QRW Cancer ORAL Abstracts
Garvan Institute Cancer Satellite Meeting
Sunday 26 August – Monday 27 August, 2018
Rydges Hotel, Queenstown, New Zealand

C1: Prostate Cancer Genomics – lessons from Africa

Jaratlerdsiri, W.[1], Gong T. [1], Blackburn J.[1], Chan E.K.F. [1], Bornman, M.S.R.[2], Hayes, V.M[1,2,3]

[1]Division of Genomics and Epigenetics, Garvan Institute of Medical Research, Darlinghurst, Sydney, Australia, [2]School of Health Systems and Public Health, University of Pretoria, Pretoria, South Africa, [3]Central Clinical School, Unbbiversity of Sydney, Camperdown, Sydney, Australia.

African American men are more likely to die from prostate cancer than any other racial group. The contribution of acquired genomic variation to this racial disparity is unknown, compounded by lack of genomic data for Africa. Here we performed the first deep tumour-normal paired whole-genome sequencing for prostate cancer in men from Africa. Comparing genome profiles for treatment-naïve high-risk African-derived tumours with tumour-matched European-Australians, we make novel African-specific observations. These include, identifying the most hyper-mutated prostate cancer genome reported to date (55 mutations/Mb). Excluding for this hyper-mutated tumour, our African-derived tumours showed a 2.2-fold increase in tumour mutational burden than the high-risk treatment-naïve Australian patients, a 4-fold increase than reported for intermediate- to low-risk prostate tumours, and 1.3-fold greater than that reported for the most hyper-variable European-derived prostate tumours reported to date, namely metastatic and castration resistant prostate cancer. Furthermore, we observed the frequency of oncogenic driver mutations to be increased overall (*P*-value = 2.92e-03), with roughly 30% of genes mutated novel to prostate cancer, while 79% of the recurrent predicted driver mutations appear early in tumourigenesis. Interestingly, complex genomic rearrangements were less frequent, with a notable absence of gene fusions, both common in European-derived tumours. Additional differences include, lack of any known oncogenic mutational signature, abundance of copy number gains later in tumourigenesis, and the identification of a uniquely hyper-duplicated genome. Our results, although preliminary, suggest that prostate cancer treatment in Africa would require a different strategy to current global practices.

C2: Precision cancer genomics for advanced childhood and rare adult cancers

John Grady[1], Marie Wong[1], Elektra Hajdu[2], Chelsea Mayoh[3], Emily Mould[3], Paulette Barahona[3], Mark Pinese[4], Velimir Gayevskiy[1], Amit Kumar[5], Subotheni Thavaneswaran[4], Dong Anh Khuong-Quang[6], Min Ru Qiu[7], Ken Doig[5], Chris McEvoy[5], Robyn Lukeis[7], Kelly Prendergast[3], Gary Pan[3], Emily Collignon[3], Lucille Sebastian[8], Katrin Sjoquist[8], Chee Lee[8], Andrew Fellowes[5], Amy Prawira[4,9], Anthony Joshua[4,9], Mandy Ballinger[4], Loretta Lau[3,10], Toby Trahair[3,10], Jamie Fletcher[3], Greg Arndt[3], Richard Lock[3], Vanessa Tyrrell[3], Marie Dziadek[11], Stephen Fox[5], David Ziegler[3,10], Marcel E Dinger[1,2,12], John S Mattick[11,12], Paul Ekert[6], Glenn Marshall[3,10], Michelle Haber[3], John Simes[8], David Thomas[4], Mark Cowley[1,2,12].

[1]Kinghorn Centre for Clinical Genomics, Garvan Institute of Medical Research, Sydney, Australia, [2]Genome Diagnostics, Garvan Institute of Medical Research, Sydney, Australia, [3]Children's Cancer Institute, Sydney, Australia, [4]Cancer Division, Garvan Institute of Medical Research, Sydney, Australia, [5]Peter MacCallum Cancer Centre, Melbourne, Australia, [6]Murdoch Children's Research Institute, Melbourne, Australia, [7]SydPath St Vincent's Pathology, Sydney, Australia, [8]NHMRC Clinical Trials Centre, University of Sydney, Sydney, Australia, [9]The Kinghorn Cancer Centre, Sydney, Australia, [10]Kid's Cancer Centre, Sydney Children's Hospital, Sydney, Australia, [11]Garvan Institute of Medical Research, Sydney, Australia, [12]St Vincent's Clinical School, UNSW Sydney, Sydney, Australia.

Patients with rare cancers (less than 5 in 100,000), including all paediatric cancers account for 30% of cancer deaths. Genome screening of rare tumours offers the opportunity to substantially improve patient care, though identifying targetable mutations linked to targeted therapies tested in other cancers, and assessing inherited cancer predisposition variants. In Australia, we are leading two national precision cancer genomics trials for adults with rare tumours, or children and adolescents with high-risk tumours. Each trial uses genomic screening in real-time to provide targeted treatment recommendations for patients. We have established molecular tumour boards, and the analytical (*refynr*, *Seave*), interpretational, and reporting frameworks (*gentian*) to make these trials feasible.

For adults with rare cancers, we have established the Molecular Screening and Therapeutics (MoST) program, where >700 patients that have exhausted all treatment options have been recruited in 21 months, and DNA + RNA is screened with the Illumina TST170 panel. For children with high-risk cancer, we have established the Lions Kids Cancer Genome Project (LKCGP), where we use deep whole genome sequencing (WGS) to comprehensively characterise each tumour (SNV, INDEL, CNV, SV). Tumours from >170 children enrolled on the Zero Childhood Cancer (ZCC) trial, are subject to additional molecular screening (targeted and RNA-Seq) *in vitro* drug screens, and *in vivo* patient-derived xenografts.

Despite each patient being enrolled onto these studies having exhausted all standard treatment options, we have identified at least one treatment recommendation in 50–58% of patients, and a significant germline risk allele at ~10%. These treatment recommendations differ in each tumour, and require a comprehensive approach that investigates SNV, INDEL, CNV, fusions, and tumour mutation burden.

Collectively the high rates of actionable variants identified in these rare cancers suggests an enormous potential for improving outcomes using existing targeted therapies developed for other cancers.

C3: Modern Drug Development in Medical Oncology

A Prawira
The Kinghorn Cancer Centre/St Vincent's Hospital Sydney, Garvan Institute of Medical Research

The past two decades have seen unprecedented development of novel therapies in oncology. Conventional drug development approaches that applied to cytotoxic drugs, therefore, are increasingly challenged. The life-threatening nature of advanced malignancies combined with increasing public awareness and interest, accentuate the need for improved drug availability and accessibility. This talk will discuss accelerating bench-to-bedside translation, modern/seamless clinical trial designs, and flexible approval pathways.

C4: Reflex genomic testing in the cancer clinic – time to make the switch in NZ?

Lawrence B.J.

Discipline of Oncology, University of Auckland, Auckland, NZ.

