QMB Abstracts 2019

Q1. MYC-driven cells need MNT to survive

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Deregulated over-expression of the transcription factor MYC is implicated in the development and malignant progression of most (~70%) human tumours. MYC drives cell growth and proliferation but also, at high levels, promotes apoptosis. We have found that MNT, a MYC-related transcriptional repressor, plays a vital role in both B lymphopoiesis and lymphomagenesis, primarily by suppressing MYC-driven BIM-mediated apoptosis. In Eμ-*Myc* mice, which model the *MYC/IGH* chromosome translocation in Burkitt's lymphoma, homozygous *Mnt* deletion markedly lowered premalignant B lymphoid populations and greatly reduced lymphomagenesis. Strikingly, when *Mnt* was deleted within established Eμ-*Myc* lymphoma cells transplanted into normal immunocompetent mice, survival of transplant recipients was significantly extended. The dependency of MYC-driven lymphomas on MNT for continued growth highlights that drugs inhibiting MNT could significantly boost therapy of MYC-driven tumours.

Q2. Building chains: regulation of ubiquitin transfer by TRAF E3 ligases

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Eukaryotic cell phenotype and function relies on the exquisite control of cell signaling pathways. Protein-protein interactions have a central role in these pathways and they are often regulated by the post-translational modification of proteins. One pervasive post-translational modification that plays a key role in regulating the logic of many signalling pathways is the attachment of ubiquitin to proteins. Ubiquitin chains linked through Lysine63 (K63) have a critical role in inflammatory signalling.

Following ligand engagement of immune receptors, the RING E3 ligase TRAF6 builds K63-linked chains together with the heterodimeric E2 enzyme Ubc13-Uev1A. The E3 ligase activity of TRAF6 is required for activation of the signalling cascade, with E2 binding and dimerisation of the TRAF6 RING domain required for assembly of K63-linked ubiquitin chains. Using structural and biochemical approaches we have established how TRAF6 builds ubiquitin chains. We have also shown that the TRAF6 RING domain can heterodimerise and some heterodimers can assemble chains.

Our study explains the dependence of activity on TRAF RING dimers, and suggests that TRAF RING homo- and heterodimers have the capacity to synthesise ubiquitin chains, and that this activity is enhanced by higher-order complexes. The implications of these discoveries for the regulation of cell signalling will be discussed.

Q3. Control of apoptosis by the BCL-2 protein family: How do BAX conformational changes impose cell death?

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Apoptosis, the major form of programmed cell death, is regulated primarily by the BCL-2 protein family[1]. Bcl-2 and its closest relatives (e.g. BCL-XL, MCL-1) promote cell survival by countering the activity of two distinct sets of pro-apoptotic relatives. Cellular stresses first activate the 'BH3-only' proteins, which share only a single domain with BCL-2 (e.g. BIM and BID). They then use their BH3 domain to engage and inactivate pro-survival relatives, and some also engage their pro-apoptotic relatives BAX and BAK to trigger cellular proteolysis. Understanding of apoptotic control has both led to recognition that impaired apoptosis is a hallmark of cancer development and underpinned development of novel potent cancer drugs that target select BCL-2 family members.

Nevertheless, the pivotal steps converting BAX and BAK from inert monomers into oligomers that perforate the mitochondrial outer membrane (MOM) remain incompletely understood. BAX and BAK are activated similarly, but BAK is normally integrated into the MOM, whereas BAX must first move from the cytosol to the MOM. We are addressing two unresolved issues about how BAX precipitates apoptosis.

One puzzle is that two distinct binding sites have been proposed for the engagement of BAX by BIM: (1) the well characterized surface groove resembling that on pro-survival relatives where BH3-only proteins insert their BH3 domain and (2) a poorly characterized site involving helices 1 and 6 on the opposite side of BAX. Our mutagenesis studies suggest that late steps in BAX activation like oligomerisation require the groove site but not early steps like MOM translocation, whereas obstruction of sites along helices 1 and 6, as well as alanine scanning mutagenesis suggest that those helices contribute to the early steps. Thus, our results[2] suggest that the alpha1-alpha 6 region drives MOM association and integration, whereas groove binding favors subsequent steps towards oligomerisation.

Another important unresolved issue is the precise nature of the oligomers that disrupt MOM integrity. Studies with our institute colleagues have established that the central unit nucleating the oligomers is a dimer involving insertion of the BAX BH3 domain (helix 2) into a groove comprising helices 3, 4 and 5, but the linkages joining the dimers remain unknown. We are attempting to gain insights on their structure by cryo-EM.

[1]Adams, J.M. and S. Cory S (2017) *The BCL-2 arbiters of apoptosis and their growing role as cancer targets.* Cell Death Differ 25: 27-36.

[2]Dengler, M.A., A.Y. Robin, L. Gibson, M.X. Li, J.J. Sandow, S. Iyer, A.I. Webb, D. Westphal D, G. Dewson, J.M. Adams (2019) *BAX activation: mutations near its proposed non-canonical BH3 binding site reveal allosteric changes controlling mitochondrial association.* Cell Reports, 27, 359-373.

Q4. Science in the media – spin, hype and the role of experts

Dacia Herbulock, Science Media Centre

The media is under pressure. Fewer reporters are creating more content to tighter deadlines than ever before. Despite our limited attention spans, media influence and audience reach remain high. Against this backdrop, time-poor journalists increasingly rely on experts to provide essential context for their reporting. The Science Media Centre has over a decade of first hand experience demonstrating how relevant, timely input from scientists can have a direct impact on the way news stories evolve. This talk will explore the challenges and opportunities facing researchers seeking to improve the ways their areas of expertise are covered in the media.

