QMB Abstracts: Cancer Satellite

C1: Systemic and intracellular cyclophosphamide bioactivation

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Cyclophosphamide is a prodrug used to treat haematological and breast cancers, as well as some autoimmune diseases. It can also induce peripheral immune tolerance by preventing the clonal proliferation of reactive T-lymphocytes, which has led to the use of a short-pulse of post-transplant cyclophosphamide to prevent graft-versus host disease. This approach is now established for allogeneic-haematopoietic stem cell transplantation. Cyclophosphamide is also used for conditioning (lymphodepletion) prior to chimeric antigen receptor-T cell (CAR-T) immunotherapy of haematological malignancies.

To elicit its cytotoxic effects, cyclophosphamide requires bioactivation to the potent DNA alkylating agent phosphoramide mustard (PAM), via a 4-hydroxycyclophosphamide (4-OHCP)/aldophosphamide tautomer. Variability in the conversion of cyclophosphamide to 4-OHCP by cytochrome P450 (CYP) isozymes, particularly CYP2B6 and CYP2C19, appears to influence both its plasma pharmacokinetics and therapeutic outcomes. However, despite cyclophosphamide having been used as a chemotherapeutic for over 60 years, the mechanism(s) by which A) 4-OHCP is subsequently converted to PAM and B) how it modulates the immune system are poorly understood.

We have undertaken a series of studies investigating cyclophosphamide bioactivation both systemically and within lymphoid cells. While the CYP2B6 and CYP2C19 pharmacogenes have well established variant alleles which are commonly investigated as therapeutic biomarkers of cyclophosphamide outcomes, phenoconversion is also common and CYP activity can vary substantially between treatment cycles. Furthermore, we have recently demonstrated that 4- OHCP undergoes enzymatic activation to PAM mustard within leucocytes, which may account for its immunomodulatory effects and efficacy for the treatment of haematological cancers.

C2: From an unsuccessful phase III clinical vascular disrupting agent to a target based immuno-modulator, the rise of xanthenones

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Modulation of the immune system has become a mainstay in cancer therapy. As a consequence the scope of anti-cancer drug targets has also expanded to include normal cells within the tumour microenvironment. Macrophages are one of the normal immune cell types, with tumour-associated-macrophages having immuno-suppressive and pro-tumourigenic functions. Macrophages and monocytes depend on signalling through the cell surface receptor tyrosine kinase colony stimulating factor 1 receptor (CSF1R) for survival and proliferation. Blocking cell signalling activity by sequestering the CSF1R ligand or inhibiting its kinase activity can deplete macrophage populations and generate an anti-tumour effect in pre-clinical models. These outcomes have driven a global race for the discovery of new CSF1R inhibitors that can be used to reprogram the tumour microenvironment.

We discovered that the unsuccessful phase III clinical agent Vadimezan had the ability to act as broad-spectrum kinase inhibitor alongside its known vascular disrupting effects and its recently established effect as a murine-immuno-stimulator. Since Vadimezan represented novel chemistry in the universe of kinase inhibitors we took on the challenge of developing its potential as a kinase inhibitor. Inspired by the 3-dimensional structure of CSF1R kinase domain, we used a combination of protein-structure guided drug design, medicinal chemistry and molecular pharmacology to transform this lead molecule into a potent, highly selective CNS sparing CSF1R blocker suitable for macrophage modulation in *in vivo* tumour biology.

C3: Cytostatic role of safe novel HDAC inhibitors in AR- prostate cancer

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Androgen receptor-negative (AR-) prostate tumours are observed in 11-24% of prostate cancer patients [1,2]. Presently, there are no clinically approved treatments for AR- prostate cancers. Histone deacetylases (HDACs) are overexpressed in late-stage prostate cancers and in AR- prostate cancer cell lines (PC3 and DU145) [3,4]. However, HDAC inhibitors, including suberoylanilide hydroxamic acid (SAHA), showed toxicity and lack of efficacy in clinical trials for prostate cancer [5]. Therefore, we synthesised novel HDAC inhibitors, N1-hydroxy-N^8-(4-(pyridine-2-carbothioamido)phenyl)octanediamide) (Jazz90) and [chlorido(η^5-pentamethylcyclopentadienyl)(N1-hydroxy-N^8-(4-(pyridine-2-carbothioamido-κ^2N,S)phenyl)octanediamide)rhodium(III)] chloride (Jazz167), which are based on SAHA, with the objective of making it more efficacious and safer. Jazz90 and rhodium-containing Jazz167 exhibited less toxicity towards non-cancerous (PNT1A and NIH 3T3) cells as compared to SAHA and were also non-toxic in BALB/c mice at the highest dose of 75 mg/kg. Specifically, Jazz90 was 2.9- and 2.5-fold more potent against PC3 and DU145 cells as compared to PNT1A cells, respectively, whereas Jazz167 was 3.5- and 1.3-fold more potent against PC3 and DU145 cells in contrast to PNT1A cells, respectively. They also effectively inhibited PC3 and DU145 spheroid growth by 78-89% and branching by ~95% in PC3 spheroids. These compounds also reduced HDAC activity by 60% at 50 nM in PC3 nuclear lysates, whereas these drugs increased acetylation by 6- to 8-fold in PC3 cells. Lastly, the drugs were concluded to be cytostatic and induced quiescence following their withdrawal. The mechanism of action by which these drugs induce cytostaticity remains unclear as different responses following drug treatment were observed on phosphatidylinositol-3-kinase/protein kinase B (PI3K/Akt) and Ras/mitogen-activated protein kinase (Ras/MAPK) pathway in PC3 and DU145 cells. Since the drugs were safe in BALB/c mice and were effective in spheroids, they should be tested further for their efficacy in vivo using orthotopic mice models.

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C4: Developing oncolytic Seneca Valley Virus for cancer treatment

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Oncolytic virotherapy is a very promising cancer treatment strategy due to its high specificity to cancer cells, low toxicity to patients, high safety profile, and the ability to trigger antitumour immune responses. Seneca Valley Virus (SVV), a novel oncolytic virus from the family Picornaviridae, is a replication competent single-stranded RNA virus that can be systemically delivered, and it is safe and well tolerated at the highest levels tested in patients. SVV's targeted oncolytic activity is mediated by its cellular receptor: Tumour Endothelial Marker 8 (TEM8). TEM8 is overexpressed in over 60% of human solid cancers and the associated microenvironment, and it is recognized as a marker for targeted therapy. Here, we will discuss different modalities to improve SVV oncolytic activity for the development of a highly efficient targeted clinical tool for cancer treatment.

C5: Anti-helmintic drugs mebendazole and albendazole cause selective apoptotic cell death in colorectal and breast cancer cell lines

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The well-known anti-pinworm drugs mebendazole and albendazole are commonly used in both children and adults, and are well-tolerated without serious adverse effects. They have recently shown promising results in pre-clinical in vitro and in vivo anti-cancer studies, as well as in a small phase IIa clinical trial for gastrointestinal cancers. Here, we compared their efficacy in breast (MDA-MB-231, MCF-7) and colorectal (HT29) cancer cell lines as well as in non-cancerous mesenchymal stromal cells, and examined their mechanism of action.

Mebendazole and albendazole demonstrated potent effects in the colorectal cancer cells, with IC50 values of less than 1 µM at early timepoints, 180-fold lower than the concentration required to kill half the non-cancerous cells. Both mebendazole and albendazole induced G2/M phase cell cycle arrest and disrupted tubulin polymerisation. They also caused classical apoptosis, characterised by mitochondrial membrane permeability, caspase-3 activation, phosphatidylserine exposure, DNA fragmentation and reactive oxygen species production.

The re-purposing of anti-helmintic drugs such as mebendazole and albendazole for colorectal cancer would appear to be a potentially promising strategy to enhance therapeutic response, since these drugs cause selective cell death at achievable concentrations, are orally bioavailable, are familiar to patients and have a low adverse effect profile.

C6: Growth hormone receptor antagonism delays tumour regrowth when combined with radiation in a lung cancer xenograft model

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Accumulating evidence supports a role for the growth hormone (GH) - insulin-like growth factor-1 (IGF-1) axis in cancer progression and resistance to radiation therapy. Lung cancer is the leading cause of cancer-related death worldwide, and patients diagnosed with advanced disease have a very low 5-year survival rate. mRNA expression of the GH receptor (GHR) is relatively high in lung cancer. In addition, epidemiological studies have linked elevated circulating IGF-1 to increased lung cancer incidence. The aim of this study was to evaluate efficacy of a GH receptor antagonist (GHA) as a monotherapy and in combination with radiation therapy in a lung cancer xenograft model. NCI-H460 xenografts grown in immunodeficient NIH-III mice were treated with vehicle or GHA (30 mg/kg/day) with or without fractionated gamma radiation (10 × 2.5 Gy over 5 days). Treatment with GHA slowed the tumour growth but the time taken for NCI-H460 tumours to 4× in size did not reach statistical significance compared to vehicle treated controls (16 days versus 13 days; $p > 0.05$). When combined with radiation, GHA delayed the tumour regrowth compared with radiation. The median time that the tumours took to reach 4× the pre-radiation treatment volume was significantly increased compared to radiotherapy (27 days versus 22 days; $p < 0.05$). Serum IGF-1 was assessed as a surrogate marker of GHA activity. IGF-1 concentrations were reduced by 56% and 59% in GHA-treated groups compared to vehicle-treated controls, with and without radiation, respectively. Our results suggest that GHA treatment sensitises NCI-H460 lung cancer cells to radiation, and may improve the response of GH- and/or IGF1-responsive lung tumours to radiotherapy.