Department of Medical Oncology, Northern Regional Cancer and Blood Service, Auckland City Hospital, Auckland. NZ

After an initial period of exaggerated expectation, and then a period of inevitable disappointment, the role of genomic testing to guide therapeutic choice has found a more realistic footing in the cancer clinic. Choice of systemic therapy for metastatic disease increasingly relies on identification of predictive somatic gene mutations and copy number changes, now most often considered within the context of each primary tumour site. In NZ, the current approach to genotyping is highly variable between hospitals – but usually based on serial testing of several individual genes cobbled together across various platforms – either inferred from immunohistochemistry (e.g., mismatch repair proteins), taken from small mass-array assays (e.g., *KRAS*, *NRAS*, *EGFR*), and occasionally single gene PCR-based assays (e.g., *BRAF*). Internationally, some centres now offer reflex NGS panel or exome testing of multiple cancer-related genes, although clinical applicability varies by primary tumour site. There is a new need for detection of predictive germline mutations to predict response to some agents (e.g., PD1 inhibitors, platinum chemotherapy). This short discussion proposes that, from an oncologists point of view, it is time to switch to NGS testing of selected cancers to improve patient care. A secondary gain is the data and tools to enable the NZ research community to improve our understanding of cancer.

C5: Genomics of Familial Breast Cancers

Nones, K.*[1], Johnson, J.*[2], Newell, F.[1], Patch, AM.[1], Thorne, H.[3], Kazakoff, S.1, De Luca, X.[2], Srihari, S.[4], Ferguson, K.[2], Reid, L.[2], Reed, A.M.[2], Lakis, V.[1], Holmes, O.[1], Xu, Q.[1], Wood, S.[1], Leonard, C.[1], Parsons, M.[1], The Kathleen Cuningham Foundation Consortium for Research into Familial Aspects of Breast Cancer (kConFab)[3], Australian Breast Cancer Tissue Bank (ABCTB)[5], Brisbane Breast Bank (BBB)[2], Harris, J.[6], Degasperi, A.[7], Nik-Zainal, S.[7], Ragan, M.[4], Spurdle, A.[1], Khanna, K.K.[1], Lakhani, S.[2], Pearson, J.[1], Chenevix-Trench, G.[1], Waddell, N.*[1], Simpson, P.*[2].
[1]QIMR Berghofer Medical Research Institute; [2]UQ Centre for Clinical Research ; [3]kConFab, Peter MacCallum Cancer Centre; [4]Institute of Molecular Biosciences - The University of Queensland; [5]ABCTB, University of Sydney at the Westmead Institute for Medical Research. 6 QUT, School of Biomedical Science, [7] University of Cambridge, Scholl of Clinical Medicine.
*These authors contributed equally to the study.

Approximately 10-15% of breast cancers are associated with a strong family history. Inherited pathogenic variants in *BRCA1* or *BRCA2* genes account for around 20% of breast cancer families and other moderate to highly penetrant susceptibility genes (e.g. *TP53*, *ATM*, *CHEK2*, *PALB2* and *PTEN*) account for a smaller percent of familial breast cancers. However, for over 50% of breast cancer families the underlying genetic contribution to their risk cannot be attributed to pathogenic *BRCA1* or *BRCA2* variants (termed here as non-BRCA1/2) and remains unknown. This has a profound impact for how these families are managed in the clinic. To better understand the aetiology of familial breast cancer we performed whole genome analysis of familial cases with *BRCA1* (n=26) or *BRCA2* (n=22) pathogenic variants together with non-BRCA1/2 (n=32) cases. Biospecimens and clinical data were obtained from kConFab, the Brisbane Breast Bank and the Australian Breast Cancer Tissue Bank. Whole genome sequencing (WGS) was performed for 80 matched tumour/normal pairs (60x and 30x coverage, respectively). Whole genome methylation arrays were analysed for 67 tumours. The complete spectrum of somatic and germline of mutations were evaluated, including SNPs, indels, copy number changes and structural rearrangements, and mutational signatures. Our aim was to identify potential mutations or mechanisms underlying familial non-BRCA1/2 breast cancers. *BRCA1*, *BRCA2* and non-BRCA1/2 tumours exhibited a different burden of mutations, different spectrum of mutational signatures and different telomere length. Somatic genomic features revealed fascinating insights into tumour development with 15% of tumours being re-classified from their original clinical diagnosis. Our results show how whole genome sequencing of both germline and tumour DNA can improve current genetic testing and clinical diagnoses of breast cancer families.

C6: A role for the Imprinted and Ancient gene in survival of cancer cells during tryptophan deprivation

Tomek, P.[1], Potts, C.L.[1], Print, C.G.[2], Black, M.A.[3], Gore, S.K.[1], Ching, L.[1]

[1]Auckland Cancer Society Research Centre, University of Auckland, Auckland, NZ, [2]Department of Molecular Medicine & Pathology, University of Auckland, Auckland, NZ [3]Department of Biochemistry, University of Otago, Dunedin, NZ

Cancers express indoleamine 2,3-dioxygenase 1 (IDO1) to undermine the host's antitumour immunity. Tryptophan depletion and the accumulation of a tryptophan metabolite kynurenine caused by IDO1, inactivate the cancer-killing immune cells, yet cancer cells in the same microenvironment appear not to be affected. Compensatory mechanisms of cancer cells are poorly understood. We hypothesise that cancer cells co-opt the Imprinted and Ancient gene (IMPACT) which has previously been shown to render human skin cells more resistant to IDO1-mediated tryptophan degradation.

To find supportive evidence for this hypothesis, we first explored genomic data of 28 human cancer types included in The Cancer Genome Atlas. *IMPACT* was found to be upregulated and frequently amplified in the majority of cancer types examined relative to their matching non-cancer tissues from the Genotype Tissue Expression portal. In a subset of cancer types expressing elevated *IDO1* levels, high *IMPACT* expression associated with decreased activity of stress response pathways and with increased activity of translational regulation such as mTOR pathway. This observation is consistent with IMPACT ameliorating stress response potentially induced by IDO1-mediated tryptophan catabolism. Subsequent laboratory studies demonstrated that GL261 mouse glioma cells engineered to overexpress IMPACT gained a survival advantage over their wild-type and IMPACT-knockout counterparts when cultured in low tryptophan (2 to 10 μM) media for 4 days. High kynurenine levels (up to 100 μM) did not significantly affect proliferation and viability of GL261-wild-type cells. After 4 days of incubation in low tryptophan (2 μM) medium, many IMPACT-overexpressing GL261 cells but not their wild-type or knockout counterparts markedly enlarged their nuclei and cytoplasmic area. This cell enlargement could be part of a yet uncharacterised adaptation of cancer cells to low tryptophan levels.

The laboratory and bioinformatics data presented herein support the hypothesis that IMPACT mediates a resistance mechanism allowing cancer cells to withstand tryptophan deficiency.