Q5. Stiff Hearts: A Modern Epidemic Driven by Metabolic Disease

John O'Sullivan
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The profile of heart failure has changed dramatically. Traditionally defined as reduced "pump function", a type of heart failure where the heart cannot relax (diastole), as opposed to squeeze (systole), has increased dramatically in prevalence such that it makes up over 50% of heart failure cases, driven by obesity and Type 2 diabetes epidemics. However, unlike the traditional type of heart failure, there are no approved therapies for this type of heart failure, despite almost identical 5-year mortality. In this talk, I will describe recent findings in our lab in this field, derived from our clinic, cardiac MRI, metabolomics, proteomics, a new functional cardiac tissue model, stable isotope tracing, and our murine model.

Q6. Cardiac troponins - targets for irreversible glycation damage

Delbridge, L.M.D

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There is increasing recognition that the formation of glycation modification of proteins has an important pathophysiological role in many tissues. Reversible and regulatory modifications involving O-GlcNAcylation have been identified as key in mediating intracellular signaling functions in numerous settings. The formation of advanced glycation endproducts (AGE) is also associated with disease progression. AGE modifications involve multiple chemical transformation steps to produce permanent and fixed molecular entities. In the heart, the presence of extracellular matrix AGE has been linked with elevation of myocardial stiffness and dysfunction. The role of intracellular AGE in disease progression is less well understood, and a focus of our cardiac research.

Dynamic movements of the cardiac troponin complex are an important component of the cardiac cycle. We have recently demonstrated that cardiac contractile myofilament troponins are susceptible to AGE modification. Using methods of mass spectrometry (LC-MS/MS) we have interrogated human and rat cardiac troponin-C, troponin-I and troponin-T to identify sites of endogenous AGE modification. Our findings indicate troponin-I has particular AGE vulnerability with occurrence of modifications situated in regions of the troponin complex known to be important in myofilament relaxation processes. This work has opened up new opportunity to identify molecular structure-function interactions relevant to cardiac disease contexts.

Q7. Glycomics: Generating new opportunities to understand, diagnose and treat disease.

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Carbohydrates are ubiquitous in all biological systems. Cells carry a carbohydrate-dense outer membrane called the glycocalyx, and many proteins and lipids are glycosylated. These carbohydrates serve as recognition sites for cells, microorganisms, toxins and biomolecules. The field of glycomics enables understanding of the structures, significance and roles of carbohydrates in interactions with biomolecules (the glycointeractome). Understanding these interactions is key for the development of novel strategies for the diagnosis, prevention and treatment of disease.

This presentation will highlight recent technological advances in glycomics and research findings on the glycoscience of human disease. Case studies will be presented that will demonstrate the application of this discovery process to deliver new approaches to the diagnosis cancer and treatment of infectious disease.

Q8. A proposal for a standardized genome-based bacterial taxonomy accurately reflecting evolutionary relationships

Philip Hugenholtz
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The great majority of microorganisms have yet to be cultured and characterised. This so-called “microbial dark matter” is now being revealed at an ever-increasing rate by sequence-based culture independent methods. In the past few years, thousands of near complete genomes of uncultured microbes have been assembled from sequence data obtained directly from environmental and clinical sources providing the opportunity to fully articulate microbial diversity for the first time. Current estimates suggest that cultured microorganisms only capture ~15% of total microbial diversity based on evolutionary divergence of marker genes. We propose a genome-based taxonomy founded on the existing classification of cultured organisms, but corrected for polyphyletic groups and calibrated to take into account relative evolutionary divergence. The result is a fully systematized classification of Bacteria in an evolutionary framework. Of ~100,000 publicly available bacterial genomes, over half required one or more changes to their existing taxonomy. These include extensive changes at both high ranks, such as amalgamation of the Candidate Phyla Radiation into one phylum and low ranks including subdivision of the genus *Clostridium* into more than 100 distinct genera.

Q9. Agricultural origins of a persistent lineage of vancomycin-resistant *E. faecalis* from New Zealand

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Enterococci are human and animal gut commensals. When vancomycin-resistant, they are also important opportunistic pathogens. Historically, agricultural use of the glycopeptide antibiotic avoparcin selected for vancomycin resistance in poultry, resulting in its discontinuation in agriculture in 2000. To better understand the phylogenetic relationships and antibiotic resistance patterns of human and animal-associated VRE strains in post-avoparcin New Zealand, we sequenced the genomes of 231 NZ VRE isolates (75 human clinical, 156 poultry) collected between 1998 and 2009. A clinically-relevant *E. faecalis* lineage (ST 108) was highly prevalent among both poultry and human isolates in the three years following avoparcin discontinuation, and has persisted for more than fifteen years since then.

Q10. Mātauranga guided biodiscovery of anti-*Phytophthora* compounds

Monica L. Gerth
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Kauri *(Agathis australis*) is an important endemic species in New Zealand. However, the survival of kauri is being threatened by the microbial pathogen *Phytophthora agathidicida*. *P. agathidicida* is a member of the oomycete genus *Phytophthora*, other members of which cause diseases in thousands of economically and ecologically important plants worldwide. Often referred to as 'fungus-like', *Phytophthora* are actually more closely related to diatoms and brown algae. Practically speaking, this means *Phytophthora* are unaffected by most available agrichemical fungicides.

There is an urgent need to discover and develop novel compounds that target the growth, survival and dispersal of *P. agathidicida*. In this talk, I'll present our work exploring the anti-*Phytophthora* potential of selected New Zealand native plants, using a collaborative approach that incorporates mātauranga Māori, microbiology and chemistry.