C7: Modelling drug-induced apoptosis to rationalise multi-agent chemotherapy in high-risk neuroblastoma

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High-risk neuroblastoma is an aggressive and invasive paediatric malignancy, with few actionable somatic mutations. As such, intense multi-agent chemotherapy remains the standard-of-care. Failure to effectively activate apoptosis, or the ability to evade apoptosis, has been established as a key mechanism of chemoresistance in neuroblastoma. Despite this, there is little understanding of the apoptotic mechanism-of-action of individual standard-of-care chemotherapeutic agents, let alone their combined mechanism of action. Here we apply a network-wide, systems level approach to identify drug-specific apoptotic signalling axes which will inform patient-specific, synergistic drug combinations.

A functional genomics screen was performed on a high content cellomics platform with a siRNA library of 200 apoptotic genes with current standard-of-care chemotherapy and preclinical drugs. Multi-dimensional analysis of this dataset elegantly demonstrated that synergy between any two chemotherapy drugs is proportional to the magnitude of divergence in apoptotic signalling between individual drugs. Identified drug-specific apoptotic signalling nodes were validated using genetically engineered stable cell lines harbouring fluorescent biosensors and/or CRISPR-Cas9 mediated endogenously tagged proteins on our high content cellomics platform. These tools have allowed us to perform high-throughput kinetic live cell analysis at the single cell resolution and will help establish how synergy arises due to differential apoptotic signalling at the single cell level.

The application of our systems biology approach to rationalise mechanism-based drug selection will address fundamental questions about the network level functioning of apoptotic signalling pathways which has clinically relevant implications. Our data has demonstrated that it is differences in single agent drug-induced apoptotic signalling that will give rise to synergistic drug combinations. This is contrary to the current dogma of utilising drugs with different molecular targets in combination chemotherapy. This research will inform the development of precision medicine approaches with the aim to improve patient outcomes for high-risk neuroblastoma.

C8: Transmissible cancers in Tasmanian devils

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Two independent transmissible cancers are currently circulating in the wild Tasmanian devil population. The devil facial tumour 1 (DFT1) was first observed in 1996 and has killed thousands of devils, pushing them onto the endangered species list. The devil facial tumour 2 (DFT2) was discovered in 2014 and to date has only been detected in southern Tasmania[1]. Both DFT1 and DFT2 are hypothesized to have originated from Schwann-like cells[2] and the tumour cells are transmitted via biting. Polymorphisms in major histocompatibility complex (MHC) antigens and mutations that are carried forward to new hosts should provide targets for anti-tumour immunity, but most devils die of from tumour-associated disease within 12 months despite genetic mismatches between the tumour cells and the host devil. A primary immune evasion mechanism of DFT1 cells is downregulation of surface major histocompatibility complex class I (MHC-I). However, MHC-I can be upregulated on DFT1 cells in response to interferon-gamma. Rare cases of immune recognition of DFT1 have shown the MHC-I is a major antibody target. Interestingly, DFT2 cells constitutively express surface MHC-I. Thus, alternative immune evasion mechanisms are present in DFT1 and DFT2 cells. Like human cancers, we have found that DFT1 and DFT2 cells upregulate programmed death ligand 1 (PD-L1) in response to interferon-gamma, suggesting a potential secondary immune evasion mechanism. Our team is currently developing a vaccine to target MHC-I alleles and neoantigens found in DFT1 cells.

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C9: Human Gene and Microbial Analyses Suggest Immunotherapy-like Mechanisms in Complete Response to Radiotherapy in Rectal Cancer

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Cases of rectal cancer have been increasing in the younger population worldwide. The treatment of rectal cancer usually involves surgical removal, although more modern treatments now include neoadjuvant radiotherapy (RT). Both treatments can cause potential morbidity and unwanted side-effects. Identifying biomarkers that predict how well a patient responds to RT could help stratify patients to the most suitable therapy. We therefore aimed to understand RT response mechanisms and identify potential predictive biomarkers of complete responders using patient gene expression and microbiome content of 39 pairs of pre-RT tumour (T) and adjacent normal (N) patient samples obtained by RNA-sequencing. We analysed differences of genes and the microbiome in tumours compared to normal samples (T/N) in complete responders compared to other responders.

We found that the majority of differentially expressed host genes (DEGs) in T/N of complete responders were involved in immunoglobulin genes. The top enriched gene-sets from these DEGs included complement activation and processes involving B-cells. The microbes identified as more abundant in T/N of complete responders include *Bacteroides thetaiotaomicron*, *Ruminococcaceae bacterium*, and *Hungatella hathewayi*. These bacteria also showed significant correlations with DEGs from the host analysis, most notably to BATF2.

We found evidence that an increase in expression of immunoglobulin genes in T/N samples is strongly associated with complete response in radiotherapy, and that these genes may be aiding in the response through complement activation and B-cell activation. These, and other enriched gene sets, indicate mechanisms that potentially recruit cytotoxic CD8+ T-cells. Abundant microbes found also included species known to augment immunotherapy effects. We therefore propose that immunotherapy-like responses are induced in complete response to radiotherapy in rectal cancer, and that these genes and/or microbes could be potential biomarkers of complete response to radiotherapy.

C10: Visualising *Clostridium* colonisation in tumours: steps towards clinical stage monitoring of cancer patients

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The non-pathogenic anaerobic bacterium *Clostridium sporogenes* has been utilised as an 'armed' vector for cancer gene therapy applications, with the ability to express prodrug activating enzymes and immunostimulatory molecules selectively in the tumour microenvironment. This selectivity results from the presence of tumour necrosis, which provides a suitable environment for germination of C. *sporogenes* from endospores, resulting in tumour-specific colonisation. Yet significant hurdles to successful clinical development of this therapy exist, many of which could be alleviated by the ability to monitor non-invasively the real-time spatial and temporal distribution of vector spread.

In this study, we identify the major oxygen-insensitive nitroreductase from *E. coli* (NfsA_Ec) as an imaging-capable reporter gene, with the ability to metabolise and trap positron emission tomography (PET) imaging agents for non-invasive imaging of gene expression. We establish proof-of-principle for the ability to detect NfsA_Ec expression by using microPET imaging with ^{18}F-HX4, a repurposed radiotracer originally developed for hypoxic cell imaging. A significantly higher tumour-to-blood ratio for NfsA_Ec expressing tumours was observed in two tumour models ($P \leq 0.03$) compared to corresponding wild-type controls.

To avoid the background signal from hypoxia whilst maintaining activity for NfsA_Ec imaging, we designed and synthesised a series of NfsA_Ec selective PET probes. Successful validation of these PET probes in NfsA_Ec expressing tumours was performed using fluorescent antibodies to detect intracellular adducts generated upon metabolism by NfsA_Ec. In wild-type control tumours where the presence of a hypoxic fraction was established using a positive control (Pimonidazole), no adducts were detected.

The PET imaging ability of the NfsA_Ec transgene is an important advantage for clinical development of replicating vectors such as *C. sporogenes*, as it eliminates the requirement for the extensive, labour intensive, and often invasive biopsy and blood sampling methods that are currently essential for clinical stage monitoring of cancer patients in this context.

C11: The immune microenvironment in progression and regression of cervical intraepithelial neoplasia grade 2

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Around 11-40% of high-grade pre-cancerous cervical lesions (CIN2/3) spontaneously regress through a process that is considered to be primarily immune mediated. The purpose of this study is to define the immune microenvironment of CIN2 lesions and establish the relationship between immune markers and progression to CIN3.

Sixty-eight patients under the age 25 with p16 positive, biopsy-confirmed CIN2 were identified from a large conservative management trial (PRINcess Study). Participants were separated into progressor (CIN3+) or regressor/persister groups based on the outcome of a two-year follow-up. Immune cells in the lesion and the dermal region beneath the lesion were enumerated following staining with antibodies against a range of immune markers.

We found that GATA3 and CD138 positive cells predominated in the lesion and included both positive keratinocytes and immune cells. CD4 and CD8 T cells were also highly represented. In the dermal region GATA3 positive, CD4 positive, IL-17 positive and HMGB1 positive cells predominated. Progression to CIN3+ was significantly associated with high numbers of Blimp1 (P = 0.0019) and low numbers of HMGB1 (P = 0.0119), CD4 (P = 0.0229) and Tbet (P = 0.0244) positive cells in the CIN2 lesion, and low numbers of CD4 (P = 0.0022), CD8 (P = 0.0036) and CD11c (P = 0.0176) positive cells in the dermal region. The presence of CD4, CD8 and T bet positive cells in the dermal region most strongly correlated with CD11c positive cells in the persister/regressor group but not the CIN3 progressor group.

This is the first report of significant differences in immune cell populations that infiltrate CIN2 and the local dermal region, depending on subsequent progression or persistence/regression. The enumeration of lesional Blimp1 positive cells and dermal CD4 T cells may have utility in determining the risk of progression to more severe disease.

C12: Biomarkers in Endometrial Cancer

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Endometrial (uterine) cancer incidence is rising in Aotearoa New Zealand, particularly in young people who identify as Māori or Pacific Islander. The current treatment pathway for people with endometrial cancer is lacking and needs to be invested in as incidence continues to rise.

