ABSTRACT BOOK TEMPLATE

ASI NZ Branch Meeting 2025 Abstracts

A1: The immune response to conception and why it matters

Robertson, Sarah[1]

[1]University of Adeleide, Australia.

Conception and progression to pregnancy depend on immune tolerance to paternally-inherited alloantigens. This state of tolerance is mediated by T regulatory (Treg) cells and initiated at conception, when T cells activated by seminal fluid and the conceptus in the mucosal surfaces of the female reproductive tract determine whether or not the uterus will allow embryo implantation and placental development. Immune cells exert tissue remodeling and immune-regulatory roles – they promote embryo-epithelial attachment competence, regulate the differentiation of uterine decidual cells, remodel the uterine vasculature, control inflammatory activation associated with placental development, and suppress immunity to paternally-inherited alloantigens. From a biological perspective, the uterine immune response appears to exert a form of 'quality control' – that is, to promote implantation success when conditions are favorable, but to constrain receptivity when circumstances are not ideal. Women with recurrent implantation failure and recurrent miscarriage often exhibit altered numbers or function of Treg cells and uterine natural killer (uNK) cells. Preclinical and animal studies indicate that deficiencies or aberrant activation states in these cells can be causal in the pathophysiological mechanisms of infertility, and mediate susceptibility to common pregnancy disorders including preeclampsia and preterm birth. Maternal immune cells are therefore targets for diagnostic evaluation and therapeutic intervention – but safe and effective treatments to modulate these cells are in their infancy, and personalized approaches matched to specific diagnostic criteria will be needed. There are several knowledge gaps to be resolved before the promise of immune therapies for infertility can be fully realized.

A2: Aspergillus exposure protects against influenza-induced lethality in mice

Brown, C.[1,2], Palmer, R.[1,3], Hilligan, K.[1,2].

[1]Malaghan Institute of Medical Research, Wellington, NZ, [2]Victoria University of Wellington, Wellington, NZ, [3]University of Otago, Wellington, NZ.

The respiratory tract is routinely exposed to a diverse range of environmental and pathogenic microbes that influence the inflammatory tone of the lung tissue and modulate immune responses. Each microbial encounter leaves a distinct transcriptional, epigenetic and cellular imprint on lung tissue that can determine host susceptibility to infection. *Aspergillus fumigatus* (*Af*) is a ubiquitous sporulating mould that regularly interacts with respiratory mucosa and can penetrate deep into the lower airways following inhalation of spores; however, how *Af* exposure affects the pulmonary microenvironment over time and host susceptibility to subsequent infectious challenge is currently unknown. To assess this, we administered *Af* spores via oropharyngeal instillation to C57BL/6J wildtype mice 28 days prior to intranasal infection with influenza A virus (IAV). Strikingly, *Af* experienced mice were protected against lethal disease (100% survival) compared to inexperienced controls (30% survival). Despite this protective phenotype, similar viral loads were recovered from the lung at 3- and 5dp challenge, showing that IAV could effectively establish an infection in *Af*-primed mice. Flow cytometric analysis of the lungs at 3dp challenge also showed that *Af* experienced mice had a significant decrease in IAV-induced inflammatory macrophage accumulation and an increase in activated regulatory T cells, suggesting that prior exposure to *Af* may promote an immunoregulatory environment that can moderate IAV-induced pathology. Further investigation is required to define the mechanisms behind *Af*-conferred protection against viral disease, which may highlight potential immunoregulatory targets than can limit IAV-induced pathogenesis.

A3: A high dimensional spectral cytometry method for comprehensive phenotyping of murine pulmonary cells

Rebecca Palmer[1,2], Katherine Pilkington[1], Kathryn Hally[2] and Kerry Hilligan[1]

1. Malaghan Institute of Medical Research, Wellington 6012, New Zealand
2. University of Otago Wellington, Wellington 6012, New Zealand

The lung is a complex organ with a deeply heterogenous cellular landscape. Routinely exposed to inhaled particulates such as microbes, allergens, and environmental pollutants, lung cells have unique characteristics that protect the organ from infection and damage. Comprehensive phenotyping of the lung is essential for a deeper understanding of respiratory diseases and the role that various cell types play in modulating lung structure, function and host protection. Spectral cytometry is an essential tool for studying tissue responses at the single-cell level; however, important spatial information is lost during sample preparation. In addition, phenotyping of the lung has predominantly focused on CD45+ 'immune cells', which represent just 60-70 percent of the total cells recovered from a naïve lung, overlooking the significant contribution of stromal cells to the orchestration of immune responses and disease processes. To address these shortcomings, we have developed a multifaceted spectral cytometry platform incorporating *in vivo* staining techniques to retain important spatial information about the immune subsets in our datasets. The platform additionally includes individual non-immune-, myeloid- and lymphoid-targeted panels, with a conserved 16-parameter backbone, to facilitate comprehensive profiling of all major lung cell subsets, including their activation status, functional properties and tissue localisation. The platform has been validated at steady-state and across an influenza A virus infection time course, canvassing peak inflammatory responses and tissue recovery. Our readouts align with current literature and reveal some previously undescribed cellular phenotypes, highlighting the platforms utility for detailed investigation of pulmonary cells in the context of a respiratory tract infection.

A4: The Hidden Identity of Th22 Cells: Stem-like Sentinels of the Mucosa in the Face of Bacterial Infection

Barnes, J.L.[1,2], Plank, M.W.[1,2,6], Asquith, K[1,2], Kim, R.Y.[1,2,3,5], Keely, S[1,2], Horvat, J.C.[1,2] and Foster, P.S.[2,4,5]

[1]The University of Newcastle, Callaghan, NSW, Australia, [2]Hunter Medical Research Institute, New Lambton Heights, NSW, Australia, [3]University of Technology Sydney, Sydney, NSW, Australia, [4]Macquarie University, Sydney, NSW, Australia, [5]Woolcock Institute of Medical Research, Macquarie Park, NSW, Australia, [6]GlaxoSmithKline Australia, Abbotsford, VIC, Australia.

CD4$^+$ T-helper 22 (Th22) cells are a major source of interleukin-22 (IL-22), contributing to host defence and tissue integrity at mucosal barrier sites during bacterial infection. Although phenotypically distinct from IL-22-producing Th17 cells by lack of IL-17A production, the identity and function of Th22 cells remain poorly defined due to challenges in isolating them from heterogeneous tissue T cell populations.

To address this, we developed unique multi-cytokine reporter mouse strains to definitively identify and isolate Th22 cells from inflamed mucosa. Following bacterial infection of the lung and gut, we observed the induction of archetypal Th22 cells (IL-22$^+$ IL-17A$^-$ IFNγ$^-$) in mucosal effector sites and associated lymphoid tissues. Interestingly, a subset of Th22 cells in the lung and colon lamina propria co-produced IFNγ—a feature absent in lymph nodes and Peyer's patches—suggesting site-specific functional heterogeneity.

Transcriptomic profiling of Th22 cells, compared with other IL-22, IL-17A, and IFNγ-producing effector Th subsets, revealed a distinct gene signature enriched for stem-like features. This 'core Th22 gene set'—including *Tcf7* (encoding TCF-1), *Cxcr5*, and *St6gal1*—was conserved between mouse and human Th22 cells, suggesting a cross-species transcriptional identity. In ex vivo chemotaxis assays, Th22 cells selectively migrated toward a CXCL13 gradient, consistent with CXCR5 expression. Immunofluorescence imaging further revealed that Th22 cells localise to discrete micro-anatomical niches within lymphoid aggregates, indicating a specialised role in orchestrating durable antibacterial immune responses.

Together, these findings provide the first comprehensive molecular characterisation of Th22 cells in inflamed barrier tissues and reveal that Th22 cells elicited by bacterial infection represent a distinct, heterogeneous subset with stem-like properties and pathogenic progenitor potential.

A5: Investigating how Interleukin-4 and 13 influence cholesterol and organelle distribution in bone marrow derived dendritic cells

Harlick, C.[1], Fabre, M.S.[1], Dabb, A.[1], Schmidt, A.[1], Ronchese, F.[1], Lamiable, O.[1].

[1] Malaghan Institute of Medical Research, Wellington, New Zealand.

Type 2 conventional dendritic cells (cDC2s) take up antigens and migrate to lymph nodes to prime CD4+ T helper cell responses. In the skin, a cDC2 subset characterised by low expression of the integrin CD11b is specialised toward the induction of Th2 cells. We showed that type 2 cytokines Interleukin 4 (IL-4) and IL-13 are necessary for the development of skin $CD11b^{lo}$ cDC2s. Our unpublished results suggest that cholesterol metabolism may contribute to the ability of $CD11b^{lo}$ cDC2 to prime Th2 cells. Cholesterol is known to regulate the function of dendritic cells, including migration, antigen presentation, and activation. However, whether IL-4 or IL-13 directly affects cholesterol distribution in cDC2s remains unknown.