Q11. How does RhsA cause bacterial contact-dependent inhibition?

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Bacteria engage in a lethal competition with each other for space and nutrition. Contact-Dependent Inhibition (CDI) is the ability of one bacterial cell to kill neighbouring bacteria by the injection of toxins,[1] which can be mediated by the type VI secretion system (T6SS).[2] Despite recent progress,[3,4] our understanding of how the T6SS selects and delivers specific protein toxins remains incomplete. The function of the conserved protein RhsA (rhs = *r*earrangement *h*otspot) has been obscure since its discovery over 30 years ago,[5] but it has recently been identified as a toxin delivered by the T6SS during bacterial competition in some species.[6,7] RhsA contains multiple copies of a conserved peptide motif, the RHS/YD repeat, which in other systems functions to encapsulate insecticidal toxins or display regulatory peptides (ABC toxins[8] and teneurins,[9] respectively). This talk will describe our progress in determining the molecular structure of RhsA using cryo-electron microscopy and X-ray crystallography, in order to understand how RhsA interacts with the T6SS machinery to cause CDI.

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(2) Journet, L., and Cascales, E. (2016) *The Type VI Secretion System in Escherichia coli and Related Species.* EcoSal Plus *7*.
(3) Nazarov, S., Schneider, J. P., Brackmann, M., Goldie, K. N., Stahlberg, H., and Basler, M. (2018) *Cryo-EM reconstruction of Type VI secretion system baseplate and sheath distal end.* EMBO J 37, e97103.
(4) Szwedziak, P., and Pilhofer, M. (2019) *Bidirectional contraction of a type six secretion system.* Nature Communications 10, 1516.
(5) Lin, R.-J., Capage, M., and Hill, C. W. (1984) *A repetitive DNA sequence, rhs, responsible for duplications within the Escherichia coli K-12 chromosome.* Journal of Molecular Biology 177, 1–18.
(6) Koskiniemi, S., Lamoureux, J. G., Nikolakakis, K. C., de Roodenbeke, C. T., Kaplan, M. D., Low, D. A., and Hayes, C. S. (2013) *Rhs proteins from diverse bacteria mediate intercellular competition.* Proc. Natl. Acad. Sci. U.S.A. 110, 7032–7037.
(7) Alcoforado Diniz, J., and Coulthurst, S. J. (2015) *Intraspecies Competition in Serratia marcescens Is Mediated by Type VI-Secreted Rhs Effectors and a Conserved Effector-Associated Accessory Protein.* Journal of Bacteriology 197, 2350–2360.
(8) Busby, J. N., Panjikar, S., Landsberg, M. J., Hurst, M. R. H., and Lott, J. S. (2013) T*he BC component of ABC toxins is an RHS-repeat-containing protein encapsulation device.* Nature 501, 547–550.
(9) Jackson, V. A., Busby, J. N., Janssen, B. J. C., Lott, J. S., and Seiradake, E. (2019) *Teneurin Structures Are Composed of Ancient Bacterial Protein Domains.* Frontiers in Neuroscience 13, 183.

Q12. Air microbiome: A missing eco-system?

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Microbial communities inhabiting terrestrial and aquatic ecosystems have long been studied. With the onset of metagenomics, the degree of diversity and abundance of these communities have become apparent, even on a global scale. In contrast, the atmosphere, despite its enormous planetary volume, has largely been neglected as a habitat for microbial communities, despite providing means of transport with an intercontinental range. We have studied the occurrence of airborne microbial organisms in the tropical climate of Singapore and found robust and persistent assemblages, both on intra-day and month-to-month time scales. Bacteria and plant-associated fungi were found to be the major constituent of the air microbiome, in addition to DNA derived from plants and insects. Besides conducting in-depth metagenomics studies that identified the diversity and abundance of airborne organisms, we have sequenced and assembled “100 genomes from air” using single-molecule real-time sequencing (SMRT). These genome data, together with organismal and habitat information, are stored in a “DNAir database”, which largely extends the organismal range of public databases and also includes previously uncultivatable organisms. The data generated for the outdoor settings now serves as a lead for the investigation of various in-door environments, well as clinical cohorts from patients with respiratory diseases.

Q13. The role of RIPK1 in controlling bacterial gastrointestinal infections

Gregor Ebert[1], Rebecca Ambrose[2], Marcel Doerflinger[1], Nikola Baschuk[2], Vik Ven Eng[2], Annabell Bachem[3], Sammy Bedoui[3], Marc Pellegrini[1], Elizabeth Hartland[2], John Silke[1], Jaclyn S Pearson[2].