Endometrial cancer is the top most associated cancer with obesity and for those with comorbidities or wishing to preserve their fertility, surgery (hysterectomy) is not a viable treatment option. Alternatives to surgery are needed for this changing population. One such alternative, the intrauterine device (Mirena) releases progesterone into the uterus to counter-act oestrogen stimulated growth. However, the Mirena may only work for 40% of people, the reasons for which are unknown.

Furthermore, for those receiving surgery, adjuvant treatment may include chemo or radiation therapy. Personalisation of adjuvant therapy by molecular subtype is lacking for this cancer, and molecular profiling is not yet carried out in NZ despite the 4 subtypes being identified nearly 10 years ago.

Biomarkers to support personalisation of treatments for endometrial cancer are critically needed in order to reduce morbidity and mortality for people in NZ.

C13: Slipping away – Is our brain's migratory machinery assisting in brain tumour malignancy?

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Glioblastoma (GBM) is the most common and fatal brain tumour in adults. Despite the many advances in science and medicine, the median survival time for GBM has only increased by four months over the past 30 years to a mere 15 months[1]. One of the greatest challenges in GBM is the rapidly migrating, treatment-resistant GBM stem cells[2]. A molecule of interest in this regard is polysialylated neural cell adhesion molecule (PSA-NCAM). When present on the cell surface, it reduces NCAM's interaction with the extracellular matrix and other cells, making the cell 'slippery'[3]. PSA-NCAM supports cellular migration during neural development[4], adult neurogenesis[5] and synaptic plasticity[6]; however, it may also aid in tumour cell migration and metastasis in brain tumours[7]. To investigate this possibility, we used immunohistochemistry and automated image analysis to analyse the expression of PSA-NCAM in over 80 donated patient brain tumour specimens and correlated these with patient data and clinical outcomes. We found that levels of PSA-NCAM was highly correlated with increasing tumour grade and consequently, negatively correlated with patient survival ($p < 0.0001$). With grade IV GBM tumours, a multivariate analysis revealed that PSA-NCAM levels carried a greater hazard ratio (HR) in relation to the time of death compared to the diagnostically relevant cell proliferation marker ki67 (2.40 vs 1.69, $p < 0.001$). In addition, volumetric analysis of pre-operative MRIs revealed that patients with PSA-NCAM-positive tumours had significantly larger tumour volumes when compared to PSA-NCAM negative tumours ($p < 0.01$). Together, we believe that PSA-NCAM can be a powerful prognostic marker for brain tumours and its role in brain tumour progression warrants further investigation.

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C14: Male, pale and stale: how to win a three minute thesis competition with ease.

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Being old and experienced is a key part of good science communication. I will provide an overview of my entire research career highlighting how tough we had it back in the old days. And how much easier things are nowadays. I will also explain the basis of each of the old methods and techniques we had to master, and how we had to use Kiwi ingenuity and No 8 fencing wire to make things work. Finally, I will end with a rant about how we could just do things because we were curious, not needing to worry about all the new buzzwords like research translation, impact and transdisciplinarity. That should be easy within three minutes.

C15: Evaluation of a membrane-based method for enriching circulating tumour cells from the blood of colorectal cancer patients

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Circulating Tumour Cells (CTCs) derived from solid tumours are requsite to metastatic tumour spread. Isolating CTCs and understanding their biology is essential for developing clinical applications for CTCs, such as diagnostic tests and therapeutic targeting. Currently, CellSearch is the only method of CTC enrichment approved by the U.S. Food and Drug Administration, which isolates only epithelial CTCs (CTCs expressing EpCAM) and is resource intensive. This has led to an interest in developing simpler size-based methods for CTC enrichment. MetaCell is a size-based method which has been previously used for enriching and culturing CTCs from various cancer types.

We benchmarked the MetaCell method by spiking healthy blood with different cell numbers (10, 100, 500 and 10,000 cells) of HCT116 and DLD1 colorectal cancer (CRC) cell lines and determined recovery and white blood cell (WBC) depletion rates using CTC (EpCAM and cytokeratins) and WBC (CD45 and CD16) markers via gene expression analysis and immunostaining techniques. Recovery rates were not affected by cell numbers and found be >85% for both CRC cell lines. The MetaCell method also yielded a CTC fraction of high purity with >95% depletion in WBC population.

We then applied the MetaCell method to analyse blood samples from 22 CRC patients. We detected CTCs in 45.5% of patients across all stages of CRC (AJCC Stage I-IV). We plan on generating methylomes and transcriptomes to better our understanding of the metastatic process which could further facilitate exploring the role of these markers in determining prognosis, risk of relapse and response to therapy in patients in the future.

C16: Rapid immunoprecipitation mass spectrometry of endogenous proteins supports a novel BCL6 function in glioblastoma

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Glioblastoma is a devastating brain cancer which has proven highly resistant to currently available therapies. The severity of glioblastoma tumours has been linked to the expression of BCL6, a transcriptional repressor which is well characterised in germinal centre B cells and in B cell lymphoma. Although the role of BCL6 in glioblastoma remains poorly understood, there is evidence that its activity in glioblastoma may differ from its usual role and that it may be involved in resistance to therapy. My research aims to clarify the activity of BCL6 in glioblastoma by identifying which proteins it associates with. BCL6-associated proteins were identified by performing rapid immunoprecipitation mass spectrometry of endogenous proteins (RIME) for BCL6 in glioblastoma cells.

This identified known binding partners of BCL6 as well as proteins whose association with BCL6 is novel. Known BCL6 binding partners identified in glioblastoma included the corepressor NCOR2, which provided confidence that RIME was able to identify BCL6-associated proteins. Most strikingly, two subunits of metabolic regulator AMPK were commonly identified across both the irradiated and untreated glioblastoma cell lines. Although AMPK is known to regulate transcription of BCL6, there is no previous evidence of their physical interaction. Additionally, other proteins were identified as BCL6-associated only in the untreated or irradiated cell lines, suggesting a change in the function of BCL6 in response to therapy. This research provides insight into the different roles of BCL6 in untreated and irradiated glioblastoma cells.

C17: Universal CAR-T cell therapy using stimuli-responsive “Tags” and “Pro-tags”

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Chimeric antigen receptor (CAR) engineered T cells have shown revolutionary success in treating refractory hematologic malignancies. However, there are still challenges in translating CAR-T cell therapy into solid tumours, for example potential on-target-off-tumour toxicity. The aim of this project is to improve the safety profile of CAR-T cell therapy via universal CAR-T cells. Universal CARs recognising a generic tag, linked to specific tumour ligands, have been proposed as an option to simplify this process. This means that only one universal CAR DNA construct needs to be developed and this can be used to transfect cells from any patient. The universal CAR T cells can then be used in combination with many different specific tumour-ligand-tag constructs to treat cancer

The first step of the development of this approach involves the design and synthesis of folate-biotin and glucose-biotin constructs containing a biotin tag attached to molecules overexpressed by tumours. To further enhance this technology and allow more precise control over our universal CAR-T cells therapeutic tumour targeting activity, we synthesised masked versions of “pro-tags”, containing a bulky group that only released upon exposure to a stimulus present specifically in the tumour microenvironment, for example hydrogen sulphide. With this approach, CAR-T cells should preferentially attack tagged tumour cells and not those healthy cells that express the same targeting ligand. The second part of this project involves in vitro killing with synthesised tags. Initial killing studies demonstrated killing with unmasked glucose-biotin tags. Overall, this project will contribute to the advancement of CAR-T cell therapy in treating solid tumours.

C18: Ex Vivo Culture of Patient-Derived Endometrial Cancer Tumour

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Intro: Endometrial cancer (EC) is the most common gynaecological cancer in the developed world and its incidence is fastest rising in Aotearoa. While the overall cure rate for early-stage EC treated with surgery is high, there is a growing patient cohort for which surgery is infeasible, such as those who wish to remain fertile, and those with a high BMI. Currently, the only surgery alternatives are the levonorgestrel-loaded intra-uterine device (LNG-IUS), or radiation therapy. This necessitates the implementation of research models that are clinically and biologically relevant to increase the translation of novel therapeutics from laboratory to clinic. The long-term ex vivo culture of patient tissue is a novel technique that has been validated in other cancers, but not EC. Typical cell culture techniques involve mechanical and enzymatic digestion, breaking down the natural 3D architecture and subsequently reducing the clinical relevance of the model. By contrast, ex vivo patient-derived explant models retain the spatial relationship of the tissue. Aim: To establish a model of early-stage EC and explore the effects of LNG and radiation therapy. Methods: Tissue explants were maintained on gelatin sponges for 21 days. IHC was used to investigate proliferation and apoptosis. Results: EC explants are capable of surviving in culture for up to 21 days, however there is intra- and inter- patient variability in explant viability and therefore the model might be better suited to a 7 or 14 day endpoint. The model may serve to investigate EC treatment, for example the effects of LNG and radiation on tissue survival, and the investigation of biomarkers that predict LNG response prior to treatment. Conclusion: Data from this project will be used to guide the feasibility of ex vivo patient-derived explant models of EC, with the goal of developing novel treatments that circumvent the need for surgery.