To begin addressing this, we generated Flt3-L bone marrow-derived DCs (BMDCs) and cultured them with or without IL-4 or IL-13 for three days. These cytokines induce the differentiation of cells resembling skin $CD11b^{lo}$ cDC2s. Those BMDCs were stained with fluorescent probes filipin III and DRAQ7 that label cholesterol and nuclei, respectively. Confocal imaging showed that IL-4 and IL-13 treatments led to the redistribution of cholesterol compared to unstimulated cells. IL-4 and IL-13 altered both the amount and distribution of cholesterol within these cells

To further dissect these changes, we plan to combine cholesterol filipin III staining with Cell Painting cytological profiling, a high-content imaging method that labels key organelles such as the nucleus, mitochondria, endoplasmic reticulum, actin cytoskeleton, RNA granules, and plasma membrane. Cell Painting analysis showed that IL-4 and IL-13 each induced substantial reorganisation of the intracellular architecture in BMDCs. Notably, only IL-13 triggered the appearance of multinucleated cells, suggesting distinct effects on cell morphology. Together, these preliminary data indicate that IL-4 and IL-13 remodel cellular structure and cholesterol distribution. Ongoing work will clarify how these changes are linked and could influence dendritic cell function.

A6: An end-to-end pipeline for designing and testing pan-virus, universal RNA vaccines

Lisa Connor

Malaghan Institute of Medical Research, Wellington, NZ

The rapid success of mRNA vaccines against SARS-CoV-2 has highlighted the power of programmable antigen design and delivery, yet the continuous emergence of viral variants underscores the need for broadly protective universal vaccines. We have built an integrated platform that combines AI-guided protein engineering, high-throughput mRNA-to-protein expression, and custom B-cell bait reagents to perform in vivo antigen-library screens. This approach rapidly pinpoints immunogens that elicit cross-reactive B cells targeting conserved viral epitopes.

As a proof of concept, an mRNA construct encoding a fusion of SARS-CoV-2 Delta and Omicron Spike receptor binding domains (RBD) elicited neutralising titres on par with full-spike controls but uniquely expanded B cells binding both variants, and even the more distantly related SARS-CoV-1, demonstrating selective enrichment of BCRs recognising shared epitopes through enhanced antigen avidity. Coupling BCR tetramer labelling with CITE-seq then allowed detailed mapping of somatic hypermutation and clonal evolution underlying broadly neutralising antibody emergence.

Applying these insights to influenza, we are using AI to guide construction of an mRNA library of haemagglutinin antigens from multiple strains and using targeted B-cell baits to enrich clones against conserved stem and receptor-binding regions. In parallel, novel RNA formats and LNP chemistries undergo iterative testing to optimise antigen expression, delivery, and tolerability. Employing these strategies our goal is to develop vaccine candidates with broader, more durable protection against influenza and pandemic threats.

A7: Teamwork for the cure: combination NK cell and CAR T cell therapy

McLellan AD.[1]

[1]Department of Microbiology and Immunology, University of Otago, Dunedin, NZ,

Chimeric antigen receptor (CAR) T cells and natural killer (NK) cells are immune cells able to target cancers. To date, seven CAR T cell products have been FDA approved for treatment of blood cancers. These products exceed the expectations of standard care for remissions. However, there are still major treatment gaps in CAR T cell treatable cancers and no FDA-approved CAR T cell treatments for solid cancers. We have found that although CAR T cells and NK cells are limited in their respective monotherapies, they display mutually beneficial traits when co-administered. I will present a novel and accessible pipeline for simultaneously manufacturing CAR T cell and NK cell products for cancer immunotherapy in a hard-to-treat solid cancer model using 'kit-set' artificial antigen presenting cells (aAPC). The results illustrate the synergy of teamwork between different immune cells to provide optimised cellular therapy for refractory cancers. To allow international researchers access to our new NK cell expansion technology,[1] our 'kit-set' aAPC vectors are publicly available:
https://www.addgene.org/Alexander_McLellan/

1. Dobson LJ, Saunderson SC, Smith-Bell SW, McLellan AD. Sleeping Beauty kit sets provide rapid and accessible generation of artificial antigen-presenting cells for natural killer cell expansion. *Immunol Cell Biol.* 2023 Oct;101(9):847-856. doi: 10.1111/imcb.12679. Epub 2023 Aug 16. PMID: 37585342.

A8: High-dimensional unbiased immune profiling reveals treatment-specific immune signatures in people with multiple sclerosis undergoing disease-modifying therapy.

Taylor, J.[1], Milner, I[1], La Flamme, AC[3,4], Robichon, K[2,3]

[1] Neurology Department, Capital Coast and Hutt Valley, Health New Zealand Te Whatu Ora, Wellington, NZ [2]Department of Pathology and Molecular Medicine, University of Otago, Wellington, NZ [3]School of Biological Sciences, Victoria University of Wellington, Wellington, NZ [4] Malaghan Institute of Medical Research, Wellington, NZ

Multiple sclerosis (MS) is a chronic autoimmune disease characterized by demyelination and neuroinflammation driven by autoreactive immune cells. Despite the growing use of disease-modifying therapies (DMTs) for relapsing-remitting MS, comparative analyses of their effects on immune cell profiles using unbiased methods remain limited. In this study, we applied high-dimensional spectral flow cytometry and advanced computational analysis to investigate immune cell composition across four DMTs—Natalizumab, Dimethyl fumarate (DMF), Fingolimod (FTY), and Ocrelizumab — in a longitudinal cohort of 70 participants, including newly diagnosed untreated RRMS patients and healthy controls.

Cell clustering using and dimensionality reduction revealed 16 immune cell subsets, with distinct abundance patterns associated with specific DMTs. Notably, FTY and DMF-treated samples displayed clear separation from other groups in both principal component analysis (PCA) and non-metric multidimensional scaling (NMDS), indicating unique immunophenotypic signatures. DMF treatment was characterized by upregulation of cluster 1 and downregulation of cluster 10, whereas FTY treatment showed downregulation of cluster 1 and upregulation of cluster 8. Ocrelizumab-treated participants exhibited a distinct decrease in cluster 13 abundance, revealed more clearly through NMDS than PCA.

These findings demonstrate that different DMTs induce distinct and measurable alterations in peripheral immune cell populations, which are not captured by conventional clinical tests. Unbiased high-dimensional profiling offers valuable insight into the immunomodulatory mechanisms of DMTs and can identify cellular signatures predictive of treatment response. Our study underscores the utility of longitudinal, unbiased immune monitoring in improving our understanding of MS therapies and guiding personalized treatment strategies.

A9: Rewiring endogenous genes in CAR T cells for tumor restricted payload delivery

Amanda X. Y. Chen1,2*, Kah Min Yap1,2*, Joelle S. Kim1,2, Kevin Sek1,2, Yu-Kuan Huang1,2, Phoebe A. Dunbar1,2, Volker Wiebking3, Jesse D. Armitage4,5, Isabelle Munoz1,2, Kirsten L. Todd1,2, Emily B. Derrick1,2, Dat Nguyen1,2, Junming Tong1,2, Cheok Weng Chan1,2, Thang X. Hoang1,2, Katherine M. Audsley1,2, Marit J. van Elsas1, 2, Jim Middelburg1,2, Joel N. Lee1,2, Maria N. de Menezes1,2, Thomas J. Cole1,2, Jasmine Li1,2, Christina Scheffler1,2, Andrew M. Scott6,7, Laura K. Mackay8, Jason Waithman4,5, Jane Oliaro1,2, Simon J. Harrison,2,9, Ian A. Parish1,2, Junyun Lai1,2, Matthew H. Porteus3, Imran G. House1,2+, Phillip K. Darcy1,2,10+, Paul A. Beavis1,2+

1Cancer Immunology Program, Peter MacCallum Cancer Centre, Melbourne, 3000, Victoria, Australia.
2Sir Peter MacCallum Department of Oncology, The University of Melbourne, Parkville, 3010, Australia.
3Department of Pediatrics, Stanford University, Stanford, CA 94305, USA.
4School of Biomedical Sciences, University of Western Australia, Perth, WA, Australia.
5Leukaemia Translational Research Laboratory, Telethon Kids Cancer Centre, Telethon Kids Institute, Perth, WA, Australia.
6Olivia Newton-John Cancer Research Institute and School of Cancer Medicine, La Trobe University, Melbourne, Victoria, Australia.
7Faculty of Medicine, The University of Melbourne, Parkville, Victoria, Australia.
8Department of Microbiology and Immunology at the Peter Doherty Institute for Infection and Immunity, The University of Melbourne, Parkville, Victoria, Australia.
9Clinical Haematology and Centre of Excellence for Cellular Immunotherapies, Peter MacCallum Cancer Centre and Royal Melbourne Hospital, Melbourne, Vic, Australia.
10Department of Immunology, Monash University, Clayton.
* Contributed equally
+ Senior authors contributed equally

The efficacy of chimeric antigen receptor (CAR) T cell therapy in solid tumors is limited by immunosuppression and antigen heterogeneityClick or tap here to enter text.. To overcome these barriers, “armored” CAR T cells, which secrete proinflammatory cytokines, have been developedClick or tap here to enter text.. However, their clinical application has been limited due to toxicities related to peripheral expression of the armoring transgeneClick or tap here to enter text.. Here, we developed a CRISPR knock-in strategy that leverages the regulatory mechanisms of endogenous genes to drive transgene expression in a tumor-localized manner. By screening endogenous genes with tumor-restricted expression, the *NR4A2* and *RGS16* promoters were identified to support the delivery of cytokines such as IL-12 and IL-2 directly to the tumor site, leading to enhanced anti-tumor efficacy and long-term survival of mice in both syngeneic and xenogeneic models. This was concomitant with improved CAR T cell polyfunctionality, activation of endogenous anti-tumor immunity, a favorable safety profile, and was applicable using CAR T cells from patients.

