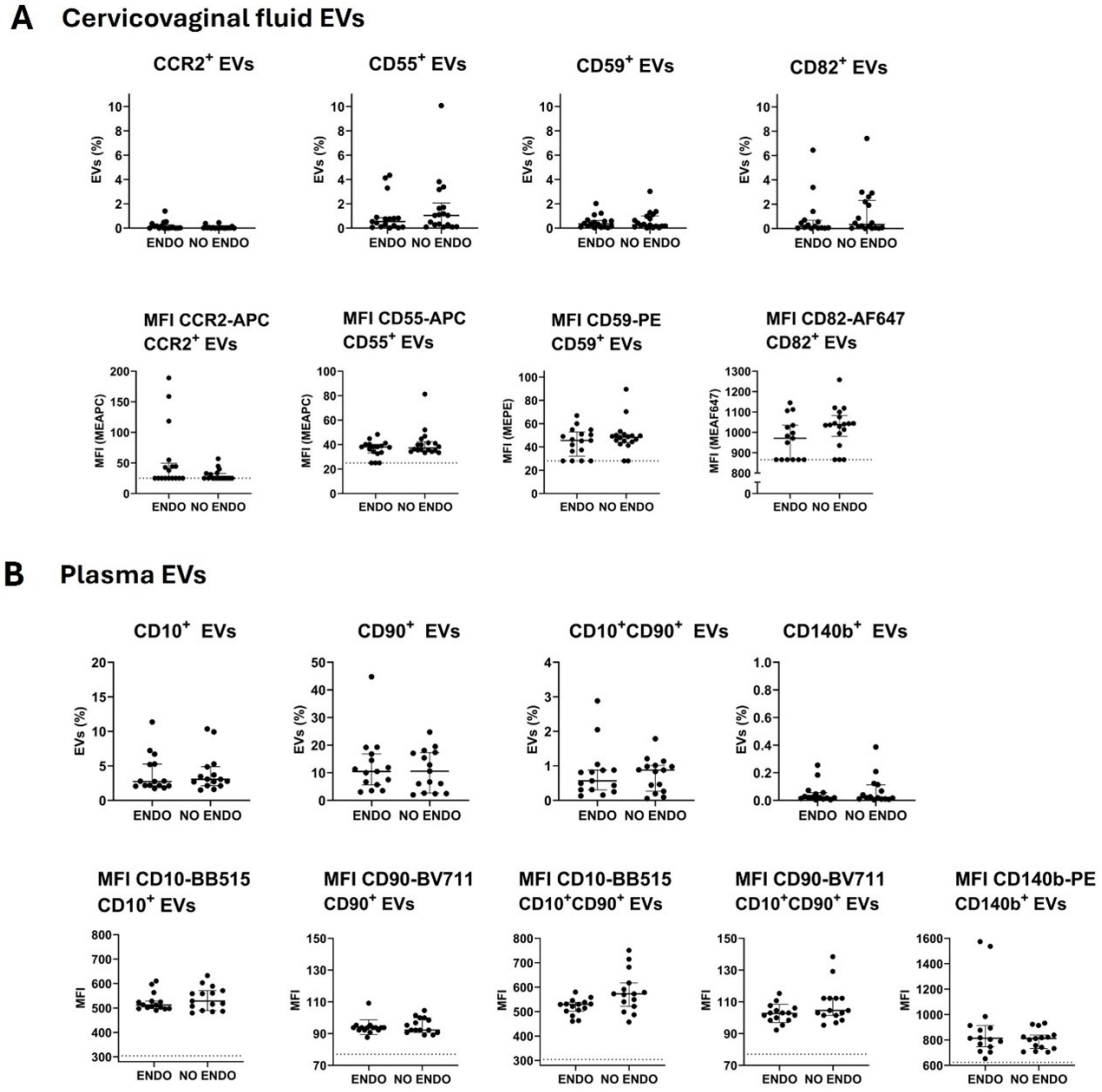

FIGURE 2: Proportions of antigen positive extracellular vesicles (EVs) and mean fluorescence intensity (MFI) in people with endometriosis and surgically confirmed controls. **(A)** Proportions and MFIs of CCR2$^+$, CD55$^+$, CD59$^+$ and CD82$^+$ cervicovaginal fluid EVs. N=17 for endometriosis, n=18 for no endometriosis. **(B)** Proportions and MFIs of CD10$^+$, CD90$^+$ CD10$^+$CD90$^+$ and CD140b$^+$ plasma. N=15 per group. Data presented as median ± interquartile range. No differences between groups were identified (adjusted p value > 0.05, Mann-Whitney tests with Holm-Šídák correction for multiple comparisons).

CONCLUSIONS

There were no differences in the antigen positive plasma and cervicovaginal fluid EVs tested between people with and without endometriosis, suggesting these EVs are not biomarkers of endometriosis. Despite this, the novel techniques for probing EV phenotype via flow cytometry developed in this study are valuable for investigating EV biology and could be applied to other endometriosis questions or disease contexts.

University of Otago, Wellington

Developing 3D models of breast cancer for therapeutic study

H ABOLINS-THOMPSON, K DANIELSON

KEY FINDINGS

This project developed three-dimensional breast cancer models, including integrated spheroid and patient-derived tumour organoid models, to study tumour cell biology and mechanisms of response to cancer therapy. There were no significant differences in response to chemotherapies paclitaxel and doxorubicin between mono- and coculture spheroid models, and scRNAseq identified moderate levels of heterogeneity within these models. These methodologies will be applied in future to optimised patient-derived tumour organoid models of breast cancer to investigate the response to clinically relevant chemotherapies.

OBJECTIVES

Breast cancer remains the most common cancer affecting women worldwide, and in New Zealand accounts for 1/3 of new cancer cases[1]. While survival rates continue to improve with advances in diagnosis and treatments, these benefits are not seen across all patient groups. Wāhine Māori are disproportionately affected by breast cancer and experience some of the poorest outcomes compared to non-Māori women in Aotearoa, including a greater likelihood of receiving chemotherapy as part of their treatment regimen[2,4]. Given the heterogeneity of breast cancer, treatment responses can vary widely between individuals. Optimising treatment regimens is therefore essential to prevent both over- and under-treatment of patients. However, traditional pre-clinical models used to test cancer therapeutics, such as 2D monolayer cultures or in vivo xenograft models in rodents, are limited. These models fail to recapitulate the complex architecture and cellular interactions of the human tumour microenvironment, contributing to the low success rate of translating therapeutics to the clinic[3,4].

There is a clear and evident need for more physiologically relevant, scalable and cost-effective in vitro models for novel therapeutic testing. Three-dimensional models, including integrated spheroid models and patient-derived tumour models, represent promising platforms that retain key aspects of tumour heterogeneity and therapeutic response[5]. This project aimed to develop and evaluate three-dimensional breast cancer culture models to study tumour cell biology and mechanisms of response to therapeutics. Specifically, we aimed to:

1. Establish and characterise tumour cell – fibroblast co-cultures in integrated spheroids.
2. Establish breast tumour organoids from patient samples (specifically recruiting Māori patients).
3. Compare response to standard chemotherapeutics (doxorubicin, paclitaxel) between the integrated spheroid models and the patient-derived tumour organoids in proof-of-concept studies.

METHODS

Integrated spheroid models: Three-dimensional spheroid models were developed using immortalised breast cancer cell lines representing distinct breast cancer subtypes: MCF7 (ER/PR+; luminal), MDA-MB-231 (triple negative breast cancer), and SKBr3 (HER2-enriched), cultured alone or in co-culture with the human breast (skin) fibroblast line CCD1128SK. Cells were aggregated using the hanging drop method to facilitate 3D spheroid formatting and then embedded in Matrigel/Collagen domes to mimic the tumour extracellular matrix in a 1:1 ratio. Chemotherapy treatment and drug response assays: Integrated spheroids were treated with standard chemotherapeutics paclitaxel and doxorubicin across a dose-response range. Cell viability was quantified using the RealTime-Glo MT cell viability assay (Promega) to generate IC50 curves. Responses were compared between 3D monocultures and 3D cocultures as a proof-of-concept study. Confocal microscopy for morphological assessment: To assess morphological changes in response to chemotherapy, integrated spheroids were

labelled with Calcein AM (for cancer cells) and CellTracker DeepRed (for fibroblasts) prior to spheroid formation. Confocal microscopy was used to visualise cell distribution, viability and co-localisation. Single-cell RNA sequencing of integrated spheroids: The 10X Genomics Fixed cell RNA profiling kit was used for preparation of single cell RNA sequencing libraries. Integrated spheroids were dissociated using dispase II to create a single cell suspension before the protocol was performed as per manufacturer's instructions. Data was analysed using ScanPy based packages on the Broad Institute's Terra platform. Integrated spheroids across the 3 cell lines, treated with either vehicle or 0.22 mM paclitaxel, were used for library preparations to investigate the effect of treatment in these models. Participant recruitment and Patient-derived tumour organoids (PDOs): Participants were recruited from Wellington and Keneperu hospitals prior to biopsy or surgery appointments. Tumour organoids were created using collagenase digestion of biopsy samples before being resuspended in Matrigel extracellular matrix medium and growth-factor containing medium.

RESULTS

Integrated spheroid models were successfully created for each cell line and imaged using confocal microscopy (Figure 1A). There was no significant difference in estimated IC50 between monoculture and coculture 3D integrated spheroids for either drug, across the 3 cell lines (Figure 2). Coculture reduced viability in MDA-MB-231 and SKBr3 spheroids, however these were insignificant (p=0.9559 and p=0.6268 respectively). No observable effect of coculture was apparent for MCF7 spheroids treated with paclitaxel. A total of 6 participants were recruited for tissue scRNAseq and tumour organoid generation. Single cell RNA sequencing of 11, 693 cells from integrated spheroid models identified 14 global cell populations, including populations able to be identified as the SKBr3 cell line, 5 populations enriched for wildtype BRCA1 and CD44, and 4 populations with increased CD44 without BRCA1 expression (Figure 3). Epithelial markers EpCAM and CDH1 were only expressed in HER2 positive populations, and the population of BRCA1-enriched cells that co-expressed ESR1 and PGR. Two subpopulation's, BRCA_5 and HER2_4 clustered separately from their two respective larger epithelial clusters. Overall, moderate heterogeneity was identified within spheroid clusters. Patient-derived tumour organoids were developed across the participant cohort (Figure 1B), however due to limited amounts of tissue from biopsy procedures IC50 curves were not able to be generated for these cultures.

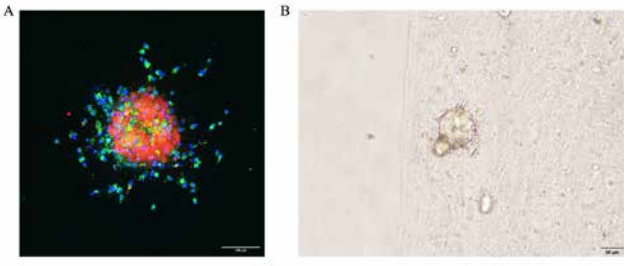

FIGURE 1: Representative images of three dimensional breast cancer cultures. **(A)** Confocal image of MDA-MB-231 (breast cancer cell line; green) and CCD1128SK (fibroblast cell line; red) after 48 hours in culture. **(B)** Patient-derived tumour organoid generated from ER/PR + HER2- breast tumour.

CONCLUSION

We have developed and characterised integrated 3D breast cancer spheroid models and early-stage patient-derived tumour organoids, with an emphasis on including samples from wāhine Māori. Although coculture with fibroblasts did not significantly alter chemotherapy responses in this proof-of-concept study, trends and transcriptional heterogeneity identified suggest stromal contributions to tumour behaviour that warrant further investigation. Future work should expand these analyses to participant tumour organoids, to investigate the response to clinically used, standard-of-care chemotherapies in a model with improved biological relevance.

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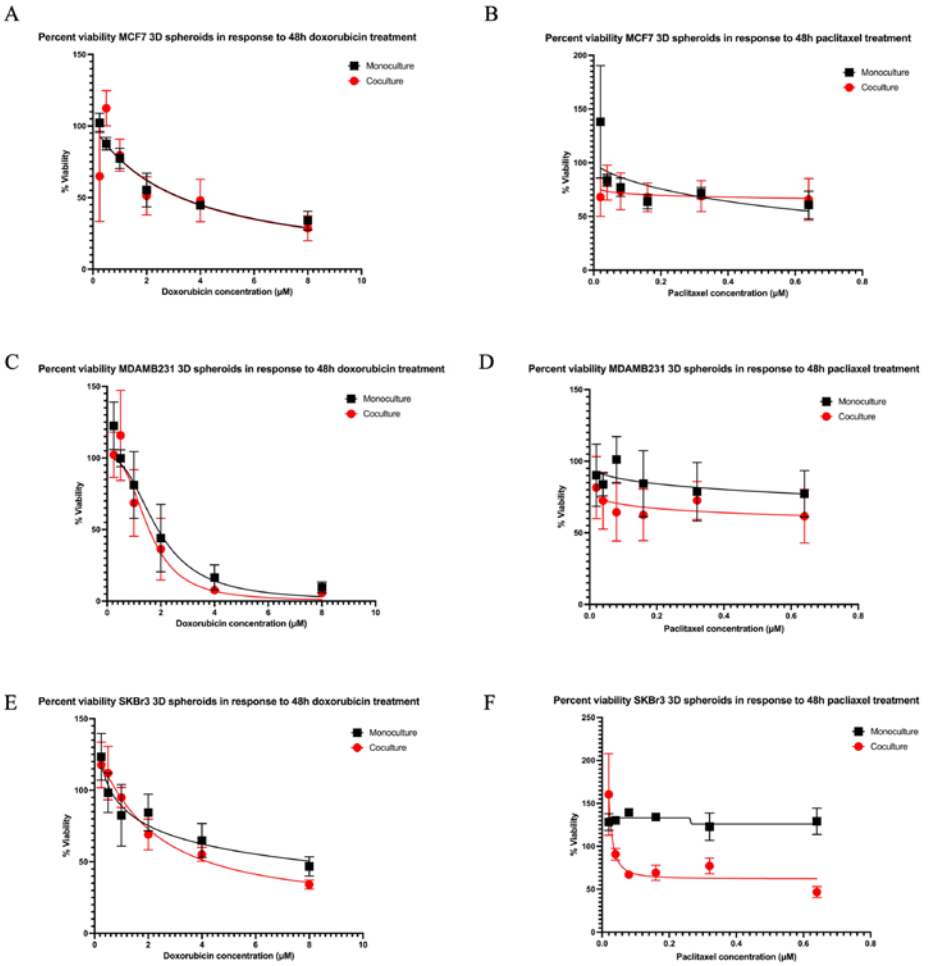


FIGURE 2: IC50 curves for integrated breast cancer spheroid models. Viability in MCF7 3D monocultures (black) or coculture (red) treated with doxorubicin **(A)** or paclitaxel **(B)**, measured with MT-RealTimeGlo. Viability in MDA-MB-231 3D monocultures (black) or coculture (red) treated with doxorubicin **(C)** or paclitaxel **(D)**, measured with MT-RealTimeGlo. Viability in SKBr3 3D monocultures (black) or coculture (red) treated with doxorubicin **(E)** or paclitaxel **(F)**, measured with MT-RealTimeGlo. Readouts for all cultures and treatments were performed at 48 hours after initial treatment onset. All samples were plated in triplicates (N =3). Error bars = SEM.

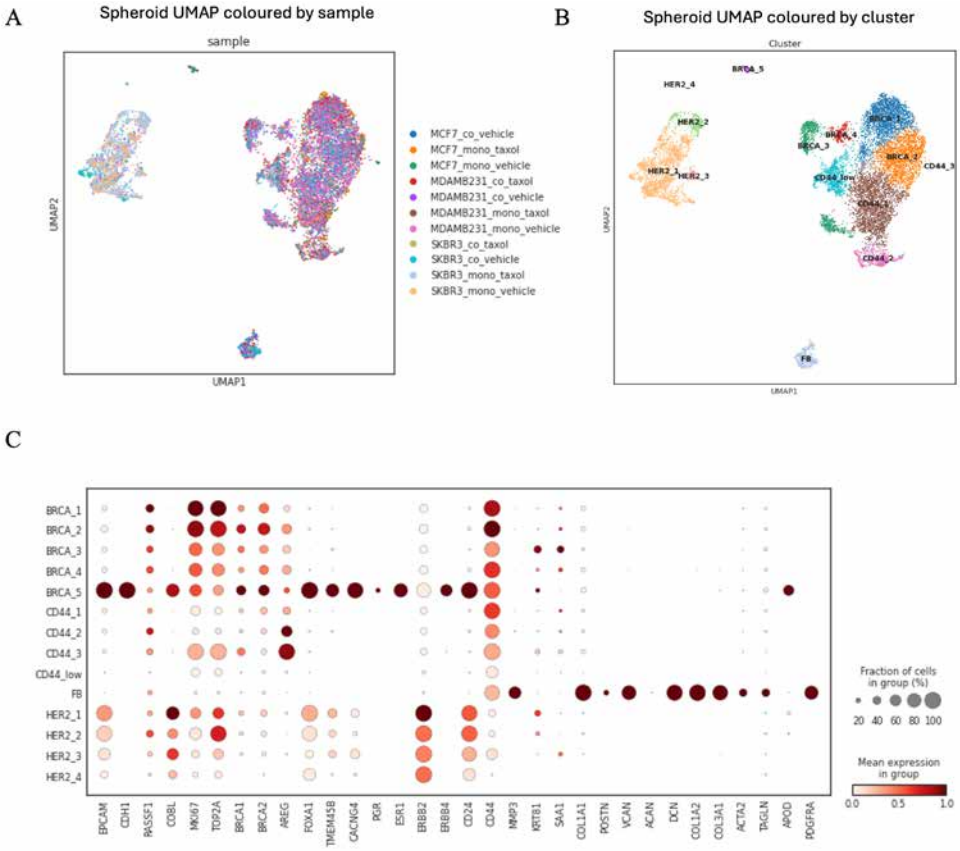


FIGURE 3: Post-quality control UMAP and gene expression in integrated spheroid models, Post quality control UMAP projection of 11,693 cells across **(A)** samples and **(B)** clusters. **(C)** Distribution of canonical cell type markers for each cluster identified in the dataset. Shade is representative of the mean gene expression in the group and size of the circle is representative of the fraction of cells expressing the gene.

University of Otago, Wellington

Characterising platelet activation in response to vascular surgery

E THOMPSON[1], A LA FLAMME[2], K HALLY[1]

[1] University of Otago Wellington (Ōtākou Whakaihu Waka)
[2] Victoria University of Wellington (Te Herenga Waka)

KEY FINDINGS

This study found that platelet behaviour in patients undergoing major vascular surgery falls into two distinct patterns. First, before surgery, patients could be clearly separated into 'high' or 'low' activators based on how activated their platelets were using our laboratory test. This difference may reflect an underlying predisposition to clotting. Second, in some patients, platelet aggregation rose during surgery and early recovery, suggesting a potential short-term period of heightened clotting risk. Others showed little change, indicating that this perioperative risk window may not apply to all patients. These findings confirm that our platelet testing approaches worked reliably in the surgical setting and provide a strong rationale for further, more detailed investigation.

AIM

Vascular surgery patients with significant non-coronary atherosclerosis are at increased risk of postoperative clotting complications, such as stroke or myocardial infarction. Currently, there is no biomarker to identify which patients are most at risk during and after surgery. Because of their underlying vascular disease, most patients are prescribed at least aspirin (a strong antiplatelet drug) and some are also on clopidogrel (another potent antiplatelet). Understanding whether platelet testing can pinpoint those most vulnerable to increased platelet activity, even despite medical therapy, could help guide future perioperative management. Here, we sought to:

1. Measure platelet function at several timepoints before, during, and after surgery.
2. Compare two complementary methods of measuring platelet function (the rapid point-of-care VerifyNow test and a detailed laboratory-based flow cytometry assay) to see how well each detects changes in platelet behaviour.
3. Explore whether pre-surgery platelet activation, or changes in activation in response to surgery, might help identify patients at higher risk of clotting during or after surgery.

METHODS

Seven patients undergoing major vascular surgery were recruited. These included operations to remove atherosclerotic plaque from the carotid or femoral arteries or involving bypass grafting for restoring lower limb blood flow. Most were male, all had significant cardiovascular risk factors, and two were taking dual antiplatelet therapy (aspirin + clopidogrel) before surgery, while the rest were on aspirin alone. Blood was collected at up to five points: before surgery, 1 hour after skin incision (during surgery), 3-6 hours after incision (late surgery/early recovery), and on postoperative days 1 and 2. Platelet function was tested in two ways:

1. VerifyNow: This is a point-of-care, cartridge-based system that measures how strongly platelets aggregate together when stimulated with a platelet activator (ADP). We defined patients as 'responsive' to surgery if their platelet aggregation changed by at least 10% at a majority of timepoints compared with the pre-surgery baseline, and 'non-responsive' if changes were smaller than this threshold.
2. Flow cytometry provides a much more detailed, multi-parameter view of platelet activation. Here, we measured four well-established platelet activation markers (CD63, CD154, PAC1, and CD62p) that reflect different aspects of platelet behaviour, including granule release, inflammatory signalling, and the ability to bind to fibrinogen. Platelets were stimulated with two common platelet agonists (ADP and TRAP-6) at doses designed to trigger a strong (but not

maximum) activation response. Antibodies were added to tag the markers of interest, and each sample was acquired using a flow cytometer that can detect each tagged marker on the surface of activated platelets. This approach allowed us to detect the proportion of platelets expressing each activation marker.

RESULTS

Six patients were fully assessed with VerifyNow; see the figure. Of these, four were defined as 'responsive' to surgery (meaning their platelet aggregation changed noticeably during surgery) and two were 'non-responsive'. This split could not be explained by obvious factors such as surgical type, duration, or pre-operative antiplatelet regimen, suggesting that unmeasured biological differences may be driving these patterns. In the responsive group, platelet activity rose sharply during surgery, with an average increase of +17% at one hour and +28% at 3-6 hours after incision. These changes could indicate a period of heightened thrombotic risk while the patient is still in theatre or in early recovery. By postoperative day 1 and 2, platelet activity had generally returned to pre-surgery levels. The non-responsive group generally maintained platelet activity within ±10% of baseline across all timepoints, suggesting that not all vascular surgery patients experience shift in platelet activation during the perioperative period.

The flow cytometry data revealed a division between 'high' and 'low' activators at the pre-surgery timepoint when platelets were stimulated with ADP. The separation was consistent across all four activation markers tested. Interestingly, this pre-surgery dichotomy was independent of the VerifyNow classification meaning that individuals with high baseline reactivity were not necessarily those whose aggregation changed the most during surgery. When platelets were stimulated with TRAP-6 at high doses, all samples showed near-maximal activation, and unstimulated platelets remained mostly inactive, so no clear separation was observed in these conditions.

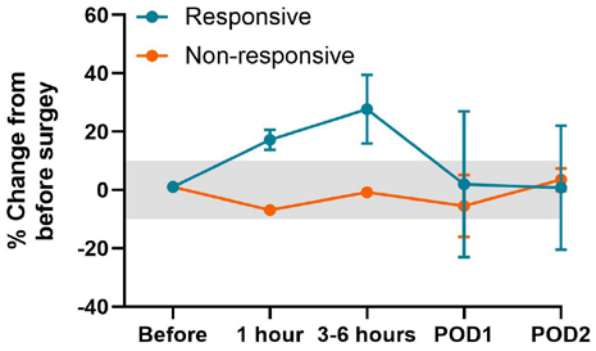


FIGURE 1: Platelet aggregation by VerifyNow can be defined as 'responsive' or 'non-responsive' to surgery. For patients defined as 'responsive', platelet aggregation increased during and shortly after surgery. Data shown are platelet aggregation during and after surgery as a relative change to each patient's pre-surgery aggregation value, mean ± standard deviation.

CONCLUSION

Our results indicate that the two tests performed here may be capturing different aspects of platelet biology: VerifyNow reflecting dynamic responsiveness to surgical stress, and flow cytometry highlighting an underlying predisposition to strong platelet activation when challenged. Patients in the 'high activator' group or within the 'responsive to surgery' group may represent those with a greater intrinsic tendency toward clot formation. Understanding why these groups exists, and whether their platelet phenotype links to postoperative thrombotic events, could open the door to more personalised perioperative management strategies.

University of Otago, Wellington

The role of colorectal tumour-derived extracellular vesicles in modulating primary human monocytes

B KENT, TEO DE REZENDE MCGUINNESS, K DANIELSON

KEY FINDINGS

This project examined the effects of small packages of cellular cargo released from colorectal cancer cells (extracellular vesicles; EVs) on circulating immune cells called monocytes. EVs from colorectal cancer cells decreased the expression of some anti-inflammatory cytokines (TGF-β), increased expression of pattern recognition receptors (TLR2 and NOD1), and decreased expression of inflammasome proteins (CASP5). EVs isolated from the blood plasma of a small group of participants with colorectal cancer (n=4) did not have the same effects, with much more varied effects on gene expression between different participants. Overall, this work has shown that colorectal cancer cell derived EVs can affect the expression of some key markers of immune activity in monocytes, but that more work needs to be done to understand differences between individuals.

PROJECT AIMS

This project investigated how small packages of cellular cargo called extracellular vesicles (EVs) could be released from colorectal tumour cells and effect the gene expression of immune cells called monocytes. Specifically, we aimed to:

1. Determine how EVs from a colon (DLD1) and rectal (SW837) tumour cell line could affect the gene expression of a panel of key inflammatory markers in primary human monocytes from healthy donors.
2. Compare the effects of EVs from cells lines to total circulating EVs from the plasma of patients with mid-stage (stage II - stage III) CRC.

METHODS

Aim 1: small EVs were isolated from the conditioned culture media of DLD1 (Stage III colorectal adenocarcinoma) and SW837 (grade IV rectal adenocarcinoma) cells and co-cultured with monocytes isolated from healthy volunteers for 24 hrs at a dose of 10^5 particle per mL. Transcriptional changes in key pro- and anti-inflammatory cytokines, inflammasome, and signalosome were measured by RT-qPCR (Table 1).

Aim 2: Total small EVs were isolated from the plasma of n=4 patients with mid-stage (stage II-III) CRC and co-cultured with monocytes as described above.

PRO-INFLAMMATORY CYTOKINES	TNF-A, IL-1B, IL-6, CXCL10
Anti-inflammatory cytokines	IL-10, TGF-β
Inflammasome	ASC, Caspase-1, Caspase-5
Signalosome	TLR-2, TLR-4, NOD-1, NOD-2
Reference genes	GAPDH, HPRT-1

TABLE 1: mRNA targets for RT-qPCR analysis.

RESULTS

EVs from the DLD1 colon cancer cell line decreased expression of anti-inflammatory cytokine TGF-β ($p<0.05$) and produced a trending decrease in IL-10 ($p<0.051$; Figure 1). Conversely, DLD1-EVs produced a trending increase in the expression of pattern recognition receptor TLR2 ($p<0.054$). EVs from the rectal cancer cell line SW837 increased expression of pattern recognition receptor NOD1 ($p<0.01$), and decreased expression of inflammasome protein CASP5 ($p<0.05$).

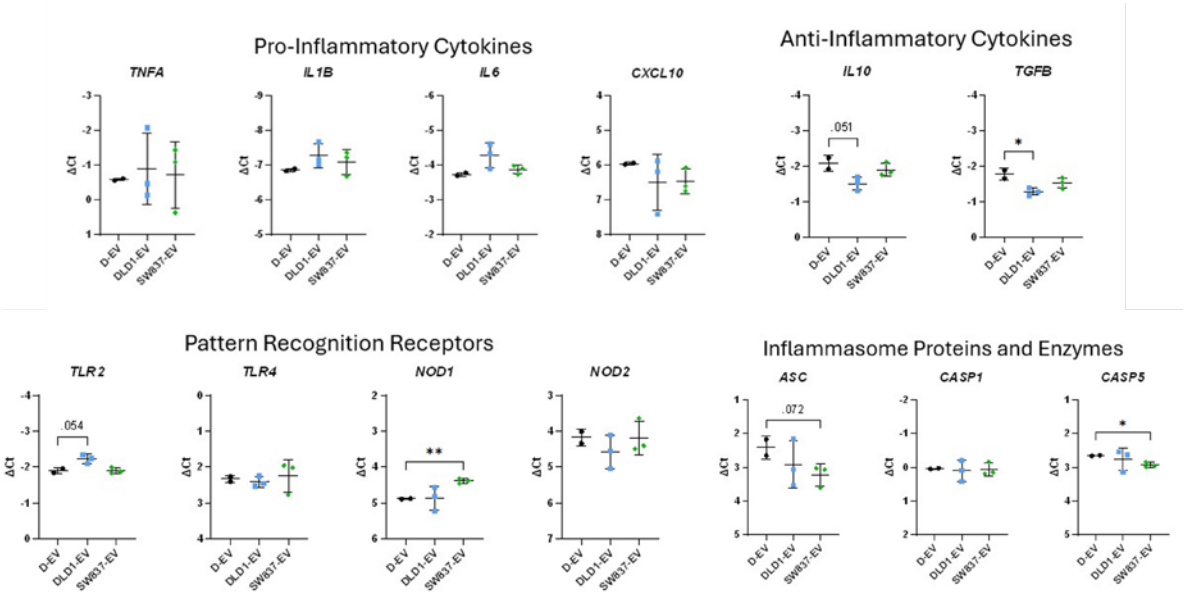


FIGURE 1: Effects of cell-derived EVs on monocytes. Monocytes were isolated and co-cultured with CRC-EVs for 24 hours. Total RNA was extracted, and the expression of target mRNA was probed using TaqMan chemistry-based RT-qPCR. Results were analysed using the ΔΔCt method, and differences between the ΔCt of D-EV treated monocytes and ΔCt of monocytes treated with CRC-EV were determined using unpaired T-tests. Points represent the ΔCt value of monocytes treated with EVs isolated from different cell line passages (n = 3). Lines represent means, and error bars represent standard deviation. The y-axis has been reversed for ease of interpretation. * $p < 0.05$. ** $p < 0.01$.

EVs isolated from the blood plasma of participants with colorectal cancer had no statistically significant effects on mRNA expression of any targets (Figure 2). The observed effects were more variable between individuals and we are continuing to investigate these effects in a larger cohort of participants.

CONCLUSION

Colorectal cancer cell derived EVs produced changes in gene expression in monocytes that could have mixed outcomes for whether they act in a pro- or anti-tumour manner. Increased expression in pattern recognition receptor mRNA and decreased expression in anti-inflammatory cytokine mRNA would indicate priming for response to tumour cells; however, decreased expression in inflammasome-related mRNA indicates a reduced immune response. Future work will investigate the functional effects of these EVs on monocytes and further explore the inter-individual variance in effects in a larger cohort of participants.

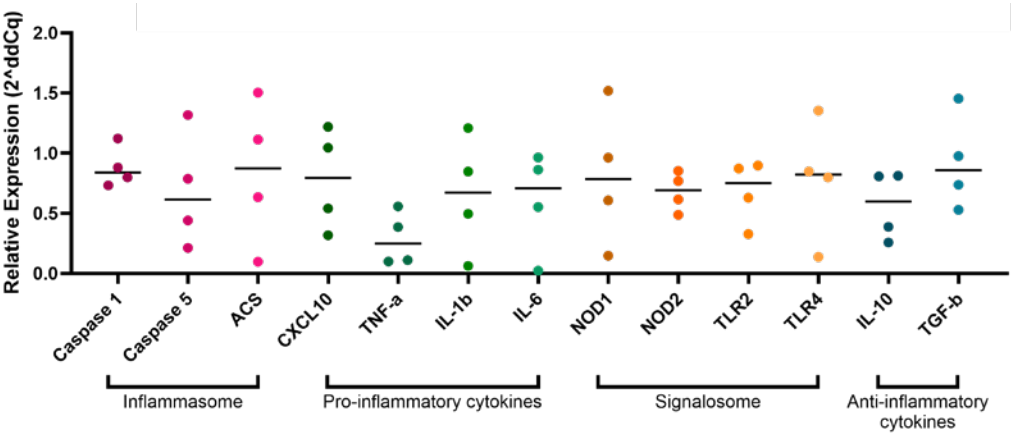


FIGURE 2: Effects of plasma-derived EVs on monocytes. CRC-EVs from participants with stage II-III had no significant effect on monocyte inflammatory marker mRNA expression compared to EVs from pooled healthy control plasma (n=4, Student's ttest).

CENTRE FOR DIABETES, OBESITY AND ENDOCRINE RESEARCH
Wellington Hospital

A comparison of post meal glycaemic excursion after ingestion of konjac rice, cauliflower rice and brown rice (control) in type 1 diabetes.

A PARRY STRONG, J KREBS, R BISHOP, R HALL

Centre for Diabetes, Obesity and Endocrine Research, Health New Zealand Capital, Coast and Hutt Valley, Wellington Hospital, Wellington.

KEY FINDINGS

Konjac may be a useful carbohydrate replacement in those wishing to reduce overall energy intake and insulin use, without causing hypoglycaemia. However it may be less palatable and satiating than other options.

AIM

The aim of this study was to investigate whether foods made from konjac are glucose lowering over and above their lack of carbohydrate in T1DM, as compared with cauliflower rice and a brown rice control.

METHODS

We conducted mixed meal tolerance tests in 15 people with T1DM and compared two-hour postprandial area-under-the-curve for glucose and GLP-1 for the three test foods.

Participants used their established insulin to carbohydrate ratio for their insulin dose prior to the brown rice meal but gave no insulin for cauliflower or konjac rice. Satiety scores were recorded.

RESULTS

Of the 15 participants recruited 6 were female, mean duration of T1DM was 14 ± 8.0 years and mean age was 46 ± 17.7 years. Post meal ingestion the konjac meal was significantly less satiating than the other meals ($p<0.001$). There was no difference in area—under-the-curve for glucose or GLP-1 for either test meal compared with brown rice (figure 1). Konjac scored lowest on the palatability scale. No hypoglycaemia was noted.

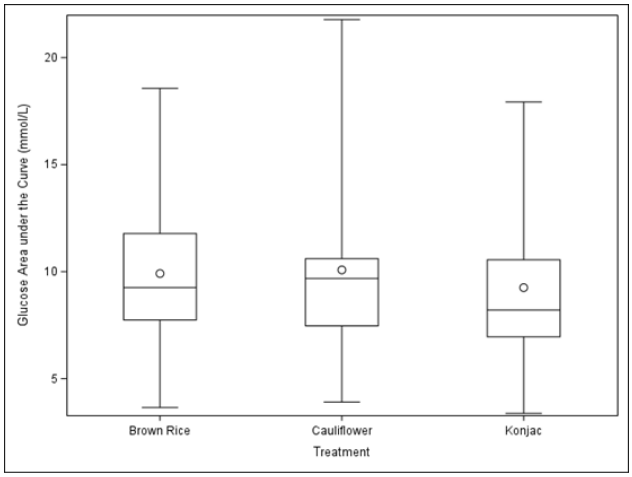


FIGURE 1: Glucose AUC for meal tests

CONCLUSION

Konjac may be a useful carbohydrate replacement in those wishing to reduce overall energy intake and insulin use, without causing hypoglycaemia. However it may be less palatable and satiating than other options.

RESEARCH PROJECT & TRAVEL GRANTS

Research Project Grants 2025

THE FOLLOWING PROJECTS HAVE BEEN APPROVED FOR FUNDING IN THE FINANCIAL YEAR ENDED 30 JUNE 2025 OR ARE LONGER TERM PROJECTS FROM PREVIOUS FUNDING ROUNDS. THESE WILL BE REPORTED ON IN SUBSEQUENT RESEARCH REVIEWS.

## Analysis of biomarkers to evaluate clinical response to treatment in multiple sclerosis **DR KATHARINA ROBICHON**	Dr. Robichon is a lecturer at the Department of Pathology and Molecular medicine at the University of Otago, Wellington. She received a research grant of up to $6,520. Her research focuses on the analysis and comparison of four selected biomarkers in the serum of naïve multiple sclerosis patients before start of treatment and after one year of treatment and relate them to treatment response. The question Dr Robichon aims to answer is how can it measured if a chosen treatment is working well for each person living with multiple sclerosis? Having specific, sensitive, and cost-effective measurements of how well a treatment is working is essential when people diagnosed with multiple sclerosis are starting treatment.
## The CINEMA study – Characterising the Immune response in Endometriosis And chronic pelvic pain **CERIDWYN JONES & EMILY PATERSON**	Ceridwyn Jones and Emily Paterson received a Research For Life grant of up to $8,500 to undertake research into the role of neutrophils in endometriosis. Endometriosis is a chronic, inflammatory condition that affects around 1 in 9 women and affects gender diverse people. Immune dysregulation is known to be a key feature of the pathophysiology of endometriosis, but neutrophils in endometriosis remain an understudied immune cell subset. Their research aims to uncover the phenotype of neutrophils to further our understanding of how the immune system contributes to the development of endometriosis. Ceridwyn is a PhD candidate with Te Herenga Waka – Victoria University of Wellington and Emily is a PhD candidate with the University of Otago Wellington.
## Using cardiac troponin to detect myocardial injury after vascular surgery **DR KATHRYN HALLY**	Dr Kathryn Hally and her team have received a Research For Life grant of up to $21,044 to understand the value of a cardiac troponin surveillance program for detecting postoperative myocardial injury in patients undergoing vascular surgery. Postoperative myocardial injury is a significant clinical event that, in international literature, happens frequently in these patients given their burden of cardiovascular comorbidities. The proposed research aims to implement a cTn surveillance program to understand the frequency and clinical risk factors for this type of injury in this clinical context. By addressing this gap, the project hopes to design cardiac troponin surveillance tools for the detection and management of myocardial injury in non-cardiac surgery.

Quantification of dietary titanium dioxide (E171) in New Zealand food products to estimate population intake and its role in metabolic dysfunction-associated steatotic liver disease (MASLD)

JANGREZ KHAN

Jangrez Khan received a Research For Life grant up to $8,934 to study the dietary exposure to titanium dioxide (E171) in New Zealand and its potential impact on Metabolic dysfunction-associated steatotic liver disease (MASLD). This research aims to fill a crucial gap by quantifying E171 exposure, correlating it with metabolic diseases such as diabetes and obesity, and investigating the additive's role in MASLD through in vitro studies. The findings will offer valuable insights into the health implications of E171. Jangrez Khan is a PhD student at School of Health Sciences at Massey University, Wellington.

Adipose-derived stem cell extracellular vesicles as cancer therapeutic delivery vehicles

DR KIRSTY DANIELSON

Dr Kirsty Danielson received a Research For Life grant of $17,700 to undertake research investigating a new type of cancer therapy delivery mechanism. Dr Danielson's research is exploring the use of a type of cell product called extracellular vesicles that can carry different drug cargo to treat breast cancer. Dr Danielson is Group Leader of the Surgical Cancer Research Group at University of Otago, Wellington.

Complication rates and metabolic health in young adults with type 2 diabetes

DR DAVID LEWIS

Dr David Lewis received a Research For Life grant of up to $14,771 to fund research examining the complications of early onset type 2 diabetes in the Wellington Region. Rates of type 2 diabetes (T2DM) are increasing internationally and in Aotearoa New Zealand. A rising concern is its increasing development in adolescents and young adults in whom a small number of studies have shown faster than expected damage to tissues such as the eyes and kidneys as well as a high rates of hypertension, lipid abnormalities and obesity. This study is recruiting young and older adults with T2DM to compare detailed screening including retinal photography, carotid ultrasound and vascular and nerve testing. Measurement of pancreatic insulin production and its efficiency will be estimated along with diabetes severity and control. Better understanding of how young onset diabetes is affecting young New Zealanders will inform patient care and planning of our health system.

Engineering imaging agents to expand function and options in cancer management

PROF DAVID ACKERLEY

Professor David Ackerley and his colleagues, Dr Sarah Messenger and Professor Rachel Codd, received a Research For Life grant of up to $18,000 that will support their development of molecules that will improve surgical outcomes by enabling surgeons to precisely locate tumours within the bodies of patients prior to surgery, and then cause cancerous tissues to be highlighted by fluorescence during surgery in a manner that enables their precise and complete removal.

Understanding the metabolic changes that drive low-grade glioma

DR MELANIE MCCONNELL

Dr Melanie McConnell received a Research For Life grant of $12,825 to study the metabolism of low-grade brain cancer. These cancers don't respond well to therapy and most people will pass away 7-10 years from diagnosis. The McConnell lab have designed and built a new model of these brain cancers to try to improve the quality of research and improve outcomes for people with brain cancer. Here they compare the metabolic changes in their model to what is seen in human cancers, in collaboration with the Galvosas lab in the School of Physical and Chemical Sciences at VUW. These data will help us better understand the biology of the low-grade brain cancers, start to tell us why therapy fails, and will act as a testing board for new therapies. Dr McConnell is an Associate Professor at Te Herenga Waka - Victoria University of Wellington.

Characterising the immunomodulatory effects of novel marine compounds

MATTHEW ELLMERS

Matthew Ellmers received a Research For Life grant consisting of up to $5,000 to aid in characterising how natural products derived from sea-sponges alter immune system responses. Diseases of the immune system are becoming increasingly common worldwide, and 1 in 1000 New Zealanders are affected by the autoimmune condition Multiple Sclerosis (MS) which causes neurological and vision issues, fatigue and can lead to paralysis.

Matthew's research focuses on how natural products impact the cellular production of immune cytokines, the "messengers" of the immune system, that are often implicated in inflammatory and autoimmune diseases. These natural products could potentially be developed as new therapies to treat immune-related diseases, with a specific focus on MS. Matthew is undertaking a master's degree under the supervision of Professor Anne LaFlamme of the School of Biological Sciences at Victoria University of Wellington.

Synthetic optimisation and structure-activity investigation of bioactive fungal metabolite (-)-TAN-2483B

BETHANY HAWKEN

Bethany Hawken received a Research For Life grant of up to $5,000 to develop analogues of the fungal natural product (-)-TAN-2483B, an inhibitor of Bruton's tyrosine kinase (Btk). Aberrant Btk signalling has been associated with a range of B-cell malignancies. In New Zealand, approximately 1000 people are diagnosed with non-Hodgkin lymphoma each year, the majority of which are B-cell lymphomas. Bethany's research will expand the structure-activity relationship for (-)-TAN-2483B which could inform the development of new Btk inhibitors. Bethany is a PhD student supervised by AProf Joanne Harvey (School of Chemical and Physical Sciences) and AProf Simon Hinkley (Ferrier Research Institute) at Victoria University of Wellington.

Whole exome sequencing in primary hyperparathyroidism: prevalence of predisposing germline mutations and application in clinical practice

DR RICHARD CARROLL

Dr Richard Carroll and his colleagues received funding of up to $5,565 from Research For Life to undertake a study assessing the prevalence of inherited genetic mutations predisposing to primary hyperparathyroidism using whole-exome sequencing in New Zealanders. Patients with primary hyperparathyroidism are at increased risk of osteoporosis and fractures, kidney stones, and cardiovascular disease. The condition is typically treated with surgical removal of the abnormal parathyroid gland. Up to 60% of selected patients with primary hyperparathyroidism will have a predisposing genetic mutation, and identification of these patients significantly changes management strategies. Early detection of these predisposing variants in New Zealand hyperparathyroidism patients will be extremely important for determining clinical treatment and type of surgical intervention, potentially saving patients an invasive operation. Results from this project have the potential to impact on clinical practice in New Zealand and overseas.

Investigating the temporality of monocyte function after acute myocardial infarcation

DR KATHRYN HALLY

Dr Kathryn Hally, Lecturer at the University of Otago Wellington (UOW), has received a $11,274 Research For Life grant to characterise the function of monocytes in response to a heart attack. Monocytes are an important circulating immune cell that migrate into the affected heart tissue and are involved in healing after a heart attack. It was previously thought that monocytes are helpful in this context by supporting tissue repair. However, Kathryn and her research team have recently demonstrated that monocytes remain activated up to 10 days after a heart attack. Constant activation of these cells may maintain an inflammatory environment within the heart tissue, and lead to harm. To strengthen these findings, this current research aims to understand how monocytes function during this time after a heart attack: are monocytes involved in maintaining inflammation or facilitating tissue repair? This research will involve analysing monocytes from a biobank of cryopreserved peripheral blood mononuclear cells (PBMCs) that have previously been collected from heart attack patients, and will be conducted by Michael Roberts, a BBioMedSc(Hons) student at UOW. Michael will be supervised by Kathryn, alongside Dr Ana Holley, Lecturer at UOW, and AProf Peter Larsen, Head of Department of Surgery and Anaesthesia at UOW.

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 Fibrosis in Liver Cells during Bartonella Infection

ZOE KING

Zoë King has received a Research For Life grant of up to $13,800 to undertake research into the mechanisms underlying development of liver fibrosis during Bartonella henselae infection. B. henselae is an underdiagnosed but prevalent bacterial pathogen in New Zealand. Transmitted to individuals from infected cats, B. henselae typically causes a self-limiting illness, cat scratch disease. However, some patients develop serious complications from the infection, including liver infection and fibrosis. Fibrosis, characterised by the proliferation of extracellular matrix proteins, such as collagen, contributes to numerous diseases. The aim of this research is to characterise the interactions between B. henselae and liver cells, including those that promote fibrosis, and to identify B. henselae virulence factors that are involved. Findings could lead to better prevention, treatment, and diagnosis of B. henselae liver infections.

Bartonella quintana interactions with the host innate immune system

BRIANNA OTTO

Brianna Mouariki Otto has received a Research For Life grant of up to $12,500 to carry out research on the interactions between the human pathogen Bartonella quintana and innate immune cells. B. quintana causes a disease known as trench fever in humans hosts but can also persist chronically in the bloodstream. It also causes serious complications, such as endocarditis, in some patients. Very little is understood about how B. quintana avoids the immune response to cause disease. Preliminary data suggests that B. quintana resides in an uncharacterised vacuole in host macrophages and that it avoids or delays the bactericidal activity of macrophages. The aim of this research is to determine how long B. quintana survive inside macrophages and to use microscopy and staining of cellular markers to characterise the vacuoles that B. quintana resides in. The function of macrophages following phagocytosis will also be assessed.

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.

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 received a grant of up to $15,000 from Research For Life 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 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 I 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.

Unlocking therapeutic potential: Repurposing drugs to treat invasive meningioma

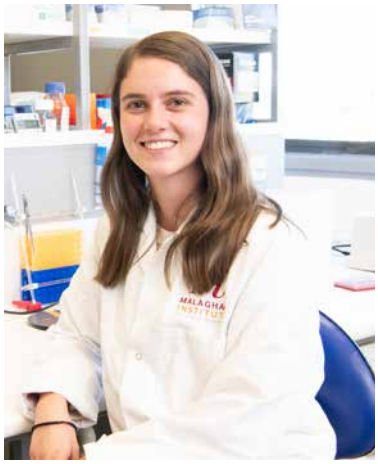
MATTHEW MONRO

Matthew Munro received a RFL grant of $ 14,000 to research meningioma, the most common brain tumour, with incidence rates three times greater in women than men. Patients with symptoms can undergo surgical removal, but high-grade cases are likely to recur due to tumour cell invasion into the brain. However, if invasion were slowed or prevented, recurrence would be less likely, and more cases could be monitored without requiring removal and associated post-surgical morbidity. We have preliminary evidence that the Pharmac-subsidised drugs sunitinib, mebendazole and everolimus can reduce meningioma invasion. In this context, I will identify differences between pre- and post-drug treated meningiomas. We will perform a thorough proteomic and metabolomic analysis to determine the mechanisms through which repurposed drugs affect cellular invasion. Understanding the unique molecular mechanisms involved in meningioma invasion and progression will help identify potential therapeutic targets. Our goal of identifying repurposed drugs that are off-patent and relatively inexpensive aims to contribute to health care equity for all New Zealanders by enabling wider access to treatment throughout the community.

Travel Grant Reports

ABBY MARTIN

OPTIONS FOR THE CONTROL OF INFLUENZA XII, BRISBANE IN SEPTEMBER 2024



Abby Martin

Thanks to the support provided by the Wellington Medical Research Foundation, I was able to attend the OPTIONS XII Conference in Brisbane. This conference series is the only global scientific meeting dedicated to research in the field of influenza and other respiratory diseases. My attendance allowed me to engage in critical future research discussions and establish networks as an early PhD student. My abstract was selected for a poster presentation, where I shared results from the first year of my PhD with prominent researchers in the field. As the first international conference I have attended, this experience was invaluable, allowing me to share my scientific contribution, receive expert feedback, and gain insight into current global efforts in respiratory disease research.

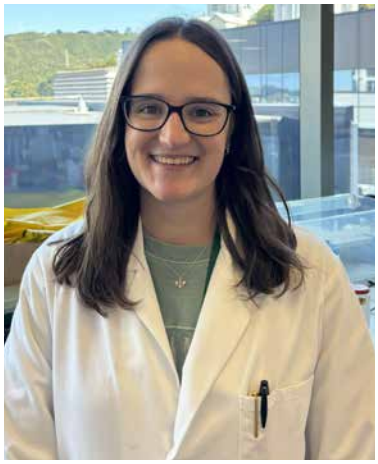
To date, my research has been focused on improving immune responses to circular RNA (circRNA) vaccines. Compared to the currently licensed messenger RNA (mRNA) vaccines, which were used during the COVID-19 pandemic, circRNA offers additional benefits which have been demonstrated in numerous studies. For example, its closed, circular structure provides greater stability compared to the linear structure of mRNA. This enhanced stability enables extended protein expression, which is important in a vaccine setting for promoting more robust immune responses. As a result, circRNA is considered a promising future vaccine candidate. However, more research is required to gain a better understanding on the specific immune responses it generates, which through my research I intend to complement.

In preparation for the conference, OPTIONS XII hosted a Mini School of Influenza for early career researchers. This provided an invaluable opportunity to engage with fellow scientists in the early stages of their research career. As a result, I was able to form meaningful connections with young researchers from all over the world, including Asia, Europe and South America. Establishing these networks was a particularly memorable and important aspect, as I endeavour to communicate my findings with peers to further my future research.

Both events were unique and unforgettable. Attending these enabled me to broaden my research horizons and further develop my scientific communication skills by liaising with experts in the field. I received valuable feedback on my work to date, and formed important connections, thus contributing to both my personal and professional development. I look forward to implementing the knowledge I have gained from this conference to shape my future scientific endeavours.

BROOKE WALDRAM

52ND ANNUAL SCIENTIFIC MEETING OF AUSTRALIAN AND NEW ZEALAND SOCIETY FOR IMMUNOLOGY (ASI), SYDNEY IN NOVEMBER 2024



Brooke Waldram

With support from the Research For Life travel grant I was able to attend the Annual Scientific Meeting of Australian and New Zealand Society for Immunology (ASI) in Sydney, Australia in November 2024. The ASI conference showcased a variety of presentations, poster sessions and workshops and was a fantastic opportunity to learn about the current advancements in immunology.

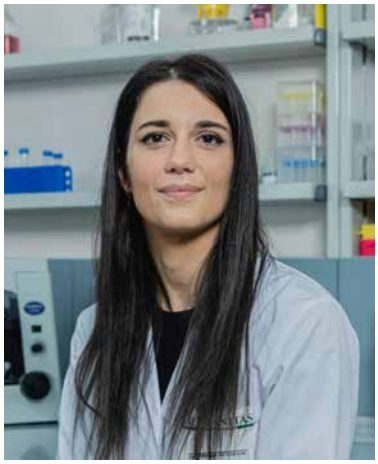
I presented a poster describing my master's research which explored a potential therapeutic for multiple sclerosis (MS). My project focused on the immunomodulatory effects of kappa opioid receptor (KOR) agonist, specifically nalfurafine, and their influence on CD4+ T helper cell subsets. These findings suggest that KOR agonists may provide a promising strategy for the treatment of MS by modifying immune responses while also addressing the need for remyelination and neuroprotection.

I also had the opportunity to give a short talk on my work during the student workshop session. This was a great opportunity to refine my presentation skills, receive feedback from my peers and engage in discussions about diverse projects and career pathways with other early career researchers.

Attending ASI was an inspiring and valuable experience, and a great conclusion to my master's experience. I am grateful to Research For Life for their support in attending this conference.

CATARINA CARCO

52ND ANNUAL SCIENTIFIC MEETING OF AUSTRALIAN AND NEW ZEALAND SOCIETY FOR IMMUNOLOGY (ASI), SYDNEY IN NOVEMBER 2024



Catarina Carco

I was fortunate to receive a $2000 travel grant to attend the Australian and New Zealand Society for Immunology (ASI) Annual Meeting 2024, held in Sydney from the 25th to the 29th of November 2024. This prestigious conference brought together over 600 immunologists, including leading experts in dendritic cell and T cell biology, to discuss groundbreaking research and foster collaborations in the field.

At the conference, I had the opportunity to present my recent findings through a poster titled: "Characterisation of skin migratory type 2 dendritic cells in wild-type and IL4RA-KO mice during Th2 immune responses."
My research focuses on the functional and transcriptomic plasticity of dendritic cells (DC2s) and their role in sustaining type 2 T cell responses in allergic skin inflammation. This work highlights how IL-4/IL-13 signalling governs the differentiation of CD11blo DC2s and its implications for Th2 immunity. The presentation was well received, sparking engaging discussions with attendees, particularly regarding the implications of these findings for chronic allergic diseases and potential therapeutic targets.

Attending the ASI Annual Meeting offered invaluable opportunities to connect with leading immunologists, including Prof. William Agace and Prof. Kenji Kabashima whose research aligns closely with my work. I received constructive feedback on my analyses and identified new perspectives on the functional roles of dendritic cell subsets in regulating T cell responses. Conversations with fellow researchers deepened my understanding of advanced techniques, which will be instrumental in refining my current projects and planning future studies.

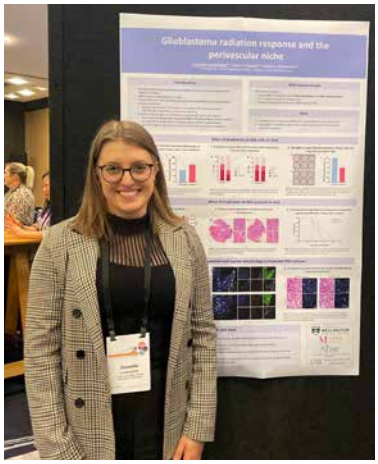
The meeting also featured plenary sessions and workshops showcasing the latest advances in immunology, from the cellular mechanisms driving allergic and inflammatory diseases to cutting-edge methodologies for studying host-immune interactions. As a new member of the ASI community, this was a unique opportunity to stay updated on emerging trends and to reconnect with collaborators, particularly in the Australasian immunology landscape.

The experience has greatly enriched my research, providing fresh perspectives on the molecular mechanisms underlying mucosal immune responses. I am immensely grateful to Research For Life for supporting my participation. This funding enabled me to share my findings with an international audience, engage in meaningful scientific dialogue, and build partnerships that will support my future work. Representing the Malaghan Institute of Medical Research at this event has not only enhanced the visibility of our research but has also reaffirmed the importance of our contributions to the global immunology community.

Thank you, Research For Life, for making this impactful experience possible!

DANIELLE LEWTHWAITE

NEW ZEALAND SOCIETY FOR ONCOLOGY (NZSO) CONFERENCE IN AUCKLAND, OCTOBER 2024



Danielle Lewthwaite

With the generous support of the Research For Life travel grant I was able to attend and present at the New Zealand Society for Oncology (NZSO) conference held in Auckland 2024. This conference brought together cancer researchers and clinicians from across Aotearoa, with a particular focus on the power of making connections.

At the poster session I was able to present my work conducted during my time as a research assistant focusing on the effect of irradiation on the glioblastoma tumour microenvironment. Glioblastoma is a deadly form of brain cancer, with tumours readily displaying both radiation and chemotherapy resistance. The work I presented looked at characterising the irradiation response *in vitro* and *in vivo* for a recently developed glioblastoma murine cell line model and also visualising the stromal components of the tumour microenvironment which may be contributing to therapy resistance.

Attending presentations over the course of three days, along with the early career researcher event, provided the wonderful opportunity to connect with other researchers and clinicians, and discuss both cancer research and treatment in Aotearoa. Two particular highlights of the conference were attending the Mārire Goodall Keynote talk by Prof Nadine Caron titled "Is the profession changing us? Or are we changing the profession?", and that from Dr Eric Sahai from the Francis Crick Institute looking at why liver metastases are so hard to treat with immunotherapy.

Thank you again to Research For Life for providing funding enabling me to attend this conference.

DEVLIN FORSYTHE

EUROPEAN ASSOCIATION OF NEURO-ONCOLOGY (EANO) CONFERENCE, GLASGOW IN OCTOBER 2024

Devlin Forsythe

With the generous support of Research For Life, I attended the 2024 European Association of Neuro-Oncology (EANO) meeting in Glasgow, an international gathering of clinicians and biomedical researchers dedicated to improving the understanding and treatment of brain cancers and related neurological diseases.

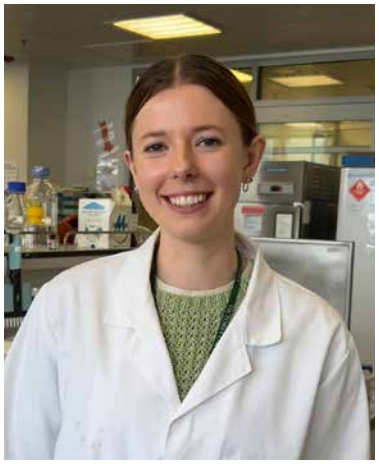
At this meeting, I presented key findings from my PhD research, which focuses on developing a new animal model for the brain cancer glioma. This model aims to improve how effectively researchers can translate promising treatments from the laboratory into therapies that benefit patients.

Presenting to an audience of world-leading experts was an invaluable opportunity. Their constructive feedback not only strengthened the quality and impact of my thesis but also challenged me to consider new directions and approaches. Interacting with researchers and clinicians from around the world, many of whom I would not normally have the chance to meet, broadened my understanding of current advances in the field.

The experience also opened my eyes to the breadth of opportunities available for postdoctoral research overseas. Attending EANO 2024 has significantly advanced my scientific work and professional network, and I am deeply grateful to Research For Life for making this possible.

ELYSHA-ROSE GRANT

52ND ANNUAL SCIENTIFIC MEETING OF AUSTRALIAN AND NEW ZEALAND SOCIETY FOR IMMUNOLOGY (ASI) CONFERENCE, SYDNEY IN NOVEMBER 2024



Elysha-Rose Grant

Thanks to the generous support from Research For Life, I was able to travel to Sydney and present at the 52nd Annual Scientific Meeting of Australian and New Zealand Society for Immunology (ASI), held in November 2024.

During my PhD, in collaboration with chemists at Te Herenga Waka, Victoria University of Wellington, I have been investigating a drug that inhibit Btk and Bmx, which are pro-inflammatory molecules found in immune cells. I presented a poster titled "Characterising the immunomodulatory properties and mechanism of action of a novel Btk inhibitor in B cells." In this poster, I presented results on the unique impact that this novel fungal natural product has on the function of B cells and I delved into the mechanism by which this inhibitor elicits its effects. My poster sparked lively discussions with experts in plasma cell biology, where I was able to make new connections and gain helpful insights which were invaluable in informing the decisions made in the following stages of research conducted in my PhD. Over the course of 5 days, the ASI conference consisted of many presentations, debates, workshops, and poster sessions. This allowed me to network with scientists across many areas of expertise and at a variety of stages in their careers, and afforded me the opportunity to learn about the vast array of research conducted in the immunology space across Australia and New Zealand.

Overall, attending this conference was an immensely rewarding experience that has helped guide and inform my research decisions in my PhD. Thank you again to Research For Life for providing the funding that allowed me to attend this conference.

EMILY PATERSON

16TH WORLD CONGRESS ON ENDOMETRIOSIS, SYDNEY IN MAY 2025

Emily Paterson

With support from Research For Life, I was able to attend and present at the World Congress on Endometriosis in Sydney, Australia in May 2025. This bi-annual conference brings together the world's leading experts from all disciplines related to endometriosis.

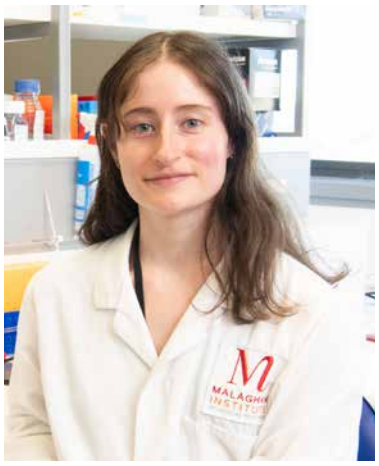
I was involved in a pre-congress Early and mid career (EMCR) workshop "Guiding and Mentoring the Next Generation". Here, I was invited to present with my mentor Dr. Thomas Tapmeier, about our experience with the World Endometriosis Society mentoring program. Along with the knowledge gained through the rest of the engaging and insightful workshop, I was able to meet many of the esteemed academics who comprise the board of World Endometriosis Society, as well as connect with many other early career researchers.

The Congress had a diverse and interesting program. I enjoyed hearing about new, cutting-edge techniques for modelling endometriosis, along with important areas outside my biomedical expertise, particularly the psychosocial considerations. I presented my research on cervicovaginal fluid and extracellular vesicles as diagnostic biomarkers of endometriosis in an oral presentation. Here, I was able to gain feedback and insights on this work, which was an incredible opportunity ahead of both my PhD defence and for future publication.

Thank you so much to Research For Life for supporting my attendance at the World Congress on Endometriosis. I gained valuable presentation experience, expanded my network widely and returned to my endometriosis research deeply inspired.

JESS COTTERELL

OPTIONS FOR THE CONTROL OF INFLUENZA XII, BRISBANE IN SEPTEMBER 2024



Jess Cotterell

I'm grateful to have received the Research For Life travel grant to attend and present my poster at OPTIONS for the Control of Influenza XII 2024 in Brisbane, Australia. This conference offered valuable insights into recent and exciting influenza research. It focused on pandemic preparedness and reported the latest influenza research, including recent zoonotic outbreaks such as the American bovine outbreak and the pan-zoonotic avian outbreak in South America.

In addition to the conference, I participated in the School of Influenza, a limited-entry course designed for early-career researchers to learn from experts. This course provided background information on the novel research presented at the conference and allowed me to meet young researchers interested in a similar field, fostering international connections to a global health issue.

My poster outlined preliminary research for my PhD project, highlighting the differences in immune responses between conventional protein and mRNA vaccination. I described how mRNA vaccines stimulate large pro-inflammatory signals, leading to limited recall of memory. Now the basis of my PhD project, I am investigating how early mRNA-induced signals shape downstream immunity, which is crucial for optimising mRNA vaccine design. mRNA vaccines are a frontrunner for the next generation of influenza vaccines, and our findings will inform strategies to overcome key challenges faced in current influenza vaccines.

Attending the OPTIONS XII conference was a great opportunity for me as an early-career researcher to interact with experts and learn about fundamental influenza virology and the latest research. It also allowed me to present the basis of my PhD project, which has guided and strengthened my ongoing research.

KAITLIN BUICK

ASI ADVANCED IMMUNOLOGY SCHOOL, SYDNEY IN JUNE 2025

Kaitlin Buick

In June 2025, with the generous support from Research For Life, I was able to attend the Advanced Immunology School in Sydney, Australia. About 40 early career immunologists attended the school along with invited faculty members from across Australia and New Zealand who are experts in their chosen immunology fields. It was an intensive four-day course that encourages the attendees to network and engage in in-depth discussions with both the faculty and other attendees. Each day was filled with talks, round table discussions and numerous opportunities to network. The school was unlike any conference I have been to as everyone, including the faculty members, stayed on site for the duration. This helped to create a collaborative and friendly environment that fostered interactions and engagement.

I had a fantastic time at the school and found it incredibly valuable. I was able to present some of my work and learn some new skills through the opportunity to chair a speaker session. The school provided a supportive and engaging environment that helped me to gain more confidence with networking, and I was awarded the Best Contributor Award at the end of the school. Attending the Advanced Immunology School has allowed me to grow my network and I hope to see the attendees and faculty I met there at future Australia and New Zealand Society for Immunology events.

KAYDEN BORCHOWSKY

RANZCOG ANNUAL SCIENTIFIC MEETING, WELLINGTON IN OCTOBER 2024

Kayden Borchowsky

With the generous support of Research For Life, I was able to attend and present my research at the Royal Australian and New Zealand College of Obstetricians and Gynaecologists Annual Scientific Meeting (RANZCOG ASM) which was held in Wellington in October 2024. This is a scientific conference with a clinical focus, proving valuable to myself as a future clinician and current Bachelor of Medical Science (honours) student. The theme for this conference was 'Partnerships in Practice', underpinning the importance of clinical relationships with colleagues, patients, and their whānau.

My abstract was chosen for an oral presentation where I presented my research investigating the role of Neutrophil Extracellular Traps (NETs) in endometriosis pathogenesis, and their feasibility as an endometriosis diagnostic biomarker. This provided useful experience in communicating complex information to both researchers and those with a clinical background, a skill needed for clinical work. I was also able to make connections with doctors and scientists who work in the endometriosis space, which will be helpful for my future study and practice.

The keynote speakers featured clinicians with a research component to their practice who discussed their clinical careers alongside their academic interests. These speakers enriched my understanding of how clinical practise can be interwoven with academia, research and teaching, broadening my view of a clinicians standing in the academic space.

I was fortunate to be able to attend a pelvic pain session which deepened my understanding of the complexity of the syndrome's aetiology and the interdisciplinary approach needed for effective management. This session brought into question the importance of a specific diagnosis, when, for example, an endometriosis diagnosis rarely changes pelvic pain management. Further, this session highlighted the central role of pelvic floor physiotherapy in persistent pelvic pain, increasing my appreciation for this important profession.

Overall, my attendance at the RANZCOG ASM was inspiring, rewarding and extremely valuable for my future medical career. This conference has added to my interest in persistent pelvic pain and endometriosis, motivating me to continue work in this field.

KIT MOLONEY-GEANY

52ND ANNUAL SCIENTIFIC MEETING OF THE AUSTRALIAN AND NEW ZEALAND SOCIETY FOR IMMUNOLOGY (ASI) CONFERENCE, SYDNEY IN NOVEMBER 2024

Kit Moloney-Geany

I am very grateful for the travel grant provided by Research For Life, which enabled me to attend the 52nd Annual Meeting of the Australian and New Zealand Society for Immunology (ASI) in Sydney, Australia. This was my first immunology-focused conference since joining the Malaghan Institute, and it provided an invaluable opportunity to understand the breadth of research being conducted across Oceania. ASI offered a fantastic overview of the various facets of immunology taking place both locally and in Australia. A particular highlight was meeting postdoctoral researchers at a similar career stage and learning how they design and conduct their work.

At ASI, I presented my research in a poster session. My work investigates how microbial infections remodel the lung microenvironment and the extent to which this remodelling influences subsequent infections. In particular, we focus on the formation of ectopic lymphocyte aggregates known as tertiary lymphoid structures. Presenting at ASI gave me a valuable opportunity to discuss my preliminary findings, which were met with encouraging feedback. In addition, hearing presentations from other researchers in the same field helped me refine future research questions and identify the methodologies best suited to address them. A particular high point was connecting with Dr. Claudia Kemper from the National Institutes of Health, USA, who shared our enthusiasm for this area of research and expressed interest in sharing data and collaborating.

Overall, ASI was an excellent experience that helped me better orient myself within the immunology research landscape in Oceania and begin building important research connections. I look forward to strengthening these collaborations as I progress in my career.

LUCY PICARD

LONDON CALLING CONFERENCE OXFORD NANOPORE TECHNOLOGIES, LONDON IN MAY 2025

Lucy Picard

Attending the London Calling 2025 conference was a valuable and inspiring experience. The event brought together leading researchers, clinicians, and innovators in nanopore sequencing, and I was honored to present my work on developing a bioinformatic diagnostic pipeline to streamline cancer treatment through precision medicine.

My poster presentation was well received. I had many engaging conversations with attendees who expressed strong interest in the clinical potential of my work. Several researchers and ONT staff commented on the practicality and timeliness of tools that support oncology treatment decisions. The feedback affirmed the relevance of my research in the broader context of precision medicine.

I participated in hands-on workshops, including one on loading PromethION flow cells, which will enhance our lab's technical capabilities. These sessions deepened my understanding of the technology and will benefit our group's projects. I also gained insights into how clinical groups globally are integrating nanopore sequencing into diagnostics, knowledge that will support its implementation in New Zealand's clinical genetics services. I connected with many researchers and clinicians, especially in cancer genomics. Notably, I had promising discussions with Australian teams about improving healthcare for Indigenous and underserved communities. There is strong mutual interest in developing culturally appropriate, community-based diagnostic tools. These collaborations could be pivotal in bringing nanopore sequencing into routine clinical care in Aotearoa. Additionally, the Australia/NZ ONT sales team is open to discussing how they can support these goals.

The conference was immensely valuable both professionally and personally. It reinforced the importance of my research and provided a platform to connect with a global community of like-minded scientists. I left with new skills, fresh ideas, and a renewed sense of purpose. Most importantly, it opened doors to meaningful collaborations that could shape the future of cancer diagnostics in New Zealand. I'm deeply grateful for the opportunity and look forward to applying what I've learned to benefit our research group and the wider healthcare community.

NGAIRE SPARKES

11TH BIENNIAL PACIFIC REGION INDIGENOUS DOCTORS CONGRESS (PRIDOC), KAURNA COUNTRY (ADELAIDE) IN DECEMBER 2024

Ngaire Sparkes

Ko Ngaire Sparkes tōku ingoa, he uri ahau nō Ngāi Tūhoe me Ngāti Ruapani. Nō Waikaremoana ahau. My name is Ngaire Sparkes, and I am a descendant of Ngāi Tūhoe and Ngāti Ruapani from Waikaremoana.

To begin, I would like to acknowledge Ngāti Toa Rangatira for allowing me to base my PhD on their amazing antenatal hub and Research For Life for the $1,500 travel grant to present my PhD at PRIDoC—tēnā koutou katoa.

I am a third-year PhD candidate and Research Fellow with Te Tātai Hauora o Hine (National Centre for Women's Health Research Aotearoa, Victoria University of Wellington). My PhD explores the acceptability, feasibility, and key learnings of an innovative Iwi-led antenatal hub (based in Porirua and called Te Puna Wairua) from the perspectives of ngā whānau hapū (pregnant families) and those involved in its design and delivery. The results from this research will help shape the hub and hopefully inform future antenatal service provision across Aotearoa.

In December 2024, I was privileged to present an overview of my PhD at PRIDoC. PRIDoC brings together Indigenous doctors' member organisations from the United States of America, Canada, Hawai'i, Taiwan, Australia, and Aotearoa. It is a forum for Indigenous peoples to gather and discuss topics of mutual interest pertaining to holistic and cultural health and wellbeing. It was interesting to observe that despite divergent geographic locations, we share many similarities as Indigenous peoples in terms of both aspirations and challenges.

My presentation was in a Pecha Kucha style category (20 slides, 20 seconds each, minimal words), created specifically to provide a space for students and junior doctors to share their projects/research/initiatives/etcetera quickly and creatively. This was my first time presenting in Pecha Kucha style, and I learnt innovative information-sharing techniques. Paradoxically, despite being the shortest presentation I have ever delivered (only 4min20sec), it was the most time-consuming to prepare!

Presenting at PRIDoC allowed me to discuss and seek feedback about my PhD project in a supportive, international, and Indigenous environment. I was also fortunate to meet other Indigenous health clinicians/researchers and develop meaningful interpersonal connections to support my current and future research endeavours. Above all, attending PRIDoC has helped further develop and refine my ideas about Māori health, research, and policy.

Ngā mihi ano to Research For Life for helping me to attend this conference.

TESSA PECK

52ND ANNUAL SCIENTIFIC MEETING OF THE AUSTRALIAN AND NEW ZEALAND SOCIETY FOR IMMUNOLOGY (ASI) CONFERENCE, SYDNEY IN NOVEMBER 2024

Tessa Peck

Thanks to the generous support of Research For Life, I was able to present my research at the annual Australasian Society for Immunology Conference in Sydney. The conference took place at the end of November in 2024 at the ICC which stands and operates on Tumbalong, the land of the Gadigal clan of the Eora Nation. The conference ran over five days, with workshops, poster sessions, seminars, and social events which gave me plenty of opportunities to meet other scientists and learn about the incredible research carried out by our Australasian immunology community.

I presented research carried out at Te Herenga Waka investigating the mechanism of action of a novel compound in a model of multiple sclerosis (MS). This work was in collaboration with The Ferrier Research Institute and aims to create a compound that could be used to treat people with MS. We have found that treatment reduces the leakiness of the blood brain barrier which, in turn, prevents the uncontrolled influx of inflammatory cells into the brain and spinal cord during disease. Many MS treatments can have serious side effects so it is important to fully understand the mechanism of action of any compound before it can be used in patient populations.

During my poster presentation I was able to discuss this work with our Australian collaborators which was hugely insightful. It is always so valuable to speak in person rather than over Zoom and our discussions sparked many new ideas on where to take the project. Overall, attendance at this conference was rewarding both personally and professionally. I would like to thank the Wellington Medical Research Foundation again for their financial support, allowing me to attend such a fantastic conference.

SOTARO OCHIAI

52ND ANNUAL SCIENTIFIC MEETING OF THE AUSTRALIAN AND NEW ZEALAND SOCIETY FOR IMMUNOLOGY (ASI) CONFERENCE, SYDNEY IN NOVEMBER 2024

Sotaro Ochiai

I was awarded a $2500 travel grant to attend the Australia and New Zealand Society for Immunology (ASI) annual meeting in Sydney, Australia, held from November 25–29. This premier event brought together over 600 researchers, including renowned leaders in immunology from Australia and around the world.

At the conference, I presented my latest findings via a poster titled "Allergic Skin Inflammation Drives IL-4-dependent Differentiation of CD11b-low cDC2s." My study explores how the IL-4 cytokine supports the allergic skin environment by promoting the generation of “pro-allergic” dendritic cells. The presentation was well-received, sparking discussions with delegates specializing in cytokines, dendritic cell biology, and allergic responses.

A highlight of the meeting was forging connections with Australian scientists, which holds particular importance due to our geographical proximity. These collaborations provide a unique opportunity to share resources, expertise, and insights, enabling more efficient and impactful progress in our shared research areas.

I sincerely thank Research For Life for enabling me to present my work and engage in meaningful in-person discussions. This experience has significantly broadened the reach of my research and strengthened its impact.

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's prize was won by Danielle Sword (centre right) for her presentation on CAR T-cell therapy in Aotearoa. Last year's winner Ellie Johnson (centre) achieved runner up for her presentation on housing as a public health intervention.

Featured competitors left to right: Samantha Murton, Thalia Heiwari, Ellie Johnson, Danielle Sword and Paul Owaci.

NIWA Science Fair

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

The following exhibits won Research For Life WMRF Special Prizes in 2025:

GURLEEN KAUR YEAR 8 STUDENT FROM CHILTON SAINT JAMES SCHOOL
Won $150 for her project called "Battle of the Brands. Which oral product cleans teeth best, and is safe for enamel?"

EMMA SMART-BALDWIN YEAR 8 STUDENT FROM QUEEN MARGARET COLLEGE
Won $150 for her project titled "Year level vs backpack weight."

Research For Life

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