C19: Hyaluronic acid biology in Pancreatic Ductal Adenocarcinoma using Clinically Relevant *In Vitro* models

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Hyaluronic acid (HA) is a major component of the stroma of pancreatic ductal adenocarcinoma (PDAC) and its high abundance is negatively correlated with patient survival. Recently, a HA-depleting drug showed promising success when combined with chemotherapies in a phase I and II clinical trials, however, in phase III trials it failed to improve patient outcome and was not well tolerated due to adverse side-effects. This is one of many stromal-targeting drugs that has failed to make its way into clinics, despite the large stromal composition (up to 80%) of PDAC. Hence, we revisited the biology of this unique phenotype in PDAC – abundant HA. We utilised bioinformatics tools, 3-Dimensional (3D) spheroid culture of PDAC cell lines (PANC1, MiaPaCa-2, BxPC-3, and HPAF-II) using the ultra-low attachment method and RNA sequencing, as well investigating the effect exogenous HA has on these models, to gain a better understanding of HA biology in PDAC. In our experiments, the addition of exogenous HA into the culture media of PDAC spheroids led to cell-line specific volumetric changes. In contrast, when cultured in 2D as monolayers with exogenous HA, minimal phenotypic effects were seen. A similar cell-specific trend was observed in our RNA sequencing data. The regulation of HA synthesis, degradation and function involves many enzymes, receptors, and binding proteins. To investigate this further, we evaluated the expression of 53 genes related to HA biology in each cell line. Patterns of gene expression for each cell line provided an explanation for cell-line specific volumetric changes after addition of exogenous HA. For instance, we found higher expression of the HA surface receptor *HMMR* in cell lines that underwent a phenotypic change. Together, our study highlights the importance of using carefully selected 3D *in vitro* models to understand the stromal biology of PDAC.

C20: Reprogrammed antioxidant defences in metastatic melanoma cells

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Melanoma is the deadliest form of skin cancer and New Zealand has one of the highest rates worldwide alongside Australia, largely due to increased UV radiation. Despite recent developments in targeted therapy and immunotherapy, melanoma cells frequently develop resistance, resulting in relapse and metastasis. Therefore, new treatment options are imperative. Metastatic melanoma cells are reported to rely on enhanced antioxidant defence systems for their ability to metastasise and survive. These same antioxidant defences can limit the effectiveness of specific treatments, thus disruption may be an effective strategy for improving treatment outcomes in treatment-sensitive and treatment- resistant cells. In this study, we monitored redox homeostasis in a panel of NZM metastatic melanoma cell lines and non-cancerous primary melanocytes by measuring peroxiredoxin oxidation status. Peroxiredoxins are a family of thiol-dependent peroxidase enzymes that are extremely sensitive indicators of the cellular redox environment. Cytosolic and mitochondrial peroxiredoxin oxidation was quantified in the panel of cell lines, with cytosolic peroxiredoxin 2 showing the most variation between cell lines. Analysis of RNA-Seq data available provides information on potential causes of redox variations across cell lines. Investigations are underway to identify the nature of the metabolic reprogramming that occurs in these cells. We are also assessing the impact of antioxidant pathway inhibitors, both alone and in combination with the B-Raf kinase inhibitor vemurafenib. Initial results indicate increased cell death at lower doses when both inhibitors are used in combination. We are further extending this study to investigate the synergistic effect of this new treatment combination on vemurafenib-resistant metastatic melanoma cell lines.

C21: ADSC-EVs and macrophage polarisation in fat grafting for breast reconstruction post-mastectomy

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Introduction: Despite autologous fat grafting (AFG) being a favourable oncoplastic breast reconstruction option, graft retention rates are variable. Enriching the grafted tissue with extracellular vesicles (EVs) released from adipose derived stem cells (ADSCs) may promote retention by interacting with macrophages resident within the breast cavity. We aimed to identify key macrophage phenotypes that are modulated by ADSC-EVs in vitro.

Methods: Fat samples were collected from three women undergoing AFG (19/CEN/23). ADSCs were cultured and EVs isolated from the media. Monocytes were isolated from two healthy volunteers (19/CEN/129) and cultured towards M0 (unpolarised), M1-like (pro-inflammatory) or M2-like (anti-inflammatory) phenotypes. ADSC-EVs were added to cultures for 48 hrs and macrophage phenotype was examined using a 10-marker flow cytometry panel interrogating antigen presentation (CD86, CD80, HLA-DR), adhesion (CD11b), pattern recognition (TLR4, CD14), Fc receptor (CD64) and scavenger receptor expression (CD36, CD163, CD206). UMAP was used to visualise cells, and phenotypically homogeneous clusters were identified using FlowSOM. A manual gating strategy was generated to recapitulate clusters, and applied to a repeat experiment. Both runs were analysed to examine the prevalence of each cluster - representing a unique macrophage phenotype - with and without ADSC-EVs.

Results: Following the addition of ADSC-EVs, M1-like macrophages demonstrated a reciprocal shift of cell distribution from a cluster with a 'high' inflammatory profile – characterized by higher expression of all markers – (46.9% without ADSC-EVs; 24.8% with ADSC-EVs) to a cluster with a lower inflammatory profile (47.7% to 67.4%). M0 macrophages shifted from an 'inflammatory' phenotype (higher expression of most markers; 35.0% to 14.4%) to a phenotype characterised as $TLR4^{low}CD206^{low}HLA\text{-}DR^{low}$ (12.9% to 25.8%). M0 and M2-like macrophages expressing TLR4, were more prevalent with ADSC-EVs (5.6% to 10.4% for M0; 11.9% to 16.7% for M2-like).

Conclusions: ADSC-EVs are complex regulators of macrophage phenotype that can shift macrophages away from a heightened pro-inflammatory state.

C22: Characterisation of single cell transcriptomics in urinary cells for the detection of bladder cancer

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Bladder cancer is a heterogeneous disease making diagnosis, prognosis, and treatment challenging. The tools available to clinicians for diagnosis, such as cystoscopy, are costly and invasive. Notably, bladder cancer has one of the highest recurrence rates of any malignancy, requiring patients to have routine cystoscopies ranging from every three months to yearly. Biomarkers found in urine offer promising opportunities for non-invasive bladder cancer detection. Urinary biomarkers that match the sensitivity and specificity of cystoscopy would reduce the need for cystoscopies leading to improved health equity.

Multiple urine tests exist; however, the overall reported sensitivities and specificities fall below the 95% benchmark suggested as being required to replace cystoscopy. Of these urine tests, targeting mRNA using RT-qPCR has the highest reported sensitivity (91-93%). Despite high sensitivity, diagnosis of early-stage bladder cancer becomes challenging due to the limited number of tumor cells exfoliated into the urine as well as false positives caused by non- urothelial cells expressing high levels of target mRNA. To overcome these challenges, detection of mRNA or protein in situ, combined with morphology data, is predicted to increase the sensitivity and specificity to detect low-grade, early-stage tumors.

This project aims to characterise mRNA biomarkers and their corresponding proteins using in situ technologies. To determine which biomarkers are most applicable, single cell RNA sequencing and genomic analysis will be used. Candidate biomarkers will then be validated using bladder cancer patient urine and tumour biopsies.

C23: Untangling the interaction between Heterochromatin Protein 1α and G-quadruplexes

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Heterochromatin Protein 1α (HP1α) is a pivotal architectural protein which establishes and maintains domains of heterochromatin. HP1α deposition must be carefully orchestrated to ensure critical regions of the genome, such as the telomeres and centromeres, are protected. The histone code proposes that the chromodomain of HP1α binds trimethylation of Histone H3 on lysine 9 (H3K9me3) to establish these constitutively condensed domains, however the DNA/RNA binding hinge domain of HP1α has also been shown to be required for targeting to heterochromatin.

Both HP1α and telomeric repeat-containing RNA (TERRA) have been shown to regulate heterochromatin at the telomeres, and previously we have shown that HP1α binds TERRA. TERRA is a long non-coding RNA (lncRNA) transcribed from the telomeres, and because of its guanine (G)-rich sequence, TERRA forms a G-quadruplex (G4) structure: a non-canonical nucleic acid formed by four guanine nucleobases bound in a tetrad, stacked to form a tetraplex. Because this RNA is possesses a structure that differentiates it from other lncRNAs and is associated with a constitutively condensed region of heterochromatin, we hypothesised that TERRA may recruit HP1α through structural recognition to ensure heterochromatin establishment at telomeres.

We show that HP1α binds with high affinity to TERRA dependent on its structure, displaying strong selectivity towards both RNA and DNA parallel G4s, not preferring anti-parallel G4s or other structures of nucleic acids. Multiple lysine patches within the hinge are essential for this binding, however the hinge alone lacks the specificity displayed by the whole HP1α protein. Further work is now underway to investigate HP1α-G4 binding and the structural complexities of HP1α that contribute to this distinct selectivity.

Understanding the intricacies of the binding between HP1α and TERRA enlightens heterochromatin maintenance at telomeres, the tight regulation of which is essential for genomic stability and greatly affected in cancer immortalisation.