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Receptor interacting protein kinase 1 (RIPK1) is an essential adaptor protein involved in inflammation and cell death signaling networks. *RipK1* deficiency is embryonic lethal in mice and humans with biallelic *RIPK1* mutations suffer severe immunodeficiency and intestinal inflammation. Lethality of *RipK1* deficiency is rescued by deletion of RIPK3 and Caspase-8 or FADD, thus the *in vitro* function of RIPK1 is to limit TNFR1-driven apoptosis. Many studies are currently focused on identifying the *in vivo* role of RIP kinases in disease. In this study, using two distinct mouse models of infection, we have shown that RIPK1 is essential for controlling systemic and gastrointestinal infection by the bacterial pathogen *Salmonella* Typhimurium, and *Citrobacter rodentium*, a natural mouse pathogen used as a model for pathogenic *E. coli* infection in humans. *RipK1*$^{-/-}$*RipK3*$^{-/-}$*Casp8*$^{-/-}$ triple knockout (KO) mice were highly susceptible to infection with both *S*. Typhimurium and *C. rodentium*, with significantly higher bacterial loads in faeces and spleen compared to wildtype mice, increased spleen weights and organ pathology scores. *RipK3*$^{-/-}$*Casp8*$^{-/-}$ double KO mice were also more susceptible to infection with both pathogens, however this was not as dramatic as in the triple KO mice. Interestingly, it appears that *RipK3*$^{-/-}$ KO mice are also susceptible to *C. rodentium* infection, however, RIPK3 alone appears to play no role in protecting against *S.* Typhimurium infection, highlighting a difference in innate responses required to combat these two gastrointestinal pathogens. Preliminary RNAseq data suggested that the *RipK1*$^{-/-}$*RipK3*$^{-/-}$ *Casp8*$^{-/-}$ triple knockout mice lack a robust Th17 response compared to wildtype mice, suggesting a potential role for RIPK1 in promoting or regulating protective Th17 responses in the gut. Overall, this study will contribute to our understanding of innate immunity in protect against bacterial infections and also contribute to the understanding of the *in vivo* role of RIPK1 in infectious disease.

Q14. Illumina Award winner

Links between the infant gut microbiome and immune development

Dr. Tommi Vatanen
University of Auckland

The human gut microbiome develops towards the adult composition during the first years of life and is implicated in overall gut health, early immune development and growth, among other things. The gut microbiome structure and functions are highly dynamic during the first year of life, reflecting changes in, for example, diet, medications and geography. I have studied the early gut microbiomes of infants who were genetically predisposed to type 1 diabetes (T1D) in multiple international cohorts involving more than 1,000 babies from Europe and North-America. In Northern Europe, my work demonstrated that differences in microbiota-derived lipopolysaccharides may preclude aspects of immune development in Finnish and Estonian children, uncovering a mechanistic link between the human gut microbiome and immune-mediated diseases. In another longitudinal study with babies from three European countries and three US states, I found that early probiotics and bacterial short-chain fatty-acids may provide protection from islet autoimmunity and early onset T1D.

Q15. Human genomics of infectious disease

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Genome-wide association studies (GWAS) have been highly successful and have led to a remarkable range of discoveries, not just the identification of genetic variants associated with disease, but in understanding disease biology with downstream translation for health benefit. The major successes are largely confined to non-communicable diseases, with the GWAS of infectious diseases lagging behind.

Progress in infectious disease GWAS, which infectious diseases have delivered, and what has been challenging will be presented, focusing on typhoid fever, malaria and tuberculosis (TB). Identfication of human genes playing a major role in TB susceptibility has been elusive, so our current approach is to investigate both bacterial and human genomic variation from the one patient cohort, to consider both varying genomes and their interaction in TB susceptibility.

Genomics of infectious disease can identify novel aspects of host and pathogen biology opening new opportunities in basic and translational research, vaccine development and host-directed therapies.

Q16. Using high throughput sequencing to diagnose New Zealanders with rare disorders: academic and clinical challenges

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The utility of whole exome and genome sequencing to diagnose rare disorders is clear, with many studies showing improvements in long term care, earlier and accurate diagnosis and net healthcare savings. Our own studies support these international trends, with an average diagnostic rate >60%, encompassing a spectrum of genetic variants (single nucleotide variants, structural variants and complex rearrangements). The biological interpretation of variant calls from this data has uncovered some interesting challenges, with both academic and clinical implications. These include the tissue source used for sequencing (e.g. contaminating reads from saliva derived DNA), interpreting variants which might be specific for our unique New Zealand populations, and understanding the biological role of variants of unknown significance. This presentation will address some of the approaches the Applied Translational Genetics group have developed in an attempt overcome these challenges, in an effort to best use this sequencing technology in research and clinical environments in New Zealand.

Q17. Origin and evolution of Tuberculosis in New Zealand

Knapp, M.[1], McDonald, S.K.[1], Buckley, H.[1], Kardailsky, O.[1], Aung, H.L.[2], Cross, H.[1], Jeunen, G-J.[1], Matisoo-Smith, E.[1], Walter, R.[3], Cook, G.M.[2]

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The origin and antiquity of tuberculosis (TB) in the Pacific is controversial. TB-causing *Mycobacterium tuberculosis complex* (MTBC) bacteria are thought to have arrived with European sailors or settlers but skeletal lesions consistent with TB have been observed in pre-European human and animal assemblages, contradicting this popular view.

We combine palaeogenetic and macroscopic analyses of pre-European human and animal remains to determine how TB arrived and evolved in the Pacific.

We found that both animal and human remains showed lesions consistent with TB infection and MTBC-specific TaqMan assays identified MTBC DNA in multiple human and animal remains. High-throughput sequencing of MTBC positive remains is underway and preliminary results confirm the presence of *Mycobacteria* in sequenced remains.

This study has the potential to fundamentally alter our understanding of how TB spread around the world and to provide new insights into how MTBC bacteria adapt to human hosts and alter their potential for causing epidemics.

Q18. Surveillance of HIV-1 Diversity by Next Generation Sequencing (NGS) of Archival Specimens from the Congo Basin

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Surveillance of human immunodeficiency virus-1 (HIV-1) strain diversity is critical to the prevention, diagnosis, and treatment globally. Since partial sequences may underrepresent recombination and diversity, complete genome sequencing is essential to advance HIV surveillance and maintain diagnostic accuracy.