C7: Functional genomic screens for mechanisms of resistance to trastuzumab emtansine

Siemens, K.N[1], Khan, A.[1], Lipert, B.[1], Tsai, P.[2], Kakadia, P.N.[2], Dixon-McIver, A.[3], Ng, D.[3], Jamieson, S.M.F.[1,4,5], Bohlander, S.K.[2,4], Print, C.G.[2,4], Wilson, W.R.[1,4], Wilson, S.[6], Harvey, V.J.[7] and Hunter, F.W.[1,4]

[1]Auckland Cancer Society Research Centre, University of Auckland, Auckland, New Zealand, [2]Department of Molecular Medicine and Pathology, University of Auckland, Auckland, New Zealand, [3]IGENZ, Auckland, New Zealand, [4]Maurice Wilkins Centre for Molecular Biodiscovery, New Zealand, [5]Department of Pharmacology and Clinical Pharmacology, University of Auckland, Auckland, New Zealand, [6]Department of Medical Oncology, Auckland City Hospital, Auckland, New Zealand, [7]Department of Oncology, University of Auckland, Auckland, New Zealand

Amplification of the *HER2* oncogene results in breast cancers with an aggressive clinical course. Therapeutic agents targeting HER2 provide objective responses in some patients and have improved survival. However, many tumours are refractory to HER2 blockade or acquire resistance during treatment. Mechanisms of resistance to these agents are poorly understood, particularly for the antibody-drug conjugate trastuzumab emtansine (T-DM1), which is comprised of the potent microtubule inhibitor mertansine (DM1) conjugated to trastuzumab.

We utilised CRISPR-mediated functional genomic screens integrated with RNA sequencing to identify genetic determinants of resistance to T-DM1 in breast cancer. A panel of human breast cancer cell lines showing varying patterns of sensitivity to the HER2 blockade was studied. *HER2* amplification and protein expression were assessed by FISH and immunoblotting, respectively. Drug sensitivity was characterised by CellTiter-Glo and clonogenic cell survival assays. *Streptococcus pyogenes* Cas9 and the whole-genome single guide RNA libraries (GeCKOv2) were stably transduced using lentiviral vectors. The resulting genome-scale knockout libraries were functionally validated using 6-thioguanine, for which resistance-conferring mutations are known. Library complexity and treatment-induced sgRNA enrichment were assessed by next-generation sequencing (Illumina NextSeq500). Saturation-scale knockout libraries were used in screens with T-DM1 and free DM1, with selected drug-resistant lines also characterised by RNAseq.

The cell lines displayed patterns of sensitivity to T-DM1, trastuzumab, lapatinib and neratinib reflective of clinical response rates. Genome-scale knockout libraries showed revealed enrichment of mutations in the 6-thioguanine sensitivity gene *HPRT1* following drug challenge. MDA-MB-453 and MDA-MB-361 libraries showed resistance to T-DM1 and DM1 after eight and thirteen weeks of escalating drug treatment, respectively. Deep sequencing of sgRNA and mRNA revealed a number of genes, including *SLC46A3*, *KEAP1*, *TRIAP1*, *ERLIN2*, and *CUL3* – in addition to complex transcriptional networks - that are potential regulators of T-DM1 sensitivity. The predictive utility of these gene sets is being explored in clinical datasets.

C8: Synergistic effects of combinations of existing drugs to treat melanoma

Khanh Tra, Cris Print and Peter Shepherd
University of Auckland and Maurice Wilkins centre

This presentation will describe studies that use combinations of widely used existing drugs that when used together achieve surprising synergistic effects on melanoma. This is only seen in syngeneic and xenograft models and not in melanoma cells in culture. This suggests these combinations work by co-ordinating effects in both the tumour cells and the microenvironment cells. Studies using IHC and species specific RNA seq to investigate this possibility will be presented.

C9 *BRCA1* mutation stabilises cyclin E1 in breast cancer to create a therapeutically targetable subset

Diar Aziz,[1] Christine S.L. Lee[2], Kristine Fernandez[2], Sarah Alexandrou[2], Niantao Deng[2], Neil Portman[2], C. Marcelo Sergio[2], Dariush Etemadmoghadam[3], kConFab Investigators, Elgene Lim[2,4], David Bowtell[3], Paul Waring[1], C. Elizabeth Caldon[2,4]

1. Centre for Translational Pathology, Department of Pathology, University of Melbourne, Parkville, Victoria 3010, Australia.
2. The Kinghorn Cancer Centre, Garvan Institute of Medical Research, Sydney, NSW 2010, Australia.
3. Peter MacCallum Cancer Institute, Victorian Comprehensive Cancer Centre, Parkville, Victoria 3010, Australia.
4. St. Vincent's Clinical School, Faculty of Medicine, UNSW Sydney, NSW 2052, Australia.

Basal like breast cancers (BLBC) are a highly aggressive subset of breast cancers that respond poorly to available targeted therapies and chemotherapies. In order to define therapeutically targetable subsets of BLBC we examined the co-occurrence of two markers that are associated with BLBC: cyclin E1 and *BRCA1*/2 loss. In high grade serous ovarian cancer (HGSOC) these markers are common, but mutually exclusive, and can be used to define therapeutic subsets. We tested the same hypothesis for BLBC.
Using a patient cohort enriched for BLBC cancers and *BRCA1*/2 loss, we identified that, unlike in HGSOC, there is convergence between *BRCA1* loss and high expression of cyclin E1. This was only rarely associated with 19q12 gene amplification to increase *CCNE1* expression. Instead we found that *BRCA1* loss stabilises cyclin E1 during the cell cycle. Increased cyclin E1 was associated with decreased phosphorylation on cyclin E1 Threonine T62 in the patient cohort. Using siRNA we show that *BRCA1* loss leads to cell cycle specific stabilisation of cyclin E1 by reducing cyclin E1 T62 phosphorylation, and conversely the overexpression of *BRCA1* increases T62 phosphorylation. Mutation of the cyclin E1 T62 site to alanine increased cyclin E1 stability and cell proliferation, and in combination with other mutations led to increased survival in Paclitaxel. Since cyclin E1/Cdk2 can protect from DNA damage and cyclin E1 is elevated in *BRCA1* null cancers, we hypothesised that CDK2 inhibition would make these cancers highly sensitive to PARP inhibition. CDK2 inhibition induced DNA damage and synergised with the PARP inhibitor Rucaparib in *BRCA1* mutated cell lines. Treatment of a Brca1 null/cyclin E1 PDX model with combination Olaparib and CDK2 inhibition severely inhibited tumour growth. We conclude that *BRCA1* status and high cyclin E1 have potential as predictive biomarkers to dictate the therapeutic use of combination CDK inhibitors/PARP inhibitors in BLBC.

C10: Identifying novel treatment options for women with aggressive, therapy-resistant ovarian carcinosarcoma

Holly Barker[1], Gwo Yaw Ho[1,2,3], Elizabeth Kyran[1], Kristy Shield-Artin[1], Cassandra Vandenberg[1], Justin Bedo[1], Jan Pyman[2], John Weroha[4], Matthew Wakefield[1], Ronny Drapkin[5], Clare Scott[1,3]

[1]Walter and Eliza Hall Institute of Medical Research, [2]Royal Women's Hospital, Melbourne, [3]Peter MacCallum Cancer Centre, [4]Mayo Clinic, USA, [5]University of Pennsylvania, USA

Ovarian carcinosarcoma (OCS) is the most lethal gynaecological cancer. Evidence-based treatment is limited to first-line platinum-based chemotherapy. Most patients develop lethal relapsed disease within one year of treatment, therefore, identifying new therapeutic strategies is vital. OCS are heterogeneous, with both epithelial (carcinoma) and mesenchymal (sarcoma) components proposed to exist in a state of stable epithelial-to-mesenchymal transition (EMT). We have developed preclinical models to identify new treatment options for OCS, including assessing the efficacy of the novel chemotherapeutic, eribulin (Halaven®), previously shown to reverse EMT.