C24: Leveraging the gut microbiome for improved cancer outcomes

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The gut microbiota sits at the interface between the host's physiology and exposome and it plays a central role in the causation of colorectal cancer. The microbiome defines the therapeutic response and toxicity of multimodal chemotherapy. This talk defines surgical / microbiome interactions in a systems biology context in cancer and it will outline how we can modulate the microbiome to reduce surgical and therapeutic risk. It provides a future map for the personalised translational application of gut microbiome metabolism in cancer surgery throughout the patient treatment journey. This talk will also define the TIMER mechanisms through which the microbiome modifies chemotherapeutic response and it will outline how translational metabonomics can be applied at the bed side to provide novel biomarkers and therapeutic targets that leverage the gut microbiome for improved patient outcomes.

C25: Radiation exposure elicits a neutrophil-driven response in healthy lung tissue that enhances metastasis

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Radiotherapy is one of the most effective approaches to treat cancer, although healthy tissue injury due to off-target radiation exposure remains a clinical challenge. In this study, a model of acute radiation injury to the lung was used to explore the biological link between tissue damage and cancer progression. Healthy mouse lung tissue was exposed to radiation prior to the induction of metastasis, resulting in a strong enhancement of cancer growth. Locally activated neutrophils were found to be key drivers of the tumour-supportive preconditioning of the lung microenvironment, governed by enhanced regenerative Notch signalling. Importantly, these tissue perturbations endowed arriving cancer cells with an augmented stemness phenotype. These findings not only reveal a novel tumour-supportive function of neutrophils in the context of tissue-injury, but also have important clinical implications by suggesting targeting their activity could maximise the success of radiotherapy for the treatment of cancer.

C26: Messengers in the microenvironment: the role of extracellular vesicles in fat graft retention for breast reconstruction.

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Autologous fat grafting (AFG) is an increasingly popular tool for oncoplastic breast reconstruction because it uses natural tissue with low donor site morbidity and can help ameliorate post-radiation and surgery-associated pain. The major caveat for AFG is the variable retention rate of the graft; in a pilot study we conducted in the Wellington Region, retention rates measured by MRI ranged from 30-100% at 3 months post-procedure. Low graft retention is largely due to a failure of the graft to integrate into the recipient environment, resulting in inadequate vascularisation of the tissue, cell death, and fibrosis. In essence, this is an issue of communication between the various cell types in the donor and recipient tissue to create a viable microenvironment in which the grafted tissue can thrive. Recently, membrane- bound packages called extracellular vesicles (EVs) have come to prominence as a key mechanism for cellular cross-talk, functioning through the delivery of active molecular cargo to neighbouring cells. EVs are important regulators within tissue microenvironments and are potential therapeutic targets or tools for promoting tissue function. This is particularly true of EVs originating from adipose-derived stem cells (ASC-EVs), which exhibit pro-angiogenic and anti-inflammatory effects, but have not yet been well explored in the context of AFG. Our current work investigates the effects of patient-derived ASC-EVs on cellular functions relevant to graft retention. Our initial findings indicate that ASC-EVs (from different donors) consistently promote tube formation in HUVEC models, modulate macrophages towards anti- inflammatory phenotypes, and do not promote the proliferation of breast cancer cells. We are also developing complex 3D multi-cellular models for testing the efficacy of ASC-EVs to promote graft retention in a system that is more reflective of the tissue microenvironment. The overall aim of this work is to develop ASC-EVs as an acellular therapeutic to improve AFG retention post-cancer treatment.

C27: Quantitative proteomics reveals cancer cell genotype can drive matrisomal changes associated with aggressive disease in pancreatic ductal adenocarcinoma (PDAC)

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Pancreatic ductal adenocarcinoma (PDAC) is highly lethal, with a five-year survival rate of ~9%. PDAC is characterised by robust stromal activation, leading to pro-tumourigenic extracellular matrix (ECM) deposition. Recently, we have shown that targeting desmoplasia can improve chemotherapy efficacy and impair metastasis in pre-clinical models[1-3]. As such, we aimed to use proteomics to dissect the matrisomal signatures of pancreatic tumours derived from the highly-metastatic KPC (Pdx1-Cre; LSL-K-rasG12D/+; LSL-p53R172H/+) and poorly-metastatic KP^{fl}C (Pdx1-Cre; LSL-K-rasG12D/+; LSL-p53fl/+) mouse models. We hypothesised that these tumours would have distinct matrisomes, with these changes revealing novel pro-metastatic matrisomal proteins involved in PDAC.

Pancreatic tissue from wildtype, KPC, and KP^{fl}C mice were collected at early (6 weeks), mid (10 weeks) and end-stage disease (12+ weeks) and were decellularised. Data-independent acquisition (DIA) liquid-chromatography tandem mass spectrometry (LC-MS/MS) was used to identify differentially abundant proteins.

LC-MS/MS demonstrated an increased abundance of Nidogen 2 (NID2) in KPC tumours at mid stage disease compared to KP^{fl}C. Interrogation of single cell-RNASeq murine and human PDAC datasets revealed that NID2 is enriched in PDAC specimens, especially at mid stage disease, mirroring our proteomics results. Immunofluorescence, western blotting, and qPCR show that NID2 is significantly enhanced in cancer-associated fibroblasts (CAFs) isolated from KPC tumours compared to KPC cancer cells (CCs). Furthermore, 3D organotypic matrices seeded with NID2-depleted CAFs had reduced desmoplasia, shown via second harmonic generation (SHG) imaging and Picrosirius-Red staining. In addition, 3D invasion assays revealed that depletion of CAF-derived NID2 impeded invasion of KPC and KPflC CCs in the presence of chemotherapy, compared to control. Furthermore, subcutaneous and orthotopic co-seeding experiments using NID2 knockdown KPC CAFs + KPC CCs show that reduced CAF-derived NID2 impedes tumour growth, invasion and metastasis *in vivo*.

1. Murphy K. et al., *Intravital imaging technology guides FAK-mediated priming in pancreatic cancer precision medicine according to Merlin status*, **2021**, (7), eabh0363
2. Vennin C. et al., *CAF hierarchy driven by pancreatic cancer cell p53-status creates a pro-metastatic and chemoresistant environment via perlecan.* Nature Communications, **2019**, 10 (1), 3637

3. Vennin C. et al., *Transient tissue priming via ROCK inhibition uncouples pancreatic cancer progression, sensitivity to chemotherapy, and metastasis*. Science Translational Medicine, **2017** (384), eaai8504

C28: An Old Drug with New Tricks; Examining the Anti-Cancer Potential of Metformin in EML4-ALK+ Non-Small Cell Lung Cancer.

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Crizotinib is an effective first-line therapy for the treatment of EML4-ALK+ lung cancer, however, resistance usually develops after one year. Epidemiological studies have shown that the hypoglycaemic agent, metformin, is associated with a reduced cancer incidence. Could metformin be used to improve the efficacy of crizotinib? Possibly; previous in vitro data has suggested that metformin provides synergistic benefit to crizotinib in lung cancer cells. But this is some distance from proof of concept in solid tumours. Therefore, this study aimed to examine if the in vitro synergism translated to an in vivo xenograft lung cancer model. In vitro mechanistic testing was also examined to determine metformin's action in ALK+ lung cancer cells.

Tumour-bearing Nu/J mice received daily administration of vehicle, metformin (100 mg/kg), crizotinib (25 mg/kg) or the combination, orally for 14 days (6-7/group). Tumour volume was measured daily. H3122 ALK+ lung cancer cells were treated with both drugs alone and in combination to study drug toxicity. Mitotracker dye with confocal microscopy was used to examine the mitochondrial membrane potential (MMP). Western blotting was used to study changes in AMPK and mTOR (known metformin targets).

Metformin, crizotinib and the combination decreased tumour volume compared to vehicle (612, 424 and 552 vs. 943 mm^3, respectively). However, the combination had no greater tumour suppression than crizotinib. All treatments also showed no toxicity in vivo, shown by low ALT and creatinine plasma levels. In vitro, metformin decreased mitotracker dye (suggesting an MMP reduction) but had no effect on AMPK nor mTOR.

Metformin was efficacious in an in vivo tumour model, but, provided no synergistic benefit in combination with crizotinib. The reduction in the MMP, but not AMPK or mTOR, suggests that metformin is disrupting the energy production in cancer cells. These findings warrant further examination of the value of metformin in cancer therapy.

C29: Evolving genomic complexity unveiled in ctDNA analysis of melanoma patients

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There is an increasing global focus on integrating liquid biopsy into routine cancer care, with many commercial tests now available. These minimally invasive blood plasma-based genomic tests identify tumour-derived DNA mutations in blood samples. They can provide information to patients and their clinicians about tumour growth, optimal treatment, treatment response, and tumour relapse after surgery, which can facilitate more informed clinical decisions. We have been developing affordable and sensitive liquid biopsies for the detection and surveillance of patients with advanced-stage melanoma, focusing on potential use in remote clinics to address health system inequities.

Circulating tumour (ct)DNA-derived mutations were identified in patients' plasma samples utilising a multi-platform approach, including next-generation sequencing and droplet digital PCR, and then mutations were tracked over time. The plasma ctDNA analysis detected complex tumour mutational patterns for many advanced melanoma patients, and here we will present the benefits and reflect on the challenges of such testing, especially in light of tumour heterogeneity. The successful implementation of liquid biopsy into cancer care in New Zealand is poised to make a significant difference to our patients in terms of improving equity of access and outcome, informing timely management, and optimising capacity-constrained care, however robust systems will be required to make this a reality.