A10: Using gene editing to fine-tune gene expression and dissect the immune checkpoint inhibitor response in melanoma

du Rand, A.[1,2], Malhi, C.[1], Verdon, D.[1,2], Didsbury, A.[1,2], Michaels, Y.[3], Knapp, D.[4], Jamieson, S.[1,5], Dunbar, R.[1,2], Sheppard, H.[1,2]

[1]School of Biological Sciences, The University of Auckland, Auckland, New Zealand, [2]Maurice Wilkins Centre for Molecular Biodiscovery, The University of Auckland, Auckland, New Zealand, [3]School of Biomedical Engineering, University of British Columbia, Vancouver, Canada, [4]Institute for Research in Immunology and Cancer (IRIC) and Department of Pathology and Cell Biology, University of Montreal, Montreal, Canada, [5]Faculty of Medical & Health Sciences, The University of Auckland, Auckland, New Zealand.

Immunotherapies employing immune checkpoint inhibitors (ICIs) have significantly advanced the treatment of several cancers including melanoma by unleashing the body's immune system to kill cancer cells. One class of ICIs targeting the PD-1/PD-L1 inhibitory signalling axis blocks the binding between the PD-1 receptor on T lymphocytes and its ligand PD-L1 on cancer cells. However, this treatment is only effective in 30% of melanoma patients, and the biological mechanisms underlying this variable response remain poorly understood.

We hypothesize that there is a specific range of PD-1/PD-L1 expression that optimally promotes cancer cell killing. To investigate this, we are applying CRISPR/Cas9-mediated genome editing to modulate endogenous levels of PD-1 in T cells and PD-L1/PD-L2 in melanoma cells, whilst maintaining expression within physiologically relevant ranges. This fine-tuning is facilitated by the insertion of synthetic microRNA (miRNA) response elements of differing strengths into the 3' untranslated regions (3'UTRs) of the target genes.

To date, we have achieved targeted insertion of a miRNA response element into the PD-1 locus in primary human T cells or the PD-L1 locus in melanoma cells with efficiencies of up to 99% and 98%, respectively. Flow cytometric analysis demonstrated a corresponding reduction of these cell surface proteins, indicative of fine-tuned gene expression. Additionally, we have successfully generated highly efficient (>90%) PD-L1 and PD-L2 knockout melanoma cells and PD-1 knockout T cells. Ongoing studies are investigating how modulation of this pathway influences the activity of melanoma-specific T cells. Ultimately, this research is intended to support the identification of patients likely to benefit from ICI therapy and to inform the development of optimized T cell-based immunotherapies.

1. Michels, Y., et al (2019). *Precise tuning of gene expression levels in mammalian cells*. Nature communications. 10(1): p. 818.

A11: Δ133p53-like isoform exerts context-dependent effects on immunotherapy and tumour survival

Authors:
Kevin Ly, Yan Wen, Antony Braithwaite, Kunyu Li
Affiliation:
Department of Pathology, Dunedin School of Medicine, University of Otago

Abstract:
Adoptive cell therapy (ACT) is a promising approach to enhance anti-tumour immunity, yet resistance mechanisms remain a significant clinical challenge. The Δ133p53 isoform, a truncated variant of the tumour suppressor p53, is frequently overexpressed in advanced cancers and associated with poor outcomes. Here, we investigate the immunological and tumour-intrinsic roles of Δ122p53, the murine analogue of human Δ133p53, in a syngeneic colorectal cancer model.

In a **prophylactic ACT study**, mice received T cell therapy prior to tumour challenge with either empty vector (EV) or Δ122p53-expressing tumour cells. Mice bearing Δ122p53-expressing tumours exhibited **significantly reduced survival**, independent of the T cell type used, suggesting tumour-intrinsic resistance mechanisms. Supporting this, **in vitro assays under nutrient-deprived conditions** showed that Δ122p53 expression enhances tumour cell survival, indicating a stress-adaptive advantage.

In contrast, a **therapeutic ACT trial**, in which mice received treatment after tumour establishment, revealed that Δ122p53 expression in the host **enhanced anti-tumour immune responses**, regardless of whether the tumour cells expressed Δ122p53. These results suggest that Δ122p53 may also modulate host immune function in a manner that enhances tumour clearance post-treatment.

Together, these findings highlight the **context-dependent effects of Δ122p53** on immunotherapy response—impairing tumour control in prophylactic settings while **enhancing immune-mediated tumour rejection** in therapeutic contexts. Further investigation into the temporal and cellular mechanisms involved will be critical for understanding how p53 isoforms shape tumour–immune interactions and influence immunotherapy outcomes.

A12: Using Advanced Genome Editing Techniques to Reprogramme T-cells for Cancer Immunotherapy

Buttle, B.[1], du Rand, A.[1], Verdon, D.[1], Dunbar, R.[1], Sheppard, H.M.[1]

[1]School of Biological Sciences, University of Auckland, NZ.

Historically, autologous T-cell receptor engineered T-cells (TCR-T) and chimeric antigen receptor (CAR) T-cells for cancer immunotherapy have been produced using retroviral gene insertion. Non-viral CRISPR-based editing may be a better approach, enabling physiological receptor expression and reducing insertional mutagenesis risk. CRISPR/Cas9 editing in primary human T-cells is in its early stages globally, and large-insert editing often yields low insertion rates and high toxicity, limiting therapeutic cell populations. The type of template DNA used in large-insert genome editing affects toxicity, insertion rates, and off-target DNA integration. We aim to assess the optimal template DNA type and optimise editing conditions to maximise the therapeutic T-cell population.

We have sequenced melanoma-targeting TCRs from a library of CD8+ T-cell clones using TCR sequencing protocols we developed. We plan to use optimised gene editing tools to insert these novel TCRs into the endogenous TCR locus. To optimise large-insert gene editing protocols, we are working with the insertion of a large green fluorescent protein (eGFP) tag. A proof-of-principle experiment yielded >40% insertion of eGFP into HEK293T cells. Now, to develop and assess efficient T-cell editing methods, we are:

- Developing protocols to generate linear and circular dsDNA and ssDNA templates to compare how template type affects insertion rates and T-cell viability after editing.
- Assessing the effect of DNA-sensing inhibition on T-cell viability following large-insert gene editing.
- Evaluating the ability of CRISPR-edited TCR-T cells to recognise target antigens and kill cancer cells.

This research could advance T-cell immunotherapy and support the development of a platform to generate T-cells targeting tumour-specific antigens. The scope of this work extends beyond melanoma, as we can sequence TCRs from our CD8+ T-cell clone library targeting viral, cancer germline, and other neoantigens. Additionally, methods developed through this research may be broadly applicable to all large-insert T-cell editing and editing in other cell types.

A13: Selecting antigenic targets for personalised, adoptive T-cell therapy based on the multiplexed immunofluorescence imaging of epithelial ovarian cancer tissues

Eom, J.[1,2], Didsbury, A.[1,2], Park, S.M.[1,2], Chen, C.J.[1], Robinson, H.[1], Charlton, A.[3], Lin, S.C.Y.[1,2], Munoz-Erazo, L.[1,2], Dunbar, P.R.[1,2]

[1]School of Biological Sciences, University of Auckland, NZ, [2]Maurice Wilkins Centre for Molecular Biodiscovery, NZ, [3]LabPLUS, Auckland Hospital, NZ

Cancer Germline Antigens (CGAs) are attractive targets for adoptive T cell therapy protocols due to low expression on normal tissues and avoidance of tolerance. However, heterogeneous expression of single CGAs within an individual's tumours means that therapeutic targeting of multiple CGAs may be necessary to reduce immune escape.

To better understand this heterogeneity, we assessed CGA expression in epithelial ovarian cancer (EOC). In New Zealand, EOC is the 5th highest cause of female cancer mortality and given the limited improvement in 5-year survival rates over the past few decades, is an area of high unmet need. We have utilised multiplexed immunofluorescent techniques, which allowed simultaneous assessment of multiple CGAs on a single slide and captured clonal heterogeneity across the whole tissue.