We have generated a genetically-engineered mouse model (GEMM) of OCS, driven by overexpression of LIN28B and inactivation of p53 in fallopian tube (FT) secretory epithelial cells. Tumours were found to express the FT marker PAX8, as well as the EMT marker HMGA2, which is over-expressed in 60% of human OCS tumours, indicating this model is representative of the human disease. *In vivo*, GEMM tumours were resistant to the current standard-of-care therapy cisplatin, as is seen in the clinic. However, eribulin demonstrated impressive anti-tumour activity in these highly therapy-resistant tumours. We have six OCS patient-derived xenograft (PDX) models, which were also found to express PAX8 and high levels of HMGA2, and contain varying ratios of carcinoma and sarcoma. A range of sensitivities to eribulin was observed in these PDX models, with the highly carcinomatous model being the most sensitive, but all being resistant to cisplatin. We have also generated a cell line from the GEMM, which was found to display reduced branching, adhesion and invasion following treatment with eribulin. Using our unique toolbox of preclinical models we have made the exciting discovery that OCS is sensitive to eribulin. Furthermore, we demonstrated that eribulin exerts its effects through reversing characteristics of EMT, suggesting similar EMT-targeting drugs may demonstrate efficacy in the treatment of OCS.

C11: Chromatin disruption and abnormal cell signalling in cohesin-mutant leukaemia

Jisha Antony[1,2], Greg Giminez[1] and Julia A. Horsfield[1,2]

[1]Department of Pathology, Dunedin School of Medicine, University of Otago, New Zealand
[2]Maurice Wilkins Centre for Molecular Biodiscovery, New Zealand

Acute myeloid leukaemia (AML) is an aggressive cancer of the bone marrow with an overall survival of ~30%. Outcomes for older patients are especially poor, with cure rates of only 10-15%. Our fundamental understanding of how leukaemia develops remains incomplete, and little is known about the founding mutations of AML. Treatment for AML has not changed substantially in more than 30 years. While exciting progress has been made in the development of drugs targeting recently identified mutations, it is unlikely any single-agent drug will cure the disease.

Over the last 5 years, exome and genome sequencing of AML samples revealed a substantial number of new mutations, including those in subunits of the cohesin complex. The rate of cohesin mutations is around 12-15%, but at least a further 15% of AMLs are additionally affected by downregulation of cohesin gene expression and copy number variations. We hypothesise that cohesin mutation leads to disruption of chromatin organisation, predisposing cells to respond abnormally to cell signals such as inflammation. Our recent research used CRISPR-CAS9 engineered cohesin-mutant leukaemia cells to interpret how cohesin mutant cells respond to cell signalling events, in the context of dysregulated chromatin organisation. We found that dramatic changes in chromatin accessibility and gene transcription coincide with altered differentiation and abnormal response to cell signalling in cohesin-mutant leukaemia cells. Our findings have implications for transition to malignancy and design of AML therapy.

C12: Systematic Functional Identification of Cancer Drug Resistance Genes

Lau, M.T.[1], Ghazanfar, S.[2], Yang, J.Y.H.[2], Pajic, M.[3], Neely, G.G.[1]

[1]The Dr. John and Anne Chong Lab for Functional Genomics, Charles Perkins Centre and School of Life & Environmental Sciences, The University of Sydney NSW 2006, Australia

[2]School of Mathematics and Statistics, The University of Sydney, Sydney, Australia

[3]Garvan Institute of Medical Research, The Kinghorn Cancer Centre, 370 Victoria Street, Darlinghurst, NSW 2010, Australia

Cancer drug resistance is a major obstacle in cancer therapy. To elucidate the genetic factors that regulate sensitivity to anti-cancer drugs, we performed whole genome CRISPR/Cas9 knockout screens for resistance to a spectrum of anti-cancer drugs of varying compositions and general or targeted mechanisms of action. In addition to known targets and resistance mechanisms, this study revealed novel insights into drug mechanisms of action, including cellular transporters, drug target effectors, and genes involved in target-relevant pathways. Anti-cancer drugs could be classified based on resistance mechanisms, and we provide the first functional "phylogenecity" for these compounds. Importantly, we identified 49 multi-drug resistance genes, including a previously uncharacterised gene we have named *Required for Drug-induced Death 1; RDD1*. Loss of *RDD1* resulted in resistance to five anti-cancer drugs, primarily anti-tubulin agents, and *RDD1* is required for an anti-tubulin drug to trigger MCL-1 degradation and cell death. Loss of *RDD1* also conferred resistance to anti-tubulin therapy in a mouse xenograft model, and clinically, low *RDD1* expression was associated with poor prognosis in multiple cancer cohorts, with the strongest association for ovarian cancer patient outcome. Together, we provide the first functional landscape of resistance mechanisms to a broad range of chemotherapeutic drugs and reveal new multi-drug resistance nodes. This information can guide personalised anti-cancer therapies or instruct rational drug combinations designed to minimise acquisition of resistance.

C13: Intravital imaging to monitor therapeutic response in moving hypoxic regions resistant to PI3K pathway targeting in pancreatic cancer

James R.W. Conway,[1] Sean C. Warren,[1] David Herrmann,[1,2] Kendelle J. Murphy,[1] Aurelie S. Cazet,[1] Claire Vennin,[1] Robert F. Shearer,[1] Monica J. Killen,[1] Astrid Magenau,[1,2] Pauline Melenec,[1] Mark Pinese,[1,2] Max Nobis,[1] Anaiis Zaratzian,[1] Alice Boulghourjian,[1] Andrew M. Da Silva,[1] Gonzalo del Monte-Nieto,[2,3] Arne S.A. Adam,[3] Richard P. Harvey,[2,3,4] Jody J. Haigh,[5] Yingxiao Wang,[6] David R. Croucher,[1,2,7] Owen J. Sansom,[8,9] Marina Pajic,[1,2] C. Elizabeth Caldon,[1,2] Jennifer P. Morton,[8,9], and Paul Timpson[1,2,10]

[1]Garvan Institute of Medical Research and The Kinghorn Cancer Centre, Cancer Division, Sydney, NSW 2010, Australia
[2]St Vincent's Clinical School, Faculty of Medicine, University of NSW, Sydney, NSW 2010, Australia
[3]Developmental and Stem Cell Biology Division, Victor Chang Cardiac Research Institute, Sydney, NSW 2010, Australia
[4]School of Biotechnology and Biomolecular Science, University of New South Wales, Sydney, NSW 2033, Australia
[5]Australian Centre for Blood Diseases, Monash University, Melbourne, VIC 3004, Australia
[6]Department of Bioengineering, University of Illinois, Urbana-Champaign, Urbana, IL 61801, USA
[7]School of Medicine and Medical Science, University College Dublin, Belfield, Dublin 4, Ireland
[8]Cancer Research UK Beatson Institute, Switchback Road, Bearsden, Glasgow G61 1BD, UK
[9]Institute of Cancer Sciences, University of Glasgow, Glasgow G61 1QH, UK 10Lead Contact