C30: Genetic Variation as a Long-Distance Modulator of RAD21 Expression in Humans

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Mutations and changes in expression in cohesin component gene RAD21 are common across cancers types and developmental disorders. As such, exploration of genetic variants that modify RAD21 expression, across the genome, may also provide insights into mechanisms by which distant variants impact healthy human development and disease. We performed a genome-wide search of 42,953,834 SNPs for an association with the expression of RAD21. For all variants, spatial regulatory connections were identified through a confirmed spatial interaction (Hi-C data) and an association with transcript levels of RAD21 (expression Quantitative Trail Locus) using the CoDeS3D algorithm. We identified 123 significant associations (FDR < 0.05), which are local (cis) or long-distance (trans) regulators of RAD21 expression. The 123 variants co-regulate a further seven genes (AARD, AKAP11, GRID1, KCNIP4, RAD21, RCN1, TRIOBP, and USP32), enriched for having Sp2 transcription factor binding sites in their promoter regions. The Sp2 transcription factor and six of the seven genes have also been previously associated with cancer onset, progression, and metastasis. Our results suggest that genome-wide variation in non-coding regions impacts on a network of genes centred on RAD21 and seven other genes, with a potential impact on oncogenesis. This identification of distant co-regulation of oncogenes represents a strategy for discovery of novel genetic regions which impact cancer onset and a potential for diagnostics.

C31: A novel mechanism for APOBEC mutagenesis in carriers of the APOBEC3B deletion polymorphism

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APOBEC3A and APOBEC3B enzymes are a significant source of mutations in breast cancer. Regulation of mRNA transcripts that encode these mutagenic enzymes has not been well characterised. A genetic polymorphism that deletes the APOBEC3B gene has been associated with hypermutation in luminal type breast cancer. We hypothesised that the lncRNA APOBEC3B-AS1 is a functional transcript that may be involved in regulation of APOBEC3 genes.

APOBEC3B-AS1 homologues were found in great ape species, but not other closely related primates, indicating recent evolution of this transcript. Using single cell datasets derived from blastocysts, several splice variants of APOBEC3B-AS1 were detected, and the transcript sequence was found to extend further than the annotated region. This transcript was found to be expressed in pluripotent stem cells in this dataset. In another scRNAseq dataset derived from normal breast reduction samples, concurrent expression of APOBEC3B-AS1 and APOBEC3B in RANK+ luminal progenitor cells was observed. Using ChIP-seq data, cohesin and ESR1 binding sites were observed in the promoter regions of both APOBEC3B-AS1 and APOBEC3B.

The sequence conservation pattern of the region encoding APOBEC3B-AS1 suggests that there are functional elements in this region. Due to the associations with pluripotent stem cells and luminal progenitor cells, we hypothesise that APOBEC3B-AS1 expression enhances expression of APOBEC3 mRNAs in these cell types. We propose a model for APOBEC mutagenesis in breast cancer, and how the APOBEC3B deletion polymorphism enhances this mutagenesis. Based on this model, deletion of cohesin binding sites in carriers of this polymorphism could lead to aberrant chromatin looping, enhancing expression of the more catalytically efficient APOBEC3A enzyme in luminal progenitors. This drives APOBEC hypermutation in luminal breast tumours in carriers of the polymorphism. This model may have consequences for both breast cancer prevention and treatment using immunotherapies.

C32: Cell-specific DNA methylation patterns of leukocytes and their implication for epigenetic analyses of health and disease

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Distinct cell types can be identified by their DNA methylation patterns. Much research over the last decade has focused on DNA methylation changes that occur in cancer or biomarkers that utilise cell-free circulating DNA. However, there has been little research into the differential methylation patterns between leukocytes and other tissues as a detection tool for inflammation in various contexts, including cancer.

We have identified several loci that are fully methylated in leukocytes but virtually devoid of methylation in a range of other mesoderm, ectoderm, and endoderm derived tissues. We validated these biomarkers using amplicon-bisulphite-sequencing on saliva and *in vitro* mixing of peripheral blood mononuclear cells and intestinal organoid cells combined at a defined range of ratios. Interestingly, these methylation biomarkers have previously been identified as altered in various inflammatory diseases. Moreover, using TCGA datasets we show a strong, positive linear relationship between infiltrating leukocytes and DNA methylation levels at the HOXA3 locus in six cancer types. We hypothesise this is due to leukocyte infiltration rather than a feature of the diseased cells themselves.

Our data emphasise the importance of considering cellular composition when undertaking DNA methylation analysis and demonstrates the feasibility of developing new diagnostic tests to detect inflammation and immune cell infiltration in both cancerous and non-cancerous diseases.

C33: The phase I ENABLE trial: Third-generation chimeric antigen receptor (CAR) T-cells for refractory B-cell non-Hodgkin lymphoma

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Patient-derived T-lymphocytes genetically modified to express a chimeric antigen receptor (CAR) directed against the B-cell antigen CD19 have become a standard of care for refractory B-cell lymphoma treatment. However, only 30 – 40% of recipients have durable disease free remissions after commercial 'second-generation' (2G) CAR T-cell products. There is an need for CAR T-cell therapies offering improved long-term outcomes.

'Third-generation' (3G) CARs employ two intracellular costimulatory domains in sequence, and result in enhanced CAR T-cell proliferation and anti-tumour activity.[1] Alongside CD28, the Toll-like receptor (TLR) 2 Toll/interleukin-1 receptor (TIR) domain can serve as a second costimulatory domain within 3G CARs.[2] To assess safety and efficacy of the TLR2-based CAR costimulatory domain, we established clinical-grade lentiviral vector and 3G anti-CD19 CAR T-cell manufacture within our cellular therapy facility. In parallel, we developed and implemented policies and training for recognition and management of CAR T-cell toxicities at Wellington Hospital.

The phase I ENABLE trial (ClinicalTrials.gov NCT04045913) is an investigator-initiated first-in-human dose escalation study, and represents New Zealand's first clinical application of CAR T-cells. Eligible participants have relapsed or refractory B-cell non-Hodgkin lymphoma, lack curative options, and have satisfactory organ function. Primary endpoint is safety. Secondary endpoints include manufacturing feasibility, overall response rate, complete response rate, relapse-free survival and overall survival. Exploratory outcomes include CAR T-cell kinetics, phenotype and serum cytokine profile.[3] At abstract submission, 13 subjects with relapsed and refractory B-cell non-Hodgkin lymphomas have been enrolled, and eight treated with 3G anti-CD19 CAR T-cells. Preliminary safety, lymphoma response and pharmacokinetic outcomes for cohorts receiving 5 x 10^4/kg, 1 x 10^5/kg, 2 x 10^5/kg and 5 × 10^5 3G anti-CD19 CAR T-cells/kg will be presented.

The ENABLE trial demonstrates the feasibility of CAR T-cell manufacture and delivery within New Zealand, and provides a platform for future clinical CAR T-cell development and delivery.

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2. Weng, J., et al (2019). *A novel generation 1928zT2 CAR T cells induce remission in extramedullary relapse of acute lymphoblastic leukemia.* J Haematol Oncol. 12(1):117.
3. George, P., et al (2020). *Third-generation anti-CD19 chimeric antigen receptor T-cells incorporating a TLR2 domain for relapsed or refractory B-cell lymphoma: a phase I clinical trial protocol (ENABLE).* BMJ Open. 10:e034629.

C34: Altered control of interleukin-6 during macrophage polarisation may contribute to colorectal cancer progression

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The single nucleotide polymorphism (SNP) rs1800795 resides within the interleukin-6 (IL-6) gene promoter and alters IL6 transcription[1]. The SNP has reported associations for multiple pathologies, including infections, inflammatory diseases, and the risk of various cancers. Recently, we found that rs1800795 genotype also associates with disease recurrence in colorectal cancer (CRC) and so may have value as a prognostic biomarker in that malignancy as well as others. IL-6 is a driver of the acute phase immune response, but within cancers growing evidence suggests it is immunosuppressive despite the key contributing mechanisms being unclear[2]. We have found in various settings that rs1800795 alters transcriptional control of IL6 by tumour proteins p63 and p73 (p63/p73) and CCAAT-enhancer binding protein β (C/EBPβ). In macrophages, C/EBPβ is a central regulator of M1/M2-alternative polarisation, which in turn is known to influence immune suppression[3]. Using a macrophage cell line model, siRNA knockdown, overexpression and RNA-Seq, we found that p73 was differentially expressed during polarisation, induced IL6 expression and promoted the M1 state, whereas C/EBPβ promoted M2-alternative polarisation as described[3]. Moreover, the wider regulated transcriptional programmes of p73 and C/EBPβ were highly integrated, with gene ontologies relating to cytokine regulation, IL-12 pathway activity, γ interferon signalling, and cell cycle control. In this context, the genes encoding p73 and C/EBPβ appeared to be subject mutual bidirectional transcriptional regulation. Together, these data suggest that P73 and C/EBPβ are primary determinants of macrophage polarisation, highly integrated with opposing functions, and that the rs1800795 SNP may facilitate cancer progression and other pathologies via this mechanism.

1. Berkovic MC, Jokic M, Marout J, Radosevic S, Zjacic-Rotkvic V, Kapitanovic S. Experimental and molecular pathology. 2007;83(3):474-9.
2. Johnson DE, O'Keefe RA, Grandis JR. Nat Rev Clin Oncol. 2018;15(4):234-48.
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C35: A Murine Immunocompetent Acute Myeloid Leukemia (AML) Model for Testing Immunotherapies

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Syngeneic murine bone marrow transplantation leukaemia models (MBMTLM), which are established in immune competent mice, usually require radiation of the recipient mouse before leukaemic cells are transplanted. This radiation suppresses the immune system and makes it difficult to study how the immune system responds to leukaemia cells. We aimed to establish immunocompetent MBMTLMs to understand how the immune system responds to leukaemia cells expressing a highly immunogenic antigen, SIINFEKL, a peptide from ovalbumin.

We first established MBMTLMs models driven by the CALM/AF10 or MLL/AF9 fusion genes. Then leukaemia cells were transduced with a SIINFEKL expressing retrovirus. All primary MLL/AF9-SIINFEKL (n=3) and CALM/AF10-SIINFEKL (n=2) developed leukaemia with a latency of 22-42 days. SIINFEKL positivity of the leukaemic cells was 77.4$\pm$1.9% in the MLL/AF9-SIINFEKL mice and 99% in the CALM/AF10-SIINFEKL mice. These leukaemic cells were transplanted into irradiated and non-irradiated mice to establish secondary leukaemias. In the irradiated recipients, five out of six of the secondary MLL/AF9-SIINFEKL and all the secondary CALM/AF10-SIINFEKL mice (n=6) developed leukaemia within 21-40 days. In the non- irradiated recipients, only four of the eight MLL/AF9-SIINFEKL mice and five out of eight CALM/AF10-SIINFEKL mice developed leukaemia within 29-45 days. Flow cytometry showed that SIINFEKL was expressed on 79.7-99% of the leukaemic cells in irradiated recipients. In contrast, fewer than 3% of the leukaemic cells in non-irradiated mice with secondary leukaemia expressed SIINFEKL.

In conclusion, we have established a syngeneic murine AML model in immunocompetent mice and have evidence that an intact immune system has the ability to suppress or even eliminate rapidly proliferating AML cells if they express a strong antigen. The SIINFEKL was presented on the surface of leukaemic cells and presented via the murine MHC class I H-2K^{b} molecule. These models should be useful for developing immunotherapy strategies for AML.

C36: Promoters to drive CAR T cell persistence and activity

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Chimeric antigen receptor (CAR) T cell therapy is an effective treatment of lymphoma and leukaemias, with emerging potential for the treatment of solid tumours. However, sub-optimal persistence and migration of the CAR-T cell infusion can impact on treatment efficacy. Expression of additional genetic elements, encoding either polypeptides or non-coding RNA represent potential strategies to improve T cell activity and life-span, or alternatively to introduce inducible death- switches. Drug or auto-inducible promoters may be preferred over constitutive promoters due to safety concerns of increasing CAR T cell viability / activity. I will outline our current work to adapt constitutive, drug and auto-inducible promoters for the expression of accessory genes including miRNA, in CAR T cells. This includes a description of modifications to improve the Tet-on system within the Sleeping Beauty transposon system for CAR T cell applications.

C37: Exploring the role of cohesin mutation and Wnt signalling in Acute Myeloid Leukaemia.

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Cohesin is a multiprotein complex that has essential roles in 3-Dimensional (3D) organization of the genome. Mutations in the subunits of the cohesin complex have been identified 10-12% of myeloid leukaemia. The frequency is high at ~50% in the Down Syndrome-associated acute myeloid leukaemia subtype (DS-AML).

We found that cohesin mutations are synthetically lethal with Glycogen Synthase Kinase 3 (GSK3) inhibition. GSK3 is a multifactorial kinase involved in wide range of cellular process including functioning as an inhibitor of wnt signalling. In cohesin mutant cells we found that GSK3 inhibition enhanced stabilization of β-catenin indicative of greater Wnt agonism. Wnt responsive genes were more sensitized in cohesin mutant DS-AML cells.

Wnt agonism by GSK3 inhibition or via Wnt3a-ligand induced stimulation have been shown to enhance spatial contacts between RUNX1 and RUNX1T1, two gene involved in the AML associated translocation, RUNX1-RUNX1T1. Cohesin mutation tend to co-occur with RUNX1-RUNX1T1 translocation. It is unclear how GSK3 inhibition would impact the chromatin structure in cohesin mutant cells. To examine how cohesin mutation and GSK3 inhibition impact chromatin state we used Cut & Run to profile the binding of histone modification repressive mark H3K27me3 and active marks H3K27ac and H3K4me3 in isogenic cells DS-AML cells with and without cohesin mutation. Our result show that cohesin mutation leads to loss of insulation around the RUNX1 and RUNX1T1 genes. Wnt agonism leads to enhanced deposition of active histone marks H3K27ac and H3K4me3 at the enhancers and promoters of RUNX1 and RUNX1T1 in cohesin mutant cells. MicroC HiC chromatin confirmation assay in cohesin wild type and cohesin mutant cells with and without GSK3 inhibition shows that cohesin mutation and GSK3 inhibition alters the 3D-chromatin organization of AML cells.

Understanding how cohesin mutations co-operate with the Wnt pathway may provide insights into how cohesin mutation progress leukaemia.

C38: Epithelial sodium channel regulates breast cancer cell proliferation

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Breast cancer is the most common cancer affecting women worldwide with the majority of breast cancer-associated deaths occurring due to metastasis of cancer cells. Ion channels have recently been recognised as novel regulators of cancer cell proliferation and metastasis. The epithelial sodium channel, ENaC, made up of 〈, ® and © subunits is well known for its role in Na^+ reabsorption in epithelia, however novel roles for ENaC have also been described, including influencing cancer progression. A role for ENaC in breast cancer has not been explored. Here the effects of ENaC level and activity on breast cancer proliferation was investigated. Using the publicly available SCAN-B dataset associations between αENaC mRNA expression and breast cancer subtypes, proliferation markers and epithelial-mesenchymal markers (EMT) were studied. Using the MCF7, T47D, BT549 and MDAMB231 breast cancer cells, 〈ENaC levels were increased through overexpression or depleted through siRNA-mediated knockdown, and ENaC activity was inhibited by the ENaC-specific inhibitor amiloride. MTT and EdU cell proliferation assays were used to determine the effect of these changes on breast cancer cell metabolic activity and proliferation. High 〈ENaC mRNA expression was associated with less aggressive and less proliferative breast cancer subtypes and with lower expression of proliferation markers. Decreased 〈ENaC expression or activity, in the mesenchymal breast cancer cell lines BT549 and MDAMB231, increased breast cancer cell proliferation. In contrast, increased 〈ENaC expression decreased breast cancer cell proliferation. 〈ENaC expression was associated with poor prognosis in breast cancer. Our results identify ENaC as a novel regulator of breast cancer cell proliferation, and as a potential therapeutic target.

C39: The tumour suppressor protein p16 forms amyloid structures

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p16 is a tumour suppressor protein that inhibits cell division by blocking CDK4 and CDK6 and is therefore frequently mutated in cancers. We present here the unexpected finding that p16 can undergo a structural change into amyloid fibril structures.

Amyloids are stable fibrillar polymers with a beta-sheet based structure and have so far been mainly associated with neurological diseases such as amyloid-beta in Alzheimer's or alpha-synuclein in Parkinson's disease. The formation of amyloids involves the conversion of a monomeric, soluble protein into large aggregates. The onset of formation is assumed in many cases to be spontaneous and can depend on a large number of factors.

We discovered that the tumour suppressor p16 converts into amyloid structures strictly upon oxidation, by a mechanism that has not been previously observed. When oxidized, the single cysteine residue forms an intermolecular disulfide bond, leading to a metastable homo-dimeric species that subsequently folds into amyloids. The strict control on the onset of amyloid formation has so far not been described and we present a thorough investigation of the molecular details leading to this dramatic structural change. We provide evidence for this transition through multiple methods, including Thioflavin T and tryptophan fluorescence, transmission electron microscopy, nuclear magnetic resonance spectroscopy, mass spectrometry and others.

Our cell biological experiments suggest that the amyloid state is not capable of inhibiting CDK4 and CDK6, and therefore seems to resemble an inactive and possibly pathological state [1]. We are currently investigating this possible loss-of-function conversion through studying multiple cancer-related mutations and we and aim to understand their role in various cancers.

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C40: The Δ 133p53 isoform enhances cell-surface trafficking to promote metastasis

Polwatta, S.N.[1], Boix De Jesús, A.N.[1], Wang, D.[1], Taha, A.[3], Kazantseva, M.[1, 2], Zhou, J.[4,5], Mehta, S.[1, 2], Saraiva, A.M.[1], Lim, L.[4], Reddy, M.[4], Wilson, B.[4], Delvisco, S.[5], Royds, J.[1], Ziad, F.[6], Thotathil, Z.[6], Gan, P.Y.C.[6], Braithwaite, A.W.[1, 2], Hung N.A.[1,] Slatter, T.L.[1,2].

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An isoform of p53 called Δ133p53 promotes cancer. Δ133p53 is increased in aggressive primary tumours, and has properties that promote invasion. We investigated the role of Δ133p53 in metastases to the brain, and if metastasis was driven through a central mechanism that increased protein trafficking to the cell surface. In a cohort of brain tumour metastases, Δ133p53 was increased in the majority of brain metastases from the lung, breast, and skin compared to the matched primary tumours, and was associated with a reduced time for the primary tumour to metastasise to the brain compared to tumours with no Δ133p53. Using cell lines with and without Δ133p53, cancer promoting proteins were increased on the Δ133p53 cell surface and these showed increased trafficking to the cell surface upon stimulation. Administration of protein trafficking inhibitors to tumour syngraft models and in-vitro blood brain barriers reduced Δ133p53 tumour growth or cell invasion. In conclusion, Δ133p53 is associated with increased cancer promoting proteins on the cell surface and is increased in brain tumour metastases. Future directions are aimed at manipulating cell surface trafficking to target Δ133p53 metastases.

Summary of Abstracts for the Poster Session

No.	Title	Presenter	Institution
C15	Evaluating a size-based method for enriching circulating tumour cells from the blood of colorectal cancer patients	Sai Shyam Vasantharajan	University of Otago, Dunedin
C16	Rapid immunoprecipitation mass spectrometry of endogenous proteins supports a novel BCL6 function in glioblastoma	Anna Tribe	Victoria University of Wellington
C17	Universal CAR-T cell therapy using stimuli-responsive “Tags” and “Pro-tags”	Liang Kooi Kok	University of Otago, Dunedin
C18	Ex Vivo Culture of Patient-Derived Endometrial Cancer Tumour	Hannah van der Woude	University of Otago, Wellington
C19	Hyaluronic acid biology in Pancreatic Ductal Adenocarcinoma using Clinically Relevant In Vitro models	Hossein Jahedi	University of Auckland
C20	Reprogrammed antioxidant defences in metastatic melanoma cells	Therese Featherston	University of Otago, Christchurch
C21	ADSC-EVs and macrophage polarisation in fat grafting for breast reconstruction post-mastectomy	Emma Symonds	University of Otago, Wellington
C22	Characterisation of single cell transcriptomics in urinary cells for the detection of bladder cancer	Kit Moloney-Geany	University of Otago, Dunedin
C41	Untangling the interaction between Heterochromatin Protein 1α and G-quadruplexes	Ruby Roach	Massey University
C42	Bioinformatic analysis of Levonorgestrel® resistant endometrial cancer cell lines.	Molly Dore	University of Otago, Wellington
C43	Epigenomic profiling of colorectal cancer to predict patients at high risk of metastasis	Priyadarshana Ajithkumar	University of Otago, Dunedin
C44	A new role for peroxidasin in modulating the invasive potential of cancer cells	Martina Paumann-Page	University of Otago, Christchurch
C45	Kinetics of ascorbate uptake and retention in breast cancer cell lines	Citra Praditi	University of Otago, Christchurch

C46	Understanding and targeting epigenetic and transcriptomic signature of drug tolerance in lung cancer	Aby George	University of Otago, Dunedin
C47	Identification and Development of Novel Drug Combinations for Diffuse Gastric Cancer	Keiran Redpath	University of Otago, Dunedin
C48	Investigation of Covalent Warheads in the Design of 2-Aminopyrimidine-based FGFR4 Inhibitors	Raquel Ortega	University of Auckland
C49	Deleterious Effect of Inhibition of Aryl Hydrocarbon Receptor Signalling on Leukaemia Stem Cells in Murine Bone Marrow Transplantation Model of Acute Myeloid Leukaemia	Olena Oryschuk	University of Auckland
C50	Spatial lncRNA signatures in colorectal cancer	Holly Pinkney	University of Otago, Dunedin
C51	Association of Cancer-Associated Fibroblast Phenotype and Frequency with Two-Point Immunoscore in Colorectal Cancer Patients	Rory Costello	University of Otago, Dunedin
C52	Exploiting the intrinsic insolubility of T cell and NK cell-activating cytokines and chemokines for Escherichia coli expression	Lydia White	University of Otago, Dunedin
C53	Novel functional links between colorectal carcinogenesis and the microbiome	Jessica Permain	University of Otago, Christchurch
C54	Kinetics of Decitabine-induced and TET-induced Demethylation in Pluripotent Cells	Cassandra (Rosie) Glanfield	University of Otago, Dunedin
C55	Enhanced interactions between breast cancer cells and MStCs in the chemotherapeutic stress response	Helena Abolins-Thompson	Victoria University of Wellington
C56	LncRNA combination therapy in triple negative breast cancer	Kaitlyn Tippett	University of Otago, Dunedin

C57	The role of host genes and microbes in mechanisms of response to radiotherapy in rectal cancer	Adele Hegoburu	University of Otago, Christchurch
C58	Development of ctDNA methylation analysis for non-invasive colorectal cancer detection	Morgan Jones	University of Otago, Dunedin
C59	Investigation of long non-coding RNAs as potential tumor suppressors in triple-negative breast cancer	Joke Christin Grans	University of Otago, Dunedin
C60	Click-to-dissolve: Stimuli-responsive hydrogels for improved drug delivery	Parul Rani	University of Otago, Dunedin
C61	Innate immune checkpoint inhibitor resistance is associated with melanoma sub-types exhibiting invasive and de-differentiated gene expression signatures	Sultana Mehbuba Hossain	University of Otago, Dunedin
C62	Alpha-ENaC overexpression in MDAMB231 breast cancer cells reduces cell migration and proliferation	Sarah McQueen	University of Otago, Dunedin
C63	Growth Hormone Receptor Antagonism in Melanoma	Minah Kim	University of Auckland
C64	Early detection of colorectal cancer through analysis of ctDNA fragment sizes	Alice McAtamney	University of Otago, Dunedin
C65	Drug and auto-inducible promoters to improve CAR T cell efficacy and patient safety in solid tumour treatment	Sam Smith-Bell	University of Otago, Dunedin
C66	Exploring DNA demethylation in naïve stem cells and cancer	Claudia Davies	University of Otago, Dunedin
C67	Investigating the relative composition of tumour infiltrating immune cells across solid tumours.	Lucy Picard	University of Otago, Wellington

C68	A role for Δ133p53 isoforms in protein turnover at the cell surface	Alexandra Boix de Jesus	University of Otago, Dunedin
C69	The Δ133p53 p53 isoform in tumour metastasis	Sasini Polwatta	University of Otago, Dunedin
C70	PD-1 and PD-L1 inhibitors in combination with anti-oestrogen treatment in an immune competent mouse model of breast cancer.	Jody Hazlett	University of Otago, Dunedin
C71	Investigating DNA methylome and transcriptome profile in aggressive sub type of prostate cancer	Xintong Zhang	University of Otago, Dunedin
C72	The Genetic Validation of a Precursor Lesion of Epithelial Ovarian Cancer in FancD2 Knock-Out Mice	Sarah Sczelecki	Victoria University of Wellington
C73	Extracellular vesicle miRNAs as biomarkers of levonorgestrel resistant endometrial cancer	Emily Patterson	University of Otago, Wellington
C74	Expression of the prolactin receptor affects the response of breast cancer cell lines to growth hormone receptor antagonism	Chantal Buckley	University of Auckland
C75	DNA methylation panel as a biomarker for prostate cancer screening	Bayley Knofflock	University of Otago, Dunedin
C76	Interpretation of noncoding mutations driving melanoma susceptibility	Michael Pudjihartono	University of Auckland
C77	The Dark Matter of Cancer Relapse: Noncoding RNAs and Drug Tolerance in Lung Cancer	William Davis	University of Otago, Dunedin
C78	Assessment of single cell DNA methylation analysis in data in cancer	Hannah O'Neill	University of Otago, Dunedin
C79	Fatty acid-related vulnerabilities in CDH1-null cells	Emily Schulpen	University of Otago, Dunedin

C80	Unraveling the mechanisms involved in the co-regulation of breast cancer associated genes at the 6q25.1 locus	Amie Simonek	University of Otago, Dunedin
C81	Epigenetic regulation of prostate cancer metastasis to the bone.	Emma Wilkinson	University of Otago, Dunedin
C82	A New Syngeneic Mouse Model of Glioblastoma	Devlin Forsythe	Victoria University of Wellington
C83	Investigating the role of immunotherapy in the treatment of metastatic oestrogen receptor positive breast cancer	Devon Bull	University of Otago, Dunedin
C84	Combination of ALK and SHP2 inhibitors produce synergistic suppression of ALK-positive lung cancer cell growth	Maddie Berry	University of Otago, Dunedin
C85	Pinpointing and targeting novel drivers of pancreatic cancer progression, invasion and metastasis using TRAP-seq.	Michael Trpceski	Garvan Institute, Sydney
C86	Successful monitoring of estrogenic activity in postmenopausal breast cancer patients undergoing aromatase inhibitor therapy	Emma Sutherland	University of Otago, Dunedin
C87	Sleeping Beauty Transposon-Based Vector Kit-Sets for the Manufacture of Artificial Antigen Presenting Cells for CAR NK or T cell therapy	Lachie Dobson	University of Otago, Dunedin
C88	The Δ133p53 isoform regulates receptor tyrosine kinase AXL expression through epigenetic mechanisms	Marina Kazantseva	University of Otago, Dunedin
C89	Site-specific decreases in DNA methylation in replicating cells following exposure to oxidative stress	Annika Seddon	University of Otago, Christchurch
C90	Investigating the influence of hypoxia on cGAS-STING-IFN signalling in macrophages	Phoebe Burns	University of Auckland
C91	Improving read accuracy for ctDNA diagnostics	Sarah Hannah	University of Otago, Dunedin