We pilot screened 10 samples from New Zealand EOC patients using our multiplexed immunofluorescent technique for the expression of three CGAs (PRAME, NY-ESO-1, and MAGE-A4), and analysed their co-expression patterns. In addition, we stained the same tissue samples with markers of immune cells (e.g. CD3, CD20 and CD68), stromal cells to characterise the tumour microenvironment (TME) in each patient. Our results demonstrated considerable variation between patients in both the distribution and expression of three CGAs, and the degree of immune infiltration and fibrosis across patients. Our data show the value of multiplexed screening for CGA expression in cancer patients in planning T cell therapy approaches for individual EOC patients and provide valuable insights into the microenvironment of EOC tumours.

A14: CAR T cells synergise with parallel-expanded autologous natural killer cells to improve solid cancer immunotherapy

Dobson L[1], McLellan AD.[1]
[1]Department of Microbiology and Immunology, University of Otago, Dunedin, NZ

Natural killer (NK) cells represent a new frontier in adoptive cell immunotherapies. NK cells possess an innate ability to recognise and kill cancer cells, and unlike CAR T cells, can be administered in an 'off-the-shelf' allogeneic setting. Supplementing NK cells with Chimeric Antigen Receptors (CAR) further enhances their therapeutic capacity, with initial trials outlining remarkable safety and efficacy.

This presentation will encompass our platform for expanding clinical doses of NK cells. In a laboratory setting, stimulating 5 million PBMC with homemade feeder cells can induce 70,000-fold expansions and yield over 7 billion NK cells in 14 days. The NK cells are highly cytotoxic and express Fc receptors for recognition of IgG.

Standard CAR T cell production involves selection of T cells from PBMC while discarding the remaining non-T cells. By applying our feeder cells to the residual non-T cell PBMC, the waste product can be derived into highly pure and functional NK cells to enable synergistic combination therapies of NK cells with CAR T cells. Furthermore, we have shown that NK cells can be successfully transduced with CAR, offering further potential to expand antigen-recognition for treatment of blood and solid cancers.

A15: Translating Immunology into Clinical Impact for New Zealand Patients

Didsbury, A J.[1,2]

[1]Te Aka Mātauranga Matepukupuku, Centre for Cancer Research, The University of Auckland, Auckland, Aotearoa New Zealand

[2] The University of Auckland, School of Biological Sciences, Auckland, New Zealand

Cell-based therapies represent a frontier in immunological medicine, offering innovative treatment alternatives for cancer, rare diseases, and infections. Yet in Aotearoa New Zealand, access to these cutting-edge treatments remains limited, with patients unable to participate in trials and often reliant on self-funding to access overseas care.

To address this gap, Te Aka Mātauranga Matepukupuku (The University of Auckland, Centre for Cancer Research) is establishing a cell therapy programme to enable local development, translation, and clinical delivery of immune-based cell therapies.

This talk will outline the structure and progress of the Te Aka CGT Programme, a collaborative initiative between the University of Auckland, Te Whatu Ora, the NZ Blood Service, and international collaborators. The programme will support end-to-end workflows including process development, GMP-grade cell manufacturing, and clinical integration to bring investigator-led therapies into early-phase trials.

Our flagship project focuses on the generation and delivery of virus-specific T cells (VST) to treat post-transplant viral reactivation. Approximately half of all HSCT recipients experience viral complications, and many are unable to tolerate available antivirals due to severe drug toxicities. VSTs can effectively treat post-transplant viral infections with minimal adverse events. Te Whatu Ora currently relies on high cost imported cells; however, sourcing matched VST products from international cell banks is challenging due to the unique diversity of HLA haplotypes, particularly among Māori and Pacific populations. In response, we are developing the necessary infrastructure, clinical pathways, and expertise to manufacture and deliver locally-sourced allogeneic VST products.

Importantly, the programme is being designed with a commitment to equity by integrating Māori partnership and prioritising local production to reduce cost and improve access. This will help ensure New Zealand patients receive timely access to potentially life-saving immunotherapies.

A16: 2025 Watson Oration: Professor Rod Dunbar.

Dunbar P.R.[1]

[1] The University of Auckland, School of Biological Sciences, Auckland, New Zealand

Professor Rod Dunbar holds both a medical degree (MBChB) and a PhD from the University of Otago. He spent 6 delightful years in Oxford as a post-doc in the labs of Jonathan Austyn at the Nuffield Department of Surgery, and Enzo Cerundolo at the Weatherall Institute of Molecular Medicine. Rod returned to NZ in 2002 under a Wellcome Trust International Senior Research Fellowship, and has been happily ensconced at the University of Auckland's School of Biological Sciences ever since. From 2009-2019 Rod served as the Director of the Maurice Wilkins Centre, and since 2019 he has led the MWC's China collaboration initiative. In 2016 he was appointed as a Fellow of the Royal Society of NZ, and in 2018 was awarded the University of Auckland's Research Commercialisation medal.

A17: Discovering how human macrophages sense and respond to viral infection

Labzin, L.[1]
[1] The University Of Queensland

Macrophages are sentinel innate immune cells whose cytokine response drives effective host immune defence many viruses, including influenza A virus (IAV). IAV can directly infect macrophages, which sense viral RNA and proteins or virus-induced cellular perturbations by pattern recognition receptors (PRRs) in strategic subcellular locations. The current dogma holds that the cell surface and endosomal toll-like receptors (TLRs) 4,7, and 8 sense incoming IAV virions in dendritic cells and macrophages to drive pro-inflammatory and antiviral cytokine expression. In contrast, the ubiquitously expressed cytosolic retinoic acid-inducible receptor (RIG-I) detects IAV replication intermediates to drive critical antiviral responses. Here, we show that IAV pH1N1 (Auck09) abortively infects primary human monocyte-derived macrophages (HMDM) and triggers a potent antiviral (*IFNB1*, *CXCL10)* and pro-inflammatory *(IL6, TNF)* gene expression program. We use a combination of pharmacological and genetic approaches to map which PRRs sense intrinsic IAV infection to drive specific cytokine responses. Uncovering the specific PRR and signalling pathways may identify new targets for improving protecting immunity to IAV or dampening pathological inflammation during severe IAV infection.

A18: Investigating characteristics of the highly fatal Yellow-Eyed Penguin Gyrovirus

Robinson, Z.[1], Taylor, H.[2], Eruera, A.[1], Kumaran, R.[1], Wierenga, J.[1], Geoghegan, J.[1], Kirman, J.[1]

[1]Department of Microbiology and Immunology, University of Otago, Dunedin, NZ, [2]School of Veterinary Sciences, Massey University, Palmerston North, NZ

Hoiho, the yellow-eyed penguin, *Megadyptes antipodes* is an endangered species forecast to become extinct on mainland New Zealand in 20 years. A newly identified yellow-eyed penguin gyrovirus (YEP-GV) is strongly associated with respiratory distress syndrome (RDS) in young chicks. RDS has resulted in mortality for up to 30% of the hatched chicks per season with a >90% mortality rate for affected chicks. Chicken Anaemia Virus (CAV) is a closely related gyrovirus with an approved vaccine that is administered to hens prior to laying to prevent chick mortality. To determine if the CAV vaccine could provide cross-protection against YEP-GV, an *in vitro* neutralisation assay is being developed to determine if sera from vaccinated hens is able to neutralise YEP-GV. If cross-reactivity is observed, it may be possible to repurpose the CAV vaccine to protect hoiho chicks from YEP-GV. YEP-GV is newly discovered, so virus culture, morphology, epidemiology and pathophysiology are unknown.

We have trialled different *in vitro* virus propagation methods including using stem cells from recently hatched hoiho eggs, primary hoiho bone marrow cells and the MDCC-MSB1 chicken cell line. We have successfully cultured primary bone marrow cells harvested from injured yellow eyed penguins for up to 14 days, which can provide a suitable foundation for a neutralisation assay. Additionally, we have purified virus-like particles (VLPs) produced after inoculating MDCC-MSB1 chicken cells with YEP-GV, and visualised these by electron microscopy. The VLP have possible future use as a vaccine component and can be used for the production of monoclonal antibodies against the YEP-GV capsid protein. Investigations into the characteristics of this highly fatal emergent virus and developing potential preventative measures are essential in preserving the population of the rarest penguins in the world.

A19: Modular design of circular RNA for optimised customisable vaccines

White, L.[1,2], Connor, L.[1], Patrick, W.[2]

[1]Malaghan Institute of Medical Research, Wellington, NZ, [2] School of Biological Sciences, Te Herenga Waka Victoria University of Wellington, NZ.