Application of novel intravital imaging facilitates dynamic monitoring of pathway activity upon therapeutic inhibition. Here we assess resistance to therapeutic inhibition of the PI3K pathway within the hypoxic microenvironment of pancreatic ductal adenocarcinoma (PDAC) identify a phenomenon whereby pronounced hypoxia-induced resistance is observed for three clinically relevant inhibitors. To address this clinical problem, we have mapped tumour hypoxia by both immunofluorescence and phosphorescence lifetime imaging of oxygen-sensitive nanoparticles anddemonstrate that these hypoxic regions move transiently around the tumour. To overlay this microenvironmental information with drug response, we applied a FRET biosensor for Akt activity, which is a key effector of the PI3K pathway. Performing dual intravital imaging of drug response in different tumour compartmentxs, we demonstrate an improved drug response to a novel combination therapy using the dual mTORC1/2 inhibitor AZD2014 with the hypoxia-activated pro-drug TH-302.

C14: MASTL kinase promotes chromosome instability and metastasis in breast cancer

Samuel Rogers[1,2], Rachael A. McCloy[1], Benjamin L. Parker[3#], David Gallego-Ortega[1,4#], Andrew M.K. Law[1], Venessa T. Chin[1], James R.W. Conway[1], Dirk Fey[4], Ewan K.A. Millar[6,7,8], Sandra O'Toole[9,10], Niantao Deng[1,5], Alexander Swarbrick[1,5], Paul D. Chastain [11], Anthony J. Cesare [2], Paul Timpson[1,5], C. Elizabeth Caldon[1,5], David R. Croucher[1,5,12], David E. James [3,13], D. Neil Watkins[1,5,14], and Andrew Burgess[15*].

[1]The Kinghorn Cancer Centre, Garvan Institute of Medical Research, Darlinghurst, NSW, 2010, Australia. [2]Children's Medical Research Institute, The University of Sydney, Westmead, Australia. [3]Charles Perkins Centre, School of Life and Environmental Sciences, The University of Sydney, Sydney, NSW 2006, Australia. [4]Systems Biology Ireland, University College Dublin, Dublin 4, Ireland. [5]St. Vincent's Clinical School, Faculty of Medicine, UNSW, Darlinghurst, NSW, Australia. [6]NSW Health Pathology, Department of Anatomical Pathology, St George Hospital, Kogarah, Sydney, New South Wales 2217, Australia. [7]School of Medical Sciences, UNSW Sydney, Kensington NSW 2033, Australia. [8]
School of Medicine and Health Sciences, Sydney Western University, Campbelltown, NSW 2560, Australia.[9]Sydney Medical School, Sydney University, Fisher Rd, Camperdown, NSW 2006, Australia.[10]Department of Tissue, Pathology and Diagnostic Oncology, Royal Prince Alfred Hospital, Missenden Rd, Camperdown 2050, NSW, Australia. [11]College of Medicine at Rockford Department of Health Sciences Education, University of Illinois, Rockford, Ill, 61107. [12]School of Medicine and Medical Science, University College Dublin, Belfield, Dublin 4, Ireland. [13]School of Medicine, The University of Sydney, Sydney, NSW 2006, Australia. [14]Department of Thoracic Medicine, St Vincent's Hospital, Darlinghurst, NSW, 2010. [15]ANZAC Research Institute, University of Sydney, Sydney, NSW 2139

MASTL kinase is essential for correct progression through mitosis, with loss of MASTL causing chromosome segregation errors, mitotic collapse and failure of cytokinesis. However, in cancer MASTL is most commonly amplified and overexpressed. This correlates with increased chromosome instability in breast cancer and poor patient survival in breast, ovarian and lung cancer. Global phosphoproteomic analysis of immortalised breast MCF10A cells engineered to overexpressed MASTL revealed disruption to desmosomes, actin cytoskeleton, PI3K/AKT/mTOR and p38 stress kinase signalling pathways. Notably, these pathways were also disrupted in patient samples that overexpress MASTL. In MCF10A cells, these alterations corresponded with a loss of contact inhibition and partial epithelial–mesenchymal transition, which disrupted migration and allowed cells to proliferate uncontrollably in 3D culture. Furthermore, MASTL overexpression increased aberrant mitotic divisions resulting in increased micronuclei formation. Mathematical modelling indicated that this delay was due to continued inhibition of PP2A-B55, which delayed timely mitotic exit. This corresponded with an increase in DNA damage and delayed transit through interphase. There were no significant alterations to replication kinetics upon MASTL overexpression, however, inhibition of p38 kinase rescued the interphase delay, suggesting the delay was a G2 DNA damage checkpoint response. Importantly, knockdown of MASTL, reduced cell proliferation, prevented invasion and metastasis of MDA-MB-231 breast cancer cells both in vitro and in vivo, indicating the potential of future therapies that target MASTL. Taken together, these results suggest that MASTL overexpression contributes to chromosome instability and metastasis, thereby decreasing breast cancer patient survival.

Cancer Plenary: Telomere Biology in Human Healthspan and Disease

Blackburn, E.H
University of California San Francisco USA, San Francisco California, United States

Accumulated data indicate that loss of telomere protection (often measured in the surrogate form of bulk shortening of telomeres) is both linked to and, in some cases, causally contributes to, human aging and aging-related diseases. These include cardiovascular disease, stroke, osteoporosis, inflammatory diseases, dementia, diabetes and certain cancers.

While new cancer treatments are imperative, telomere biology is highly relevant to the emerging importance of cancer prevention and interception. However, it is too simplistic and even dangerous to suggest that extending telomere length will invariably extend healthspan. Disease mechanisms are interconnected and manipulating one mechanism has the potential to positively or negatively impact other mechanisms. Telomerase is highly active in many human malignancies, and a potential target for anti-cancer stratagems. But conversely, genetic deficiency of telomerase causes certain hemopoietic, skin, and gastrointestinal cancers. Hence human telomerase activity and telomere maintenance present delicate balancing acts throughout human life. Further understanding about their control via molecular and environmental switches are anticipated to lead to applications in disease treatment and interception

1. Blackburn E.H., Epel E.S., Lin J. (2015). *Human telomere biology: A contributory and interactive factor in aging, disease risks, and protection.* Science 350: 1193-1198

C15 Targeting growth hormone signalling in cancer

Dr Jo Perry
Liggins Institute, University of Auckland, New Zealand

Human growth hormone (GH) is a classical pituitary endocrine hormone that is essential for normal postnatal growth and has pleiotropic effects across multiple physiological systems. GH is also expressed in extra-pituitary tissues and has localised autocrine/paracrine effects at these sites. Localised expression of GH is detectable in a variety of different human cancers, including breast and endometrial cancer, and this is associated with poor survival outcomes for patients. Results obtained from preclinical studies in different cancer models have been promising and provide evidence in favour of further testing the hypothesis that GH receptor antagonism could be effective in treating tumours that are GH or insulin like growth factor-1 (IGF-1) dependent. Consistent with this we have demonstrated that a clinical GH receptor inhibitor suppresses endometrial xenograft regrowth after radiotherapy. However, preclinical studies investigating the therapeutic potential of GH receptor antagonism have been limited due to difficulty accessing therapeutic tools to study the pharmacology of the receptor *in vivo*. Our progress towards developing novel GH receptor antagonists will be discussed.