HIV-1 specimens were collected at two rural hospitals in the DRC from 2001-2003. Out of 172 HIV-1 specimens classified by phylogenetic analysis of the envelope immunodominant region sequence, 18 rare and unclassified subtypes were selected for next generation sequencing (NGS) using an established HIV-specific primer approach. The viral load for 9 of these specimens was less than 5 log10 copies/ml, and therefore NGS library preparation conditions were optimized to enable sequencing of these specimens. Genomes read by alignment to references were assembled *de novo* using CLC Bio software. Strain classification was determined by phylogenetic and recombination analysis.

For low viral load specimens, the highest genome coverage obtained was by concentrating the total nucleic acid extract before reverse transcription at 42^{o} C using a SMART cDNA kit (Clontech). Genome sequences with >99.5% genome coverage, an average read depth of >10, and a sequence length of >9500 nucleotides were obtained for 14 HIV-1 specimens. The remaining four genomes had coverage of >60%. Phylogenetic and recombination analysis of the 14 complete genomes identified pure subtypes D (n=1), H (n=3), and CRF25 (n=1). The remaining genomes were simple recombinants of a single subtype with unclassified or CRF sequence (n=6), or complex recombinants of 4 or more subtypes, including A, G, H, K, and unclassified forms (n=3). Two of these complex recombinants shared 97% sequence identity.

The sequence complexity demonstrates a high level of diversity in HIV-1 strains circulating in the Democratic Republic of Congo. These complete genomes are a valuable contribution towards HIV-1 surveillance and maintenance of HIV diagnostic tool performance.

Q19. Modelling Development and Disease using Human Pluripotent Stem Cells

Ed Stanley[1,2,3]

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Problem: Models of human development and disease are required to understand human biology and to formulate and test new therapeutics. Pluripotent stem cells (PSCs) are an immortal cell type that can be differentiated into analogues of any cell type found in the human body, and thus, represent a platform to explore development and model disease.

Methods: We have developed methods for genetically modifying PSCs and for directing their differentiation to a variety of lineages, with particular emphasis on cell types related to dysfunction of the immune system. As such, recent work has focussed on understanding how different blood cells can be produced in vitro, how these cells function, and how they could be harnessed to understand or intervene in disease process. In this presentation I will outline the concepts underpinning human pluripotent stem cell technologies, including the use of genetic manipulation, reporter lines and directed differentiation.

Results: We have used PSC technology to generate insulin producing cells, T-cells and macrophages in vitro. Using reporter line technology, we have identified a novel developmental pathway in which early T-cell commitment occurs independently of haemopoietic stem cells and the thymus. Similarly, reporter lines have been used to investigate the differentiation of pancreatic endocrine cells producing Insulin and Glucagon. Last, we generated antigen presenting cells from individuals who had type 1 diabetes and have used this to examine immune interactions relevant to the genesis of this condition.

Conclusions: Pluripotent Stems represent an accessible system for creating human cell types that can inform our understanding of human development and also provide a tractable experimental framework for dissecting human disease mechanisms.

Q20. Modelling Huntington's Disease Using Direct Cell Reprogramming Technology

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Centre for Brain Research and Department of Pharmacology and Clinical Pharmacology, School of Medical Science, FMHS, University of Auckland.

Huntington's disease (HD) is an autosomal dominant neurodegenerative disorder characterised by the progressive loss of GABAergic medium spiny neurons (MSNs) in the striatum. The study of neurodegenerative disorders such as HD has been impaired by limited access to live human disease-affected neurons. Cellular reprogramming of patient-derived somatic cells now offers an opportunity to generate live human neurons for the study of neurological conditions. We have developed a highly efficient protocol for direct reprogramming of adult human fibroblasts (HDFs) to induced neural precursor cells (hiNPs) by co-transfection of chemically-modified mRNA (cmRNA) encoding the pro-neural transcription factors *SOX2* and *PAX6* in a defined reprogramming medium. Using this technology, HDFs from patients with HD (n=4; CAG repeat lengths 41 – 57) and from normal subjects (n = 4; CAG repeat lengths 18 – 34) were directly reprogrammed to hiNPs and subsequently differentiated to MSNs using a combination of growth factors and small molecules. Following reprogramming, hiNPs expressed a range of pro-neural genes including *NESTIN, FOXG1, FOXP1/2*, *MEIS* and *CTIP2*. Differentiation of hiNPs generated a high yield of DARRP32+ neurons (~30-45% total cells). To confirm our directly reprogrammed HD lines generate neurons exhibiting a disease pathology, we compared the morphology of HD-derived neurons to normal neurons. We observed that HD-derived neurons exhibited a smaller cell soma and reduced neurite length compared to normal neurons. In addition, HD neurons had a lower proportion of branched neurites per neuron compared to normal. HD-derived neurons also exhibited an alteration in BDNF and HAP1 expression compared to normal neurons. These results demonstrate that HD-derived neurons exhibit a distinct disease pathology. This provides an invaluable *in vitro* platform for studying the pathophysiology of HD and to identify and screen potential therapeutic compounds.