Circular RNA (circRNA) is a novel RNA vaccination technology, offering enhanced stability and sustained protein/antigen expression relative to industry standard messenger RNA vaccines. circRNA design is critical to address key biological questions and develop a well-performing vaccine. We aim to dissect how the different sequence elements required in a circRNA vaccine impact both circRNA production processes and key vaccine parameters including antigen expression, reactogenicity and pathogen protection. To this end, we are adopting a programmable molecular 'plug-and-play' circRNA design to allow systematic screening and optimisation of each individual circRNA element. We chose to produce circRNA using a Permuted Intron-Exon (PIE) arrangement, using self-spicing catalytic type I introns sourced from various species. We have assembled circRNA templates using different permuted self-splicing introns, molecular scars, internal ribosome entry sites and antigens. We are optimising in-house circRNA production and quality control protocols and we can successfully manufacture and purify circRNA using different self-splicing introns. In our proof-of-principle experiments, purified circRNA has been used to transfect HEK293FT cells and drive mNeonGreen reporter protein expression. The type I intron from the Anabaena leucine tRNA gene outperforms the intron from the T4 phage td gene in terms of circRNA yield across tested conditions and protein expression in vitro. Our flexible circRNA platform will allow innovative approaches to producing, testing, and utilising circRNA in vaccine development. We aim to use this technology to create highly customisable vaccines that address and overcome the limitations of current mRNA-based approaches.

A20: Development of Vaccines against Gonococcal Disease using the PilVax Platform

Mejo Chiratteparambil Korah[1,2], Catherine Tsai[1,2], Thomas Proft[1,2]

[1]Department of Molecular Medicine and Pathology, School of Medical Sciences, University of Auckland, Auckland, New Zealand, [2]Maurice Wilkins Centre for Molecular Biodiscovery, Auckland, New Zealand

Gonorrhoea is a sexually transmitted disease caused by *Neisseria gonorrhoeae*, with a high prevalence across the world. There is currently no licensed vaccine against gonorrhoea, but vaccine development is a top priority, particularly with the rise of antimicrobial resistance (AMR). Pilvax is a novel peptide vaccination platform that can trigger systemic and mucosal immune responses[1]. Various gonococcal peptide vaccine epitopes were bioengineered into the Group A Streptococcus (GAS) M1T1 or M6T6 pilus structure and expressed on the surface of the food-grade bacterium *Lactococcus lactis.* This strategy offers peptide amplification, stabilisation and enhanced immunogenicity [1,2]. The correct insertion of the peptides within GAS pili and expression on the surface of *L. lactis* were confirmed by Western blot and flow cytometry. BALB/c mice were immunised intranasally with the modified *L. lactis strains* and systemic (serum) and mucosal (nasal and vaginal) antibody responses were analysed. Antisera were then used for functional assays including a bactericidal assay and a ME-180 cell association assay. Significant serum titers of peptide-specific immunoglobulins were detected in vaccinated mice. Serum bactericidal assay and cell association assay revealed antibody-mediated killing and inhibition of gonococcal adherence to ME-180 cells, respectively. Immunisation of mice with PilVax constructs generated systemic and mucosal peptide-specific antibodies, which were bactericidal and able to neutralise gonococcal binding to host cells. Currently, cellular responses in immunised mice are being analysed, and the efficacy of the vaccine candidates will be further evaluated in a gonococcus mouse infection model.

References

1. Wagachchi, Dasun, et al. "*PilVax–a novel peptide delivery platform for the development of mucosal vaccines*." Scientific Reports 8.1 (2018): 2555.
2. Blanchett, Samuel, et al. "*Intranasal immunization with Ag85B peptide 25 displayed on Lactococcus lactis using the PilVax platform induces antigen-specific B-and T-cell responses*." Immunology and Cell Biology 99.7 (2021): 767-781.

A21: TRANSLATION OF A POTENTIAL VACCINE FOR *STAPHYLOCOCCUS AUREUS* FROM PROTEIN TO mRNA

Langley, R.J., Zandvakili, N., Chan, J., Peterken, K., Clow, F., Gibson, M., Fraser, J.D., Radcliff[1], F.J.

[1]Department of Molecular Medicine & Pathology, University of Auckland, Auckland, NZ.

Staphylococcus aureus is the leading bacterial cause of mortality world-wide and is particularly problematic in the hospital setting, where it can cause complications post-surgery or with interventions such as in-dwelling catheters. Antibiotic resistance is diminishing effective treatment options and forecast to continue growing. To date prospective vaccines have shown promise in rodent models but failed in clinical trials - a lack of known correlates of protective immunity poses a major challenge. We have combined mutated versions of three secreted immune evasion factors common to all *S. aureus* strains - Staphylococcal Superantigen-like (SSL) proteins 3, 7 and 11 – into a novel fusion PolySSL vaccine that elicits a robust IgG response with neutralising activity, attenuates signs of disease, and significantly reduces bacterial tissue burden in a mouse model of *S. aureus* peritoneal infection. Analysis of cross-strain serum antibody responses indicate a multivalent vaccine will be necessary. The capacity to deliver multiple transcripts in one package and the flexibility to modify transcripts in response to changes in pathogen characteristics makes an mRNA vaccine an attractive proposition. Vaccination of mice with an mRNA version of the PolySSL mRNA encapsulated in lipid nanoparticles (LNPs) leads to a robust serum IgG including strong serum-mediated neutralisation responses and a Th1-skewed $CD4^+$ and $CD8^+$ response. Despite induction of a significant specific immune response, suggesting high production and availability of the PolySSL protein *in vivo*, the mRNA vaccine does not confer significant protection from *S. aureus* challenge. Our current focus is now on identification of alternative transcripts and LNP formulations that can skew immunity towards a Th17 response, which is linked with positive outcomes after infection in both mice and humans. mRNA vaccines have huge potential, but further refinement of this technology is required to defeat difficult to target bacterial pathogens such as *S. aureus*.

A22: MAIT cell modulation of dendritic cells promotes regulatory immune responses in the lung

Boswell, H.E.[1,2], Hermans, I.F[1], Connor, L.M[1]

[1]Malaghan Institute of Medical Research, Wellington, NZ, [2]School of Biological Sciences, Te Herenga Waka - Victoria University of Wellington, Wellington, NZ.

Mucosal associated-invariant T (MAIT) cells are innate-like lymphocytes enriched at the lung mucosa that recognise conserved B vitamin derivatives presented by the MHC class I–like molecule MR1. When appropriately activated, MAIT cells can modulate immune responses and hold promise as therapeutic targets against respiratory pathogens. In particular,intranasal administration of the MAIT cell agonist 5-(2-oxopropylideneamino)-6-D-ribitylaminouracil (5-OP-RU) together with influenza hemagglutinin protein enhances virus-specific antibody production and protects mice against lethal influenza challenge. Intranasal administration of 5-OP-RU induces dendritic cell (DC) activation in lung draining lymph nodes. However, the mechanism of how these DCs are phenotypically altered to induce mucosal immune responses is not understood.

Single-cell RNA sequencing of lung-draining lymph nodes after intranasal 5-OP-RU revealed an enriched subset of DCs with a regulatory signature. These DCs upregulated genes associated with maturation *Ccr7, Cd86*, and MHC class II genes, as well as immunomodulatory genes *Cd200* and *Cd274*, but did not express genes associated with proinflammatory activation such as type I interferons. This phenotype suggests that DCs acquire antigen and migrate to the lymph nodes without delivering strong stimulatory signals. Following this, 5-OP-RU treatment increased the proportion of regulatory T cells (Tregs) and elevated interleukin-10 levels in the lung, suggesting an immunomodulatory role. Ongoing mechanistic studies aim to identify the MAIT–DC signalling pathways critical for Treg expansion. Elucidating how MAIT cells shape pulmonary immune responses will inform future therapeutic strategies targeting MAIT cells in respiratory infections.

A23: Death Becomes Them – Host Cells and their viruses

James P. Cooney[1,2], Ashley Hirons[5,6], Natasha Jansz[5,6,7], Cody C. Allison[1,2], Peter Hickey[1,2,3], Charis E. Teh[1,2], Tania Tan[1,2], Laura F. Dagley[2,3], Jumana Yousef[2,3], David Yurick[5,6,9], Georges Khoury[5,6], Simon P. Preston[1,2], Kathryn A. Davidson[1,2], Lewis J. Williams[1,2], Stefanie M. Bader[1,2], Le Wang[1], Reet Bhandari[1,2], Liana Mackiewicz[1], Merle Dayton[1], William Clow[1], Geoffrey J. Faulkner[7,8], Daniel H. Gray[1,2],Lloyd Einsiedel[4§], Damian F. J. Purcell[5,6§], Marcel Doerflinger[1,2§], Marc Pellegrini[1,2,10§*]

1Division of Infectious Diseases and Immune Defence, Walter and Eliza Hall Institute of Medical Research, Parkville, VIC, Australia. 2Department of Medical Biology, The University of Melbourne, Parkville, VIC, Australia.3Advanced Technology & Biology, The Walter and Eliza Hall Institute of Medical Research, Parkville, VIC, Australia. 4Department of Medicine, Alice Spring Hospital, Alice Springs, Australia. 5Department of Microbiology and Immunology, The University of Melbourne at The Peter Doherty Institute for Infection and Immunity, Melbourne, VIC, Australia.6The Peter Doherty Institute for Infection and Immunity, The University of Melbourne and Royal Melbourne Hospital, Melbourne, VIC, Australia.7 Mater Research Institute - University of Queensland, TRI Building, Woolloongabba, QLD 4102, Australia.8Queensland Brain Institute, University of Queensland, Brisbane, QLD 4072, Australia9UCB Pharma, Smyrna, GA, USA10Centenary Institute Medical Research Foundation, Sydney Camperdown 2050, NSW, Australia § Senior authors contributed equally to this work. * Corresponding author

Satisfactory preventative or therapeutic drugs are lacking for Human T cell lymphotropic virus type -1 (HTLV-1), an infection which lags several decades behind its distantly related cousin HIV-1 in this regard. Consequentially, 5-10 percent of the approximately 10 million people infected with HTLV-1 will progress to serious complications.