C16: Novel role for CBFβ as a regulator of breast cancer phenotype, progression and metastasis

A-M. Money[1], P. Aubel[1], E. Neist[1], T. Owens[1], I. Taniuchi[2], C.J. Ormandy[3], P.T. Simpson[4], M.J. Naylor[1,3,*]

[1]School of Medical Sciences, University of Sydney, NSW, Australia. [2]RIKEN Center for Integrative Medical Sciences, Yokohama City, Kanagawa, Japan. [3]Cancer Research Program, Garvan Institute of Medical Research, Darlinghurst, NSW, Australia. [4]Centre for Clinical Research, University of Queensland, Herston, Queensland, Australia.

Regulators of mammary cell fate play a critical role in the development, phenotype and progression of breast cancer. Whilst we and others have previously demonstrated a role for Runx2 as a driver of mammary basal cell and breast cancer phenotype[1], little is known about the potential role of its heterodimer binding partner CBFβ in these processes. Using both in vitro and in vivo preclinical breast cancer models, along with clinical assessment of patient cohorts, we now demonstrate a novel role for CBFβ as a key driver of human breast cancer. Forced CBFβ expression enhanced luminal breast cancer proliferation and migration while CBFβ deletion in basal breast cancers inhibited these phenotypes. During development mammary specific deletion of CBFβ impaired mammary cell proliferation, but unlike Runx2 deletion did not affect lobuloalveolar development. In a luminal breast cancer model mammary specific deletion of CBFβ inhibited breast cancer progression and metastasis to the lungs. In patient cohorts high CBFβ levels were associated with aggressive disease and poor patient prognosis. Together these data point to a novel and untested role for CBFβ as a potential therapeutic target for the treatment of aggressive breast cancer.

[1] Owens et al., (2014) *Runx2 is a novel regulator of mammary epithelial cell fate in development and breast cancer.* Cancer Research 74(18):5277-86.

C17: Can the wild type p53 tumour suppressor cause cancer?

Antony W. Braithwaite
Department of Pathology, Dunedin School of Medicine, and Maurice Wilkins Centre, University of Otago, Dunedin, New Zealand

Before p53 became famous as a tumour suppressor it was regarded as an oncogene. However, by the 1990s when it was clearly established that p53 is a tumour suppressor, much of the early work was dismissed as being due to mutant p53. However, not all the data can be dismissed so easily. Furthermore, with the discovery of p53 isoforms that appear to have transforming ability, the question of p53' s contribution to cancer has again arisen.
We created a transgenic mouse model of the 133p53 isoform (designated 122p53) mice). These mice are tumour prone, but unlike other p53 mutant mice, 122p53 mice show widespread inflammation and elevated levels of pro-inflammatory serum cytokines, notably IL-6 [1]. IL-6 was shown to be important by crossing Δ122p53 mice with *IL-6* deficient mice. Loss of *IL-6* reduced tumour incidence, metastasis and the levels of cytokines in the JAK-STAT3 pathway and that blocking this pathway prevented cell migration and invasion [2]. The studies on these mice led us to explore a role for 133p53 isoform in human cancers. We show that prostate cancers with substantially elevated levels of *133TP53* mRNA grow faster, have a high immune cell content and are more aggressive. We find a similar pattern in a subset of glioblastomas [3]. Moreover, elevated isoform expression was found to only occur in tumours with a wild type p53 gene. These studies provide strong evidence that 133p53 isoform can function as an oncogene, suggesting that wild type p53 can contribute to oncogenesis.

1. Slatter, T.J., Hung, N.A., Campbell, H.C., *et al*. (2011). *Hyperproliferation, cancer, and inflammation in mice expressing a Δ133p53-like isoform.* Blood 117, 5166-5177
2. Campbell, H.G., Fleming, N., Roth, I. *et. al.* (2018). *133p53 isoform promotes tumour invasion and metastasis via interleukin-6 activation of JAK-STAT and RhoA-ROCK signalling.* Nature Comm. DOI: 10.1038/s41467-017-02408-0 |
3. Kazantseva, M., Eiholzer, R., Mehta, S. *et al* (2018). *Elevation of the TP53 isoform 133TP53 in glioblastomas: an alternative to mutant p53 in promoting tumour development*. J Path. (in press).

C18 BCL6 – an essential role in glioblastoma therapy resistance and survival

Fabre, M.S.* [1,2], Jones, N.M* [1,2] Slatter, T.L.[3], Rowe, M.R.[1,2], Gordon, R.A.M[1], Castro, M.L.[1], Hung, N.[3], Melnick, A.M[4], and McConnell, M.J.[1,2]

[1]Centre for Biodiscovery and School of Biological Sciences, Victoria University of Wellington, NZ, [2] Malaghan Institute of Medical Research, Wellington, NZ, [3]Department of Pathology, Dunedin School of Medicine, University of Otago, NZ, [4]Weill Cornell Medical College, NY, USA.

The brain tumour glioblastoma is notoriously resistant to therapy. Even after surgical resection followed genotoxic chemotherapy and radiation treatment, most people develop recurrent disease, and die within two years of diagnosis. If the mechanism of resistance to DNA damaging therapy can be understood and overcome, therapy could be made effective, which would improve the outcome for people with this malignancy. We have shown that BCL6, a transcription factor and driver oncogene in lymphoma, is ectopically expressed in glioblastoma. BCL6 drives survival in these cells, and loss of BCL6 activity decreased the long-term proliferative potential. Further, BCL6 is induced in response to the DNA damaging therapy used to treat glioblastoma, but inhibition of BCL6 in combination with radiation and chemotherapy improved therapeutic efficacy. These data indicate that BCL6 has an essential role in survival of glioblastoma cells, and that it drives resistance to therapy.

C19: Systematic Profiling of Proteostasis and Ubiquitin Signaling in Cancer.

Robert F. Shearer[2], David R. Croucher[2] ,Kari-Anne Myrum Frikstad[3], Sebastian Patzke[3] , Trond Stokke[3] , Mary Iconomou[2], Jessie McKenna[1], Darren N. Saunders[1]

[1]School of Medical Sciences, UNSW Sydney, Australia; [2]Kinghorn Cancer Centre, Garvan Institute of Medical Research, Darlinghurst, Australia; [3]OUH - Norwegian Radium Hospital, Institute for Cancer Research, Department of Radiation Biology, Oslo, Norway.

Ubiquitylation is one of the most abundant protein modifications in cellular signaling, controlling numerous cellular pathways, and is a key regulator of proteostasis – the maintenance of cellular protein homeostasis. Ubiquitin labels substrate proteins via a highly ordered multi-step enzymatic cascade, with diversity in Ub chain topology controlling the fate substrate proteins, analogous to a cellular code. Numerous components of the Ub system have been implicated in cancer, and identification of Ub substrates is key to defining biological function of the system and understanding disease mechanisms. However, substrate identification remains a significant challenge.