Q21. iPS cells, the long QT syndrome, and the neurocardiac approach

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Long QT Syndrome, the most common cause of sudden death in autopsy negative New Zealand youth, is characterised by life-threatening arrhythmias triggered by the sympathetic "fight-or flight" response, however, the underlying mechanism is unknown.
There are few physiologically relevant tools to model neurocardiac arrhythmia mechanisms, as heterologous expression systems suffer from lack of clinical concordance, and the molecular contributors of repolarisation differ between species. Human induced pluripotent stem cells (iPS cells) have emerged as a promising tool to model inherited arrhythmias. Long QT Syndrome iPS-cardiomyocytes emulate the disease phenotype, including a pathological phenotype when challenged with catecholamines. Human stem cells can also be differentiated into catecholamine secreting sympathetic neurons, capable of coupling with murine cardiomyocytes when co-cultured in vitro. We have modified a published protocol to fit our feeder-free culturing technique. Using our modified protocol, control neurons cultured >48 days from pluripotency exhibit electrophysiological properties of mature sympathetic neurons, including the ability to fire action potentials with fast kinetics, spontaneously, and when triggered. Moreover, our iPS-derived sympathetic neurons present with large inward and outward currents, and the ability to form functional synapses with each other, as evident by frequent inward synaptic currents during baseline recordings.
Importantly, we have been able to establish a functional neurocardiac model, using human blood derived iPS cells to grow sympathetic neurons and cardiomyocytes together in coculture. After two weeks in coculture, nicotinic activation of sympathetic neurons caused beating rate increase in coupled iPS derived cardiomyocytes, while no beating rate increase was seen in iPS derived cardiomyocytes exposed to nicotine when in monoculture.
We hypothesize that this human in vitro co-culture model will be a valuable tool to directly study how sympathetic neurons regulate heart rate and heart cell activity in Long QT Syndrome patient cells, using cellular electrophysiology techniques.

Q22. Drug screening in human pluripotent stem cell-derived cardiac organoids.

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Human pluripotent stem cell-derived cardiomyocytes are emerging as a powerful platform for cardiovascular drug discovery and toxicology. However, standard 2D cultures are typically immature, which limits their capacity to predict human biology and disease mechanisms. To address this problem, we have recently developed a high-throughput bioengineered human cardiac organoid (hCO) platform, which provides functional contractile tissue with biological properties similar to native heart tissue including mature, cell cycle-arrested cardiomyocytes. Here, we take advantage of the screening capabilities of our mature hCO system to perform functional screening of 105 small molecules with pro-regenerative potential. Our findings reveal a surprising discordance between the number of pro-proliferative compounds identified in our mature hCO system compared with traditional 2D assays. In addition, functional analyses uncovered detrimental effects of many hit compounds on cardiac contractility and rhythm. By eliminating compounds that had detrimental effects on cardiac function, we identified two small molecules that were capable of inducing cardiomyocyte proliferation without any detrimental impacts on function. High-throughput proteomics on single cardiac organoids revealed the underlying mechanism driving proliferation, which involved synergistic activation of the mevalonate pathway and a cell cycle network. In vivo validation studies confirmed that the mevalonate pathway was shut down during postnatal heart maturation in mice and statin-mediated inhibition of the pathway inhibited proliferation and heart growth during the neonatal window. This study highlights the utility of human cardiac organoids for pro-regenerative drug development including identification of underlying biological mechanisms and minimization of adverse side-effects.

Q23. Function and regulation of Class 1 CRISPR-Cas systems

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Bacteriophages are viruses that infect bacteria, with 10^{25} infections occurring every second. To prevent infection by phages and other mobile genetic elements, bacteria have developed resistance strategies. These include innate immunity such as restriction-modification and adaptive immunity provided by CRISPR-Cas systems. By acquiring "genetic memories" of prior exposures, CRISPR-Cas machinery can identify and destroy the invading element upon subsequent infection. CRISPR-Cas immunity is present in about half of sequenced bacteria and is mediated through three stages. During adaptation, fragments of phage DNA are incorporated into the CRISPR array as spacers. During expression, CRISPR arrays are transcribed and processed into crRNAs. Cas proteins form a complex with crRNAs during interference that guide these complexes to the complementary phage 'protospacer' DNA or RNA sequences which are cleaved by Cas nucleases. However, phages have come up with strategies of their own to overcome CRISPR-Cas systems. In this talk, I will present our research into these bacterial immune systems carried out by our group at Otago.

Q24. CRISPR-based whole genome screening as a functional genomics tool

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CRISPR/Cas tools have wide application in manipulating gene sequences and gene expression in diverse biological systems. The efficiency of gene targeting with CRISPR guide RNAs (gRNA), coupled with the advantages of nucleic acid over protein-based sequence recognition for multiplexed applications, has made CRISPR/Cas9 the preferred technology for forward genetic screens in higher eukaryotes. These screens typically exploit non-homologous end-joining repair to generate loss-of-function (knockout) mutations at Cas9-induced DNA double strand breaks.

Our group has interests in cancer therapeutics and diagnostics, which has led us to establish CRISPR knockout screens for the identification of novel drug targets (e.g. discovery of genetic dependencies in cancer cells that can be exploited by inhibitors that phenocopy synthetic lethal interactions), for critical evaluation of mechanisms of action of new drugs, and for identification of predictive biomarkers.

This talk will outline our current wet lab and bioinformatics pipeline for CRISPR screens. We use tumour cell lines transduced with lentiviral vectors expressing *S. pyogenes* Cas9 and the Brunello gRNA library, which targets 19,114 human genes. Transduced populations are grown at scale to identify essential genes (for which the cognate gRNAs drop out of the population) or are subjected to selection by drug exposure or tumour microenvironment stresses to identify genes that contribute to sensitivity/resistance. Typically, large populations (~10^8 cells) are required to minimise bias from genetic bottlenecks during selection. PCR amplification of gRNA sequences in genomic DNA from these pools, for NGS sequencing, enables evaluation of changes in gRNA distribution at gene level using software tools such as MAGeCK. The highest-ranking hits are validated using customised gRNA screens in additional cell lines, and single gene knockout pools or clones for confirmation of phenotype and mechanistic studies. These approaches will be illustrated with examples from studies with armed antibodies (trastuzumab emtansine; Kadcyla) and DNA-crosslinking anticancer agents.