This study investigated preventative and therapeutic agents against Human T cell lymphotropic virus type-1 subtype-C (HTLV-1c) infection. We established and characterised a humanized mouse model of HTLV-1c infection and identify that HTLV-1c disease appears slightly more aggressive than the prevalent HTLV-1a subtype, which may underpin increased risk for infection associated pulmonary complications in HTLV-1c. Combination antiretroviral therapy with tenofovir and dolutegravir at clinically relevant doses significantly reduced HTLV-1c transmission and disease progression *in vivo*. Single cell RNAseq and intracellular flow cytometry identified that HTLV-1c infection leads to dysregulated intrinsic apoptosis in infected cells *in vivo*. Pharmacological inhibition using BH3 mimetic compounds against MCL-1, but not BCL-2, BCL-xL or BCL-w, killed HTLV-1c-infected cells *in vitro* and *in vivo*, and significantly delayed disease progression when combined with tenofovir and dolutegravir in mice. Our data suggests combination antiretroviral therapy with MCL-1 antagonism may represent an effective, clinically relevant, potentially curative strategy against HTLV-1c.

A24: The Pathway to Veterinary RNA Vaccines

Heiser, A.[1]

[1] Animal Health Solutions Team, Bioeconomy Science Institute | AgResearch, Palmerston North, NZ.

The emergence of mRNA vaccine technology has revolutionized approaches to infectious disease prevention, with the RNA Development Platform in New Zealand providing a foundation for innovation across both human and veterinary medicine. The potential of this platform to accelerate the development of RNA-based vaccines for animal health is increasingly recognized, particularly in the context of rapid antigen design, synthesis, and preclinical evaluation.

Key considerations for the development of veterinary RNA vaccines will be discussed:

Immunological Aspects: The selection of immune pathways and correlates of protection remains a challenge in veterinary species, with significant variation across target animals and pathogens. Advances in antigen design, including strategies to enhance antigen presentation and stimulate both humoral and cellular immunity, are critical for effective vaccine outcomes.

Antigen and Species Selection: The identification of immunogenic and protective antigens relies increasingly on genomic and proteomic tools yet must account for species-specific immune system diversity and practical constraints within production environments.

Technical and Manufacturing Challenges: The translation of RNA vaccine technology to veterinary applications requires scalable manufacturing, adaptable delivery systems, and solutions for storage and administration in diverse agricultural settings. The RNA Platform provides integrated support for these technical requirements, though further adaptation is necessary for field deployment.

Economic and Regulatory Considerations: Veterinary vaccines must achieve a balance of efficacy, safety, and cost-effectiveness to ensure adoption by the livestock sector. Regulatory pathways are complex, varying by region and species, and require comprehensive demonstration of safety and efficacy. The development of DIVA (Differentiating Infected from Vaccinated Animals) strategies is also desirable if not essential for disease management and trade.

Future Directions: RNA technology offers opportunities for rapid response to emerging animal diseases, personalized vaccine design, and integration with digital agriculture for enhanced disease surveillance. Realizing these benefits will depend on continued collaboration among researchers, industry, regulators, and producers.

A25: Metabolic and Neural Regulation of Immunity and Cancer

Mak, Tak W.[1,2]

[1]Princess Margaret Cancer Centre, University of Toronto, [2]Centre of Oncology and Immunology, University of Hong Kong

Checkpoint inhibitors has accelerated the clinical implementation of a vast mosaic of single agents and combination immunotherapies. However, the lack of clinical translation for immunotherapies as monotherapies or in combination emphasized the importance of discerning investigation. Multiple molecular mechanisms, such as metabolic alteration, genomic instability and neural regulation converge to propel tumour development are engaged. Mutations in enzymes, such as IDH, gives rise to gliomas, leukemia (AML) and lymphoma (AITL). Another component is a seemingly unconnected biological process and immunity is genomic instability. Unexpectedly, recent findings support the intriguing proposition that neurotransmitters, such as acetylcholine, tie the neuronal system to immune responses. I will discus metabolic elements and neurotransmitters in immune cell regulation of infections, autoimmune diseases, thymic selection, liver regeneration and cancer development.

A26: IL-4 driven differentiation of dermal CD11b-low cDC2s during skin allergy

Ochiai, S.[1], Larson, A.R.[1], Yang, J.[1], Lamiable, O.[1], Ronchese, R.[1]

[1]Malaghan Institute of Medical Research, Wellington, NZ

Type 2 conventional dendritic cells (cDC2s) are essential for initiating immune responses against extracellular pathogens. cDC2s are functionally and phenotypically heterogeneous, and in the skin they can be subdivided into CD11b high (CD11b-hi) and low (CD11b-low) cDC2 subsets. We recently reported that IL-13 secretion by ILC2s is essential for homeostatic CD11b-low cDC2 differentiation, and for optimal induction of T helper type 2 (Th2) differentiation against allergens and parasites. In the current study, we explored the cytokine requirement for CD11b-low cDC2s during acute allergic skin inflammation and its resolution.

Using an MC903-induced atopic dermatitis (AD) model, we examined the expression of two related Th2 cytokines, IL-13 and IL-4, by skin immune cells during skin allergy. We observed stable IL-13 expression by ILC2s at steady state and throughout inflammation. We also observed an upregulation of IL-13 in CD4+ T cells after MC903 application. In contrast, basal IL-4 expression was not detected. However, transient IL-4 expression by basophils and long-lasting IL-4 expression by T cells were observed after MC903 treatment. Hence, we further examined whether IL-4 could mediate CD11b-low cDC2 differentiation.

At early stages of AD skin inflammation, minimal CD11b-low cDC2s were detected in the skin of WT mice due to their migration to lymph nodes. By day 18, the number of skin CD11b-low cDC2s had completely recovered. Interestingly, this replenishment of CD11b-low cDC2s also occurred in IL-13 KO mice, in which CD11b-low cDC2s are absent at steady state. This replenishment was not observed in IL-4RA-deficient mice and in mice treated with basophil and CD4+ T cell depleting antibodies, suggesting an IL-4-dependent differentiation of CD11b-low cDC2s. This result was confirmed by in vivo administration of recombinant IL-4 into IL-13-KO mice.

Our data identify IL-4 as an important mediator of CD11b-low cDC2 differentiation during allergic skin inflammation to maintain the skin Th2 environment.

A27: Characterising the host macrophage response to *Bartonella quintana* using an *in vitro* cell line model

Otto, B.M[1,2], MacKichan, J.K[1,2]

[1] Victoria University of Wellington, Wellington, New Zealand
[2] Centre for Biodiscovery, Victoria University of Wellington, Wellington, New Zealand

Bartonella quintana is a pathogenic, vector-borne, Gram-negative bacillus that infects the bloodstream of its human host, causing both acute infections, and in 5-10% of cases, chronic bacteraemia. Acute infections, known as trench fever, are characterised by the periodic invasion of erythrocytes from an unknown primary niche, most common among immunocompromised, homeless, and poverty-stricken populations. Acute infections are cleared by the adaptive immune system after some months[1]. However, chronic *B. quintana* infection results in a long-term asymptomatic persistence with lack of host immune response and suppressed immune profile. Infections are not prevented by the innate immune system suggesting the bacteria are capable of evading, dampening, or actively modulating the innate immune system.

Macrophages are a key player of the innate immune system but may be hijacked by intracellular bacterial pathogens. My project aims to better understand the pathogenic interactions leading to these subversions of normal macrophage processes in the human host. My research uses the THP-1 macrophage cell line to assess their interaction with *B. quintana*, with a focus on phagocytosis, bacterial intracellular survival, and cytokine profiles, in an *in vitro* context.

An initial exploration of these interactions using flowcytometry, ELISAs, and an intracellular viability assay, has allowed us to begin characterising the early THP-1 cell responses post *in vitro* infection. While the exact mechanisms still require elucidation, *Bartonella quintana* persists intracellularly for up to 48-hours in THP-1 cells and induces several changes in phenotype. These changes to the macrophages vary between bacterial strains, indicating potential genetic differences which may confer virulence.

These results give some insight into the previously uncharacterised interplay between the host innate immune system and invading *Bartonella quintana* pathogen, directing future work to identify the molecular mechanisms responsible.