We have developed proteomics approaches to define the ubiquitin-modified proteome and identify Ub substrates in breast cancer. We have identified numerous novel substrates of the E3 ligase UBR5, including establishing a novel role for UBR5 in primary cilia formation, which is crucial for signal transduction in a variety of pathways. Further, we have mapped the specific interactomes of different ubiquitin linkage types by combining BiCAP enrichment of poly-Ub chains with lysine mutagenesis of Ub for selective imaging, isolation and characterization of specific Ub chain topologies in cells. This highly novel technique is providing unprecedented insight into the basis of signalling diversity in the Ub system and regulation of cellular proteostasis in cancer.

C20: Massively multiplexed targeted long-read single cell sequencing for deep phenotyping of tumour and immune cell repertoires

#Ghamdan Al-Eryani[1,2], #Mandeep Singh[1,2], Shaun Carswell[1,2], Katherine Jackson[1,2], James M. Ferguson[1,2], Kirston Barton[1,2], Daniel Roden[1,2], Sunny Z. Wu[1,2], Chia-Ling Chan[1,2], David Koppstein[2,3], Fabio Luciani[2,3], Tri Phan [1,2], Joseph Powell[1,2], Simon Junankar[1,2], Chris C. Goodnow[1,2], *Martin A. Smith[1,2], *Alex Swarbrick[1,2]. Garvan Institute[1], Darlinghurst, NSW, Australia; St Vincent's Clinical School[2], UNSW Sydney, Austalia; Kirby Institute for Infection and Immunity[3], UNSW Sydney, Australia.

High throughput single cell RNA sequencing (scRNAseq) is providing revolutionary insights into biological systems and disease. Most scRNAseq protocols are designed for short read sequencing and, thus, only produce information from one end of cDNA molecules. This is often sufficient for transcriptome profiling and 3' or 5' end annotation, but restricts other research applications, including splicing, adaptive immune response, or somatic variation studies. Long-read sequencing can theoretically overcome these limitations, but the higher per-base error rate makes it difficult to accurately resolve cell barcodes and unique molecular identifiers, which are essential to deconvolute scRNAseq data.

We have developed a method that overcomes these challenges called Receptor And Gene Expression sequencing (RAGEseq). After partitioning single cells with the 10X Chromium platform, cDNA libraries are split and undergo hybrid capture for cDNAs of interest followed by Oxford Nanopore sequencing. Libraries are analysed in parallel with Illumina sequencing to perform transcriptome profiling and accurately resolve cell barcodes and unique molecular indices. The short read data is used to demultiplex the long read data, which in turn is used to resolve the sequence of full-length transcripts via *de novo* assembly, error correction, and polishing.

After rigorous benchmarking on Jurkat T-cells, Ramos B-cells and peripheral blood monocytes, we used RAGEseq to characterise T-cell (TCR) and B-cell (BCR) receptor transcripts and full transcriptomes of over 1000 lymphocytes sampled from the primary tumour and draining lymph node of a breast cancer patient. We use this data to phenotype and track clonally related lymphocytes between tissues and identify BCR transcripts encoding antibodies destined for secretion versus membrane localization.

C21: The highs and lows of targeting tryptophan dioxygenases to overcome immune suppression in cancer

Ching, L-M.[1], Palmer, B. D.[1], Tomek, P. [1].

[1]Auckland Cancer Society Research Centre, Faculty of Medical and Health Sciences, University of Auckland, Auckland, NZ.

Whilst immune cells can seek out and destroy cancer cells, tumours have evolved a number of cunning ways to evade or to suppress the host's immune system. Accelerated conversion of tryptophan along the kynurenine pathway, initiated by increased expression of tryptophan dioxygenases, has emerged as a key mechanism that many cancers use to inactivate anti-cancer immune T cells. There is compelling clinical evidence showing that patients whose tumour express high amounts of the inducible tryptophan catabolising enzyme, indoleamine 2,3-dioxygenase 1 (IDO1) have a poorer prognosis than those whose tumours express low or no IDO1. These clinical observations have propelled, in the last decade, a frenzy of activity in the discovery and development IDO1 inhibitors and their application in combination with immune checkpoint blockades for the treatment of cancer. In 2015, four IDO1 inhibitors were in human clinical trials, but by May 2018, testing of these initial four had been suspended, resulting in the current hiatus in clinical studies of IDO1 inhibitors. This presentation will overview the key studies that validate IDO1 as target for cancer therapy; and then the preclinical studies of some of the investigational IDO1 inhibitors, including our own from the ACSRC, that provided such strong indication of anti-tumour benefits in combination with immune checkpoint blockades. I will conclude with a discussion of some of the lessons learnt from the early clinical trials of IDO1 inhibitors and the thoughts for the future development of tryptophan dioxygenases for the treatment of cancer.

C22: Restoring interactions between tumour and immune cells to block metastatic progression

Brockwell, N.K[1], Zanker, D.[1], O'Toole, S.[2,3],Ganju, V.[4], Parker, B.S.[1]

[1]Department of Biochemistry and Genetics, La Trobe Institute for Molecular Sciences, Melbourne, VIC, Australia [2]Sydney Medical School, University of Sydney, NSW, Australia, [3]Garvan Institute of Medical Research, Darlinghurst, NSW, Australia, [4]Hudson Institute of Medical Research, Clayton, VIC, Australia.

Triple negative breast cancer (TNBC) accounts for 15-20% of all breast cancers and has an increased risk of rapid metastasis within the first two years compared to other subtypes. Increased tumour infiltrating lymphocytes in this subtype, along with the lack of targeted therapeutic options for TNBC, has triggered interest in trialling checkpoint-targeted immunotherapy. However, responses to date to PD1-targeted immunotherapeutics have been underwhelming. Our work has shown that tumour inherent IFN signals are closely associated with the "heat" of a tumour and that loss of these signals dampens the anti-tumour immune response[1] and reduces benefit of conventional therapies and immunotherapeutics, including anti-PD1. Our recent studies have demonstrated that poly (I:C), a potent type I interferon inducer, induces a T cell inflamed tumour that sensitises preclinical TNBC models to anti-PD-1 and induces a tumour specific T-cell response that extends metastasis-free survival[2]. Using a neoadjuvant sequential biopsy cohort linked to multiplex IHC, we have now built on these findings to profile the immune landscape of TNBC to develop immune markers that predict poor chemotherapeutic response, a poor prognosis, and patients that may benefit from immunotherapeutic intervention. Utilizing multiplexed immunohistochemistry we have demonstrated that immune cell characterisation and activation status is a superior prognostic for chemotherapeutic response and risk of relapse than standard TIL characterization. Furthermore, we identified a novel prognostic marker that indicates presence of an intact tumour-intrinsic type I IFN signalling pathway which is superior to TIL characterisation and predicts survival in 3 independent TNBC cohorts. Based on these findings, we are currently trialling new intratumoral IFN inducers in our preclinical models to work towards clinical trials of immunoactivating therapies in patients with IFN and immune cell cold tumours.

1. Bidwell, B. N. *et al. Silencing of Irf7 pathways in breast cancer cells promotes bone metastasis through immune escape*. *Nat Med* 18, 1224–1231 (2012).
2. Brockwell, N. K. *et al. Neoadjuvant Interferons: Critical for Effective PD-1-Based Immunotherapy in TNBC*. *Cancer Immunol Res* 5, 871–884 (2017).

C23 Engineering memory, survival and safety into chimeric antigen receptor (CAR) T cells

McLellan A.D. Rad, S.M.A.H., Muhsin-Sharafaldine M.R, Poudel A., Tan G.M.Y., Steens J., Halpin J.C., Matheson J.A., He K., Goy H.E., Dinh T.N., Haw R.T., Saunderson S.C.

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

Chimeric antigen receptor (CAR) therapy CAR T cell therapy combines the specificity of antibodies with the cytolytic activity of T cells to create potent, tumour-killing lymphocytes and has been shown to be efficacious in the treatment of B cell lymphomas – with expanding potential for treatment of solid tumours. This talk will outline our new strategies employed to enhance CAR T cell performance and survival. Our laboratory uses the Sleeping Beauty transposon-based gene transfer systems with dual constitutive and inducible promoter and bi-cistronic expression. These systems maximise the utility of CAR genetic cassettes for improving T cell memory and activity, yet are compact enough to limit loss of transposition efficiency due to vector size increases. To mitigate immune responses to self-tissues, scFv engineering and phage display platforms, along with genetic forecast technologies, are currently in development to detect and respond to systemic and tumour-derived signals.

C24: The role of ascorbate in modifying the hypoxic response in cancer

Dachs, G.U.[1], Vissers, M.C.[2]
[1]Mackenzie Cancer Research Group and [2]Center for Free Radical Research, Department of Pathology and Biomedical Science, University of Otago, Christchurch, NZ.

Interest in the potential use of ascorbate (vitamin C) as an adjunct to cancer therapy has been revived with the identification of a number of plausible mechanisms by which it could influence tumour growth and patient outcome. In particular, ascorbate is an essential cofactor for a family of >60 dioxygenase enzymes, including those that regulate the hypoxia-inducible factor (HIF). HIF is the main transcription factor that regulates tumour growth, metabolism and metastasis.
We hypothesise that an increase in intracellular ascorbate reduces levels and activity of the HIF transcription factors, and that this in turn would reduce markers of cancer aggression. We have data ranging from cell culture models, tumour studies in ascorbate-dependent mice, analyses of tumour samples from patients with a range of different cancers, and a clinical trial in patients with colorectal cancer that support this hypothesis. However, only robust, controlled clinical trials will be able to provide evidence on whether or not ascorbate plays a role in cancer therapy and patient outcome.

C25: The immune microenvironment in normal breast tissue at risk of developing breast cancer

Cecilia W. Huo[1], Helen Tower[2], Genevieve Dall[2], Ashleigh Unsworth[2], Serene Yeow[2], Prue Hill[3,4], Grace Chew[1], Paul J Neeson[2], Heloise Halse[2], Elizabeth D. Williams [5,6], Michael A. Henderson [1,2], Nicole Haynes[2], Erik W. Thompson [5,6], Kara Britt[2,7]

[1]University of Melbourne Department of Surgery, St. Vincent's Hospital, Melbourne, Australia; [2] Peter MacCallum Cancer Centre, Melbourne, Australia
[3]Department of Pathology, St Vincent's Hospital, Melbourne, Australia;
[4] Pathology Department, University of Melbourne, Australia;
[5]Institute of Health and Biomedical Innovation and School of Biomedical Sciences, Queensland University of Technology, Brisbane, Australia;
[6]Translational Research Institute, Brisbane, Australia;
[7]The Sir Peter MacCallum Department of Oncology, University of Melbourne, Melbourne, Australia.

Breast cancer (BCa) has historically not been considered immunogenic, as the incidence is not increased in immune suppressed patients (eg: transplant recipients or HIV patients). However, there are now irrefutable data demonstrating the immune cell infiltrate of a breast tumour affects growth and metastasis. BCa has more tumour infiltrating lymphocytes (TILs) than normal breast and patients with high TILs have better response to chemotherapy. The immune system is also active in benign proliferative and in-situ lesions, where T cells, B cells and macrophages are increased compared to normal breast. We wanted to know if normal breast tissue at risk of cancer also has an activated immune system.

Increased mammographic density is a strong risk factor for breast cancer and we have shown that the number of $CD45^+$ leucocytes is higher in tissue from areas of high mammographic density (HMD), compared to low (LMD). Here we investigated the immune cell phenotype according to density in 54 women undergoing prophylactic mastectomy. The HMD and LMD areas were resected under radiological guidance in collaboration with Breast Screen Victoria, and were processed into FFPE. Fifteen paired HMD and LMD specimens were further selected (reasonable amount (>20%) of tissue /per block on H&E staining) for subsequent immunohistochemical analyses of immune cell infiltration. Macrophages, dendritic cells (DCs), B lymphocytes, and programmed cell death protein-1 (PD-1) expression were significantly increased in HMD epithelium compared to LMD. Moreover, a significantly higher level of DCs, $CD4^+$ T cells and PD-1 were also observed in HMD stroma compared to LMD stroma. The increased expression of interleukin (IL)-6 and IL-4, with unaltered interferon gamma (IFN-□), indicate a pro-inflammatory microenvironment. This indicates that the immune system may be activated very early in BCa development and may in part underpin the BCa risk associated with HMD. In line with this, we also show that mice treated with estrogen (which also increases BCa risk) have similar immune cell changes (increased myeloid derived suppressor cells, CD4+ T cells and PD1+ T cells). Thus, normal breast tissue at risk of cancer development shows pro-inflammatory immune cell changes.

QRW Cancer POSTER Abstracts

Garvan Institute Cancer Satellite Meeting

Sunday 26 August – Monday 27 August, 2018

Rydges Hotel, Queenstown, New Zealand

Summary of abstracts for Poster Session

No.	Title	Presenter	Institution(s)
C26	Long non-coding RNAs as drivers of tumor progression and metastasis	Sarah Diermeier	University of Otago
C27	Cancer Stem Cell Subpopulations in Colon Adenocarcinoma	Matthew Munro	Gillies McIndoe Research Institute, Victoria University of Wellington
C28	Proteomic analysis of breast cancer brain metastasis microenvironment in-vivo reveals a unique milieu intérieur of metabolic reprogramming	Priyakshi Kalita-de Croft	University of Queensland, [2]Queensland Institute for Medical Research (QIMR) Berghofer
C29	Development of a 3D breast cancer-adipocyte model: a tool for studying tumour microenvironment interactions *in vitro*	Jessika Wise	University of Otago
C30	The Role of Pseudokinase *TRIB1* in Breast Cancer	Hamish David Mcmillan	University Of Otago
C31	p63 promotes proliferation in Melanoma cell lines via regulation of AQP3	Luke Henderson	University of Otago
C32	The TP53 isoform Δ133p53β in glioblastoma development.	Tania Slatter	University of Otago, Maurice Wilkins Centre for