Q25. Modelling bowel cancer using CRISPR/Cas9 genome engineering and personalised medicine for advanced disease

Susan Woods
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Colorectal cancer (CRC) is one of the most prevalent forms of cancer, with well over 1 million new cases diagnosed worldwide each year. Population screening strategies have played an important role in preventing CRC in developed countries, with detection and removal of lesions at early stages resulting in excellent 5-year survival rates. Despite this, many patients still present with advanced disease for which treatment options are limited, resulting in about 700,000 deaths annually. Serrated pathway tumours account for up to 30% of CRC, and unlike the conventional form of CRC, are not effectively prevented by current population screening methods. We use our knowledge of serrated pathway specific DNA alterations, and combine it with recent advances in organoid culture, CRISPR genome engineering and colonoscopy to fast track complex, preclinical models of serrated CRC.

We have also developed a personalised medicine screening platform to guide patient-specific treatment for patients with CRC peritoneal metastases (CRPM). CRPM organoids from each patient are subjected to next-generation sequencing (WES, RNAseq) to identify actionable genetic alterations. We combine this knowledge with high-throughput drug testing to identify specific drug sensitivities for each patient. In general, drug sensitivities have been consistent with known genetic susceptibilities for each sample, but we have also revealed both private and shared drug sensitivities that were unexpected. This opens new treatment options for patients that have exhausted standard care options.

Q26. Genome Editing of Somatic Stem Cells: Creating a New Class of Medicines

Matthew Porteus

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For most diseases, particularly genetic diseases, there are no curative therapies. Instead therapies modify the disease course in some way. One possible explanation for this observation is that the medicines we give (such as small molecules, biologics and even vaccines), while having biologic activity, are static themselves and thus have to be repeatedly administered to have affect. In contrast, cells are living systems that can migrate, divide, respond to their environment and in certain circumstances persist for the lifetime of the patient and have been called “living drugs.” Because of the profoundly different pharmacokinetics and pharmacodynamics, cells as medicines have the potential to cure a range of diseases that are not curable by other modalities. Genetic engineering of cells has the potential to both increase the safety and potency of living drugs. There are a number of different approaches to genetically engineer cells but genome editing provides the most precise method. We have developed a highly active ex vivo genome editing system using the CRISPR system to precisely engineer primary human somatic stem cells. We are using this strategy to correct genetic variants that cause disease and using it to provide new biologic functions to cells (a form of synthetic biology). I will discuss our progress towards developing genome edited cells as a new class of medicines to treat patients with severe unmet medical needs.

Q27. Male germline complementation in genome edited chimaeric sheep

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Male germline transmission depends on spermatogonial stem cells to produce functional sperm. Ablating the RNA-binding proteins DAZL or NANOS2 results in spermatogonia-deficient male mice and pigs [1-3]. We show that the *DAZL*- and *NANOS2*-deficient phenotype is conserved in sheep and can be rescued by complementation with wild-type embryonic cells.

Using CRISPR/Cas9 editing, we disrupted either *DAZL* or *NANOS2* in male ovine fetal fibroblasts (OFFs). For each gene, a homology-directed repair (HDR) template was designed to introduce a premature stop codon and *Taq1* restriction site. Mutant and wild-type variants were quantified by droplet digital PCR (ddPCR) with Taqman hydrolysis probes. Following single cell seeding of putative edited cells, clonal strains were confirmed as $DAZL^{-/-}$ and $NANOS2^{-/-}$ by sequencing. Validated OFF strains were used as donors for somatic cell cloning to generate embryos for transfer into surrogate ewes. Following lambing, testes samples were collected. $DAZL^{-/-}$ and $NANOS2^{-/-}$ testis cord sections comprised histologically normal somatic support cells but lacked DDX4-immunreactive spermatogonia. Compared to wild-type, expression of spermatogonia-specific marker genes was significantly down-regulated in mutant testis samples, while somatic cell markers were not affected.
Cloned wild-type embryos, derived from OFFs constitutively expressing red fluorescent protein to trace donor contribution, were aggregated with *NANOS2* null host embryos. Following embryo complementation and transfer, we obtained four lambs that were analysed for wild-type vs mutant *NANOS2* contribution by ddPCR quantification of biopsied tissue samples. One lamb showed variable somatic chimaerism and intact spermatogonia, indicative of germline complementation. Our finding provides a basis for generating genome-edited chimaeric absolute transmitter rams as an alternative to artificial insemination, potentially accelerating genetic gain in extensive farming systems.

References

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3. Park, K.-E., et al., *Generation of germline ablated male pigs by CRISPR/Cas9 editing of the NANOS2 gene.* Sci Rep, 2017. 7: p. 40176.

Q28. Thermo fisher scientific award winner

Understanding epigenetic memory in the germline and during sex determination

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The vast majority of inherited biological information is hard-coded in DNA sequence; however, epigenetic modification provides an additional conduit for intergenerational transmission of molecular memory. In mammals, inheritance of epigenetic memory in the form of DNA methylation is largely prevented by extensive erasure and reprogramming early in germline formation. We recently discovered that, unlike mammals, the germline of zebrafish does not undergo genome-wide erasure of DNA methylation during development. The significance of epigenetic memory preservation and its consequences for basic biological processes such as sex determination, is discussed.