1. Harms A, Dehio C. Intruders below the radar: molecular pathogenesis of *Bartonella spp*. Clin Microbiol Rev. 2012;25(1):42-78.

A28: How Interleukin 13 conditions skin migratory dendritic cells toward Th2 priming.

Lamiable O.[1], Tang SC.[1], Old S.[1], Carco' C.[1], Hyde E.[1], Morgan C.[1], Eccles D.[1], Palmer R.[1], Fabre M-S.[1], Webb G.[1], Heiser A.[2], Ronchese F.[1]

[1]Malaghan Institute of Medical Research, Wellington, NZ, [2]AgResearch Ltd., Grasslands Research Centre, Palmerston North, NZ.

Type 2 conventional dendritic cells (cDC2s) play a pivotal role in initiating $CD4^+$ T helper responses. In the murine dermis, we found that homeostatic IL-13 production by type 2 innate lymphoid cells (ILC2s) drives the development of a distinct $CD11b^{lo}$ cDC2 subset. In the absence of ILC2s or when cDC2s cannot respond to IL-13, $CD11b^{lo}$ cDC2s fail to develop, skewing the balance toward $CD11b^{hi}$ cDC2s. This shift impairs Th2 priming after allergen exposure while enhancing Th17 responses to fungal antigens. IL-13 signalling in cDC2s is also necessary for allergic airway inflammation and anaphylaxis, underscoring the critical role of IL-13-conditioned cDC2s in type 2 immunity.

To explore the molecular basis of the IL-13 programming, we performed bulk RNA sequencing on antigen positive and negative migratory $CD11b^{hi}$ and $CD11b^{lo}$ cDC2s isolated from skin draining lymph nodes after immunization with fluorescently labelled pathogens inducing Th1 (*Mycobacterium smegmatis*), Th2 (*Nippostrongylus brasiliensis*), or Th17 (*Candida albicans*) responses. $CD11b^{lo}$ cDC2s displayed a transcriptional profile specialized for Th2 priming. They failed to upregulate Th1/Th17-associated cytokines (*Il12a*, *Il12b*, *Il6*, *Il23a*) during bacterial and fungal immunizations in contrast to $CD11b^{hi}$ cDC2s. These cells also showed reduced expression of pattern recognition receptor signalling genes suggesting IL-13 suppresses microbial sensing. Instead, $CD11b^{lo}$ cDC2s expressed high levels of transcriptional repressors (*Prdm1*, *Sox4*, Polycomb repressive complex genes), suggesting an active silencing program. Single-cell RNA-seq of IL-4Rα-deficient cDC2s lacking the $CD11b^{lo}$ subset showed an accumulation of $CD11b^{hi}$ cDC2s that carried Nb antigen but exhibited a diminished activation profile, suggesting that they cannot efficiently respond to Th2 antigens.

Together, our data support a model in which IL-13 actively reprograms cDC2s by suppressing Th1 and Th17-promoting pathways to enable efficient Th2 priming. This reveals a central immunoregulatory role for IL-13 in shaping dendritic cell function and guiding allergic immune responses.

A29: Large extracellular vesicles derived from keratinocytes modulate Langerhans-like cell activation of T cells

Vaughn Ticar[1], Betina Nair[1], Michelle Wilson[1], Allison Tschirley[1] and Merilyn Hibma[1]

[1]Department of Pathology, Dunedin School of Medicine, University of Otago, Dunedin, New Zealand

Mucosal epithelial cells are exclusively infected by the cancer-causing human papillomavirus (HPV) type 16, and expression of the HPV16 E6 and E7 proteins is increased following integration of the viral genome into host DNA. E6 and E7 have immune regulatory functions, including the regulation of Langerhans cells (LCs), the major antigen presenting cell subset in the epithelium. Epithelial cells can shed large extracellular vesicles (LEVs), which are small (<1 μm diameter), cell-derived particles. The aim of this study is to determine if HPV16 E6/E7+ epithelial cells release LEVs that regulate LC priming of T cells.

E6/E7-PDV cells were produced following lentivirus transduction. LEVs were purified using differential centrifugation. *In vitro* cross-presentation of OVA by bone marrow-derived LC-like cells (+/-TAP1) and priming of OT-1 cells was measured, as was expression of surface molecules and cytokine secretion by the LC-like cells.

We found that epithelial cells shed annexin positive LEVs, and that threefold more LEVs were shed from E6/E7 positive cells. Furthermore, Ctrl-LEV treatment of LC-like cells significantly increased TAP1-dependent T cell priming. In contrast, E6/E7-LEV treatment of LC-like cells did not increase T cell proliferation compared to untreated cells. While treatment with Ctrl- and E6/E7 LEVs similarly upregulated expression of MHC-I and costimulatory molecules on the LC-like cells, IL-12 secretion was only increased following treatment with Ctrl-LEVs.

Our results show that LC priming of T cells is enhanced following treatment with Ctrl-LEVs and suggest that the lack of enhancement by E6/E7-LEVs is through a lack of upregulation of IL-12 expression. Functional impairment of LC priming of T cells by E6/E7-LEVs released in the epithelium may contribute to viral persistence in HPV16-infected skin.

A30: Mapping the Spatial Niches of Antigen-Presenting Cell Subsets in the Human Lymph Node

Ooi, M.P.Y[1], Chen, C.J.[1], Park, S.M. [1,2], Dunbar, R. [1,2]

[1] School of Biological Sciences, University of Auckland, Auckland, New Zealand, [2] Maurice Wilkins Centre, University of Auckland, Auckland, New Zealand

Antigen-presenting cells (APCs) play a crucial role in both innate and adaptive immunity. Recent high dimensional flow cytometry and single cell studies have identified several distinct APC populations present in human tissues. However, our understanding of the distribution of these subsets in situ and their spatial relationship with T cells are still incomplete. Following on from our earlier description of the distribution of APCs in human lymph node (LN) ((Angel et al., 2009)), we have used whole slide imaging of 7-colour multiplexed immunofluorescence panels to map the spatial relationships of APC subsets in human LN. We utilised a whole slide digital quantification workflow to classify regions within the LN where specific APC and T cell subsets are enriched. We then implemented a downstream R script pipeline for spatial analysis and visualization of the distinct cellular neighbourhoods and their localisation to the LN tissue structures. We have identified distinct regions within the LN where different dendritic cell and macrophage subsets inhabit and interact with specific T cell subsets. Spatial analysis of key chemokines and their receptors reveal how these signals drive the colocalization of specific APC and T cell subsets. Taken together, our results highlight the spatial heterogeneity of the populations within human LN and suggest their distinct roles in modulating various aspects of T cell responses.

Angel, C. E., Chen, C. J., Horlacher, O. C., Winkler, S., John, T., Browning, J., MacGregor, D., Cebon, J., & Dunbar, P. R. (2009). *Distinctive localization of antigen-presenting cells in human lymph nodes*. *Blood*, *113*(6), 1257–1267. https://doi.org/10.1182/blood-2008-06-165266

A31: Control of skin DC2 development by IRF4

Larson, A.R.[1], Ronchese, F.[1]

[1]Malaghan Institute of Medical Research, Wellington, New Zealand.

IRF4 is a well characterised transcription factor which also has an essential role in the development of type 2 dendritic cells (DC2s). Mice with total or conditional deletion of IRF4 in DCs show reduced numbers of SIRPα+ DC2s in the spleen as well as the lung, small intestine and their draining lymph nodes (dLNs). By contrast, while numbers of DC2s in the skin dLN are severely decreased, numbers of DC2s in the skin were reported to be normal or increased, suggesting a role of IRF4 in DC2 migration. Recent studies have begun to document a substantial phenotypic and ontogenetic heterogeneity within the DC2 subset, yet the role of IRF4 in supporting the development of these different subsets is not fully understood. In this study, we wished to compare the numbers and phenotypes of residual DC2s in the skin and skin-dLN of wild-type and IRF4 KO mice to establish how the lack of IRF4 affects their differentiation and ability to migrate to the dLN.

Ex vivo assessment of ear skin, ear-dLN and spleen DC2s from naïve IRF4-KO and WT mice showed that total DC2s were reduced in number in IRF4-KO mice, and expressed the early myeloid marker CX3CR1 to suggest defective development. The proportions of different DC2 subsets in lymphoid and non-lymphoid tissues was also altered in IRF4-KO compared to WT mice. Experiments using cultured bone marrow DC2s labelled with CellTrace Violet proliferation dye showed that the lower IRF4 KO DC2 numbers were not due to either reduced proliferation or apoptosis. Our data suggest that IRF4 differentially controls the development of distinct DC2 subsets, leading to variably reduced DC2 numbers and clear defects in their maturation.

A32: Harnessing the cell's inbuilt suicide switches to combat cancer

Teh, C.E.[1,2]
[1]Immunology Division, The Walter and Eliza Hall Institute of Medical Research, VIC, Australia.
[2]Department of Medical Biology, University of Melbourne, VIC, Australia.

Dr Charis Teh is a Victorian Cancer Agency Mid-Career Research Fellow at WEHI. She holds degrees from the Australian National University and the University of Sydney, with postdoctoral training at Stanford University and WEHI. An emerging leader in cancer, cell death, and immunology, Charis investigates cellular kill switches to enhance immune responses. Her research has resulted in 36 publications and attracted over $5 million in funding. Committed to inspiring women in STEM and fostering global collaboration, she has received numerous accolades, including a Fulbright Scholarship, recognition as a Superstar of STEM, and the ASI Margaret Baird Women in Immunology Award.

In her work, Charis explores how harnessing cell death pathways can improve immune responses against cancer and infections. All cells are hardwired to die, and since the first descriptions of cell death in the early 1900s, the field has rapidly progressed from bench to bedside. A major milestone was the clinical adoption of cell death inhibitors such as Venetoclax and Emricasan. A key challenge now is to understand how to better utilise these therapies. Using murine genetic models of cell death and mass cytometry (CyTOF) technology on patient samples, Charis and her team deeply profile the cell death machinery to identify ways to enhance immune function. Recently, her team has uncovered a novel cytokine–pro-survival axis that can be targeted to improve outcomes in leukaemia[1], and identified "suicide genes" in regulatory T cells that can be manipulated to boost immune responses against infections and cancer[2].

1. Luo, M.X., Tan, T., et al. (2024). Venetoclax dose escalation rapidly activates a BAFF/BCL-2 survival axis in chronic lymphocytic leukemia. Blood *144*, 2748-2761.
2. Teh, C.E., Preston, S.P., et al. (2022). Caspase-8 has dual roles in regulatory T cell homeostasis balancing immunity to infection and collateral inflammatory damage. Sci Immunol *7*.

A33: Unique Pacific gene variants and impacts on immunity and infection.

Puli'uvea, C.[1,2], Duffy, E. [1,2], Komlósi, Z.I.[3,4], Shepherd, P.R. [2,5], Merriman, T.R.2[,6,7], Netzler, N.E.[2,5]

[1]School of Science, Auckland University of Technology, NZ, [2]Maurice Wilkins Centre for Molecular Biodiscovery, Auckland, NZ, [3]Department of Genetics, Cell and Immunobiology, Faculty of Medicine, Semmelweis University, Budapest, Hungary, [4]Swiss Institute of Allergy and Asthma Research (SIAF), Davos, Switzerland. [5]Department of Molecular Medicine and Pathology, Faculty of Medical and Health Sciences, University of Auckland, NZ, [6]Department of Microbiology and Immunology at the University of Otago, [7]Immunology and Rheumatology, University of Alabama at Birmingham, Birmingham, AL, USA,

Waves of infectious diseases were introduced into naïve indigenous communities of the Pacific nations between 1700-1900s, decimating up to 94% of the local population [1-2]. These catastrophic epidemics would have driven significant selective pressures on the immunological gene pool, potentially selecting for resistant phenotypes, which is likely to have contributed to modern day health across Polynesia. Sequencing data from a recent cohort of Polynesians in Aotearoa NZ identified thousands of unique high impact changes in genes that play central roles in the immune response to infection, but are not detected in European populations. In light of the recent findings that genetics can influence up to 75% of immune function [3], coupled with a lack of representation of Pacific peoples in genetic studies, there is still much to learn about gene function and variation in Pacific populations, including genetic drivers of immune cell composition and potential areas for precision medicine. Therefore, this study examines a cohort of Pacific gene variant carriers versus matched controls for impacts on immunity. PBMCs isolated from participants were profiled using flow cytometry to identify differences between genotypes in cellular populations and activation markers. PBMCs will be stimulated with toll-like receptor agonists to detect potential impacts on the immune response driven by the variants. This study aims to identify any key differences in the immune response to infection associated with selected Pacific gene variants.

1. Martin P, Combes C. 1996. Emerging infectious diseases and the depopulation of French Polynesia in the 19th century. J Emerging Infectious Diseases 2:359.
2. Kaholokula J, et al.,. 2020. COVID-19 special column: COVID-19 hits native Hawaiian and Pacific Islander communities the hardest. J Hawai'i Journal of Health Social Welfare 79:144.
3. Mangino M, et al., 2017. Innate and adaptive immune traits are differentially affected by genetic and environmental factors. Nat Commun 8:13850.

Summary of Abstracts for the Poster Session Template

No.	Title	Presenter	Institution(s)
	System Serology Reveals Correlates of Functional Strep A Antibody Responses In Children With Pharyngitis	Renee Shields	The University Of Auckland
	Temperature matters: exploring the impact of fever on T cell metabolism and function in infectious disease.	Rosemary Jackson	[1]Malaghan Institute Of Medical Research, [2]Victoria University of Wellington,
	Characterising the Immunomodulatory Effects of Novel Marine Natural Products	Matthew Ellmers	Victoria University Of Wellington
	High-Resolution 3D Mapping of Lymph Node Microanatomy and Antigen Distribution	Dr. Inken Kelch	[1]Maurice Wilkins Centre for Molecular Biodiscovery, [2]School of Biological Sciences, The University of Auckland,
	Investigating Cellular Immunity in Clear Cell Ovarian Carcinoma	Heidi Robinson	School of Biological Sciences, The University Of Auckland
	Spatial Analysis of Dendritic cell subsets in Human Cancers	Rachel Huang	School of Biological Sciences, The University Of Auckland
	Investigating Immune Complexity in Older People with Colorectal Cancer	Emma Casey	University of Otago
	Immuno-FlowFISH for Diagnosis of Multiple Myeloma at Remote Sites	Lisa Chalmers	University of Otago
	Impact of Obesity-Associated Factors on Monocyte Priming and Initial Response to Pembrolizumab Monotherapy	Ben Topham	Mackenzie Cancer Research Group, University of Otago
	The placenta as a controlled tumour model: parallels in immune cell recruitment	Georgia McKendry	University of Otago
	The evaluation of markers of disease progression and persistent/regression in human papillomavirus-positive cervical intraepithelial neoplasia	Dr. Filipina Amosa-Lei Sam	University of Otago, Department of Pathology
	Targeting Metabolism and Redox Homeostasis to Promote Differentiation and Epigenetic Reprogramming in Acute Myeloid Leukaemia	Ella Simpson	Mātai Hāora – Centre for Redox Biology and Medicine Group, Department of Pathology and

			Biomedical Science, University of Otago
	Behaviour of Antigen Presenting Cells within the G-rex™ Bioreactor system.	Ash Sargent	School of Biological Sciences, The University Of Auckland
	Optimisation of an IFN-γ ELISA for functional assessment of CAR T-cells for the ENABLE-2 Phase II Clinical Trial	Annie Bai	Cancer Immunotherapy Programme, Malaghan Institute Of Medical Research
	Developing a novel mucosal adjuvant based on pilus proteins from Group A Streptococcus	Ianah Rae Clarice Guasch Bitac	Department of Molecular Medicine and Pathology, University Of Auckland
	Small extracellular vesicles as indicators of Mycoplasma bovis infection: Insights from in vitro and in vivo models	Joel Pratt	Queensland University of Technology
	Unlocking tuatara immunity: a window into ancient vertebrate defence mechanisms	Dr. Danielle Middleton	Manaaki Whenua Landcare Research
	Heparan Sulfate Mimetic Tet-29 in Attenuating Extracellular Histone Cytotoxicity	Xueling Guo	Victoria University of Wellington
	The role of host histone deacetylase 6 in Influenza virus-induced interferon regulatory factor 3-mediated innate antiviral signalling	Wenlong An	University of Otago
	Tracking Immune Ageing in Midlife: Mitochondrial Stress and Myeloid Population Shifts	Dr. Annika Seddon	Mātai Hāora – Centre for Redox Biology and Medicine Group, Department of Pathology and Biomedical Science, University of Otago
	Ascorbate uptake and its effects on phenotype and cytokine expression in human monocyte-derived macrophages	Dr. Stephanie Bozonet	Mātai Hāora – Centre for Redox Biology and Medicine Group, Department of Pathology and Biomedical Science, University of Otago
	A novel vaccine approach for preventing RSV infection	Yuexiao Zhang	The University of Auckland
	Pilot Study on the Immunogenicity of a Novel mRNA-Based Vaccine	Dr. Mallory Ross	Bioeconomy Science Institute, AgResearch

	Targeting Bovine Viral Diarrhoea Virus (BVDV) in Calves (BoViDx)		
	An Anti-Virulence Vaccine for Staphylococcus aureus	Dr. Ries Langley	Department of Molecular Medicine & Pathology, University of Auckland
	Expanding immunological testing capacity for Strep A vaccine evaluation in Aotearoa New Zealand	Kelly Peterken	Faculty of Medical and Health Sciences, The University of Auckland
	iSOoTH: A study on children's sore throats to inform the development of a Group A Streptococcal vaccine	Ciara Ramiah	Department of Molecular Medicine & Pathology, University of Auckland