QMB Meeting Poster Submission – Genomics

No.	Title	Presenter	Institutions
Q29	Using a cutting-edge molecular weapon to fight tuberculosis (TB) health inequalities within Pacific communities.	Jordan Taylor	University of Otago
Q30	Pooled CRISPR-Cas9 knockout screens to identify genes that confer tolerance to the acidic tumour microenvironment	Hanting Yong	University of Auckland
Q31	Ancient balancing selection maintains incompatible versions of a conserved metabolic pathway in yeast	James Boocock	Department of Human Genetics, University of California, Los Angeles
Q32	Genetic dissection of eukaryotic protein phosphorylation	Bede Busby	European Molecular Biology Laboratory (EMBL)
Q33	Classification of the Bacteroidetes family Muribaculaceae: application of Nanopore long read sequencing to link 16S rRNA gene amplicon and metagenome assembled genome-derived taxonomies.	Maximillian Lacour	Australian Centre for Ecogenomics
Q34	CRISPR/Cas9 knockout screens for identifying predictive biomarkers and therapeutic targets in cancer	Tet-Woo Lee	Auckland Cancer Society Research Centre, University of Auckland
Q35	Highly accessible translation initiation sites are predictive of successful heterologous protein expression	Chun Shen Lim	University of Otago
Q36	When can't you use homology search algorithms to infer homology?	Stephanie McGimpsey	University of Otago
Q37	Using comparative RNASeq to identify small non-coding RNAs in bacterial clades	Thomas Nicholson	University of Otago
Q38	Expression of ribosomal RNA sequence variants in human cells	Megan Schischka	University of Auckland
Q39	Engineered A.s. Cas12a (Cpf1) variant with enhanced activity	Aira Yuzawa	Integrated DNA Technologies
Q40	Tongan marine sponge microbiomes as an unprecedented source of microbial and natural product diversity	Vincent Nowak	Victoria University of Wellington

QMB Meeting Poster Submission – Microbes

No.	Title	Presenter	Institutions
Q41	Investigations into a novel hypothetical virulence factor from Group A streptococcus	Haniyeh Aghababa	University of Auckland
Q42	So you want to build a genome? Completing de novo Escherichia coli genomes with long read data	Georgia Breckell	Massey University
Q43	Investigation of malate metabolism in mycobacteria reveals insight into the energetic role of malate quinone oxidoreductase	Liam Harold	University of Otago
Q44	The formation and function of hybrid CRISPR-Cas modules from divergent system types.	Simon Jackson	University of Otago
Q45	Novel ncRNAs of the kiwifruit pathogen Psa	Bethany Jose	University of Otago
Q46	Characterisation of FseA a novel regulator that controls excision of the Mesorhizobium loti integrative and conjugative element (ICE) ICEMlSymR7A	William Jowsey	University of Otago
Q47	Comparing *in vivo* and *in silico* techniques to map the immunodominant B and T cell epitopes of a Methanobrevibacter ruminantium M1 adhesin protein	Sofia Khanum	AgResearch
Q48	Mapping the transcriptional network of the smaIR quorum sensing system	Howard Maxwell	University of Otago
Q49	Composition and Nucleic Acid Binding of the Type I-D CRISPR-Cas Complex	Tessa McBride	University of Otago
Q50	Investigating the Dual-Target Antiactivator QseM, a Novel Regulator of ICE Transfer.	Calum Morris	University of Otago
Q51	Understanding the Innate Immune Response to Group A Streptococcus Pili	Risa Takahashi	University of Auckland
Q52	A Novel Peptide Delivery Platform for Mucosal Vaccination Based on Group A Streptococcus Pili	Catherine Jia-Yun Tsai	University of Auckland

Q53	Effect of single SNPs on expression from *E. coli* lac operon	Marketa Vlkova	Massey University
Q54	GASPEL- A mucosal vaccine against Group A streptococcus	Adrina Hema Khemlani	University of Auckland

QMB Meeting Poster Submission – Human Disease

No.	Title	Presenter	Institutions
Q55	Inflammation triggers retinal metabolic changes in a hyperglycaemic microenvironment	Gaganashree Shivashankar	School of Optometry and Vision Science, University of Auckland
Q56	Biosynthetic Production of 3-Methyl Glutamate for the Generation of new Peptide Antibiotic Derivatives	Shayhan Chunkath	University of Auckland
Q57	Defining the cellular uptake of lipoprotein(a)	Nikita Deo	University of Auckland
Q58	T cell activation with CD28 enhances tumour-cell killing.	Alicia Didsbury	University of Auckland
Q59	Role of Host Exocytosis in Internalin A-mediated entry of Listeria monocytogenes	Gaurav Chandra Gyanwali	University of Otago
Q60	*In vitro* models of insulin resistance in adipose and muscle indicate differences in glucose uptake response between IGF-II and vesiculin.	Kate Lee	University of Auckland
Q61	A Validated Assay Platform for Group A Streptococcus Vaccine testing in New Zealand	Reuben McGregor	University of Auckland
Q62	Differential internalisation of the CGRP and AMY1 receptor	Tayla Rees	University of Auckland
Q63	A Multi-Platform Approach for the Discovery of Autoantibody Targets as New Biomarkers for Acute Rheumatic Fever	Mei Lin Tay	University of Auckland
Q64	Phomaketide A inhibits angiogenesis in human endothelial progenitor cells in vitro and in vivo	Shih-Wei Wang	Mackay Medical College, Taiwan
Q65	MCL-1 antagonism is effective in sensitizing pancreatic adenocarcinoma to SFK inhibition.	Samantha Oakes	Garvan Institute of Medical Research, Australia

Q66	Novel dual-targeting antibody-drug conjugates for improved tumour selectivity	Wouter Franciscus van Leeuwen	University of Auckland
Q67	Improving antibody drug delivery to the brain via surface sugars	John Finke	University of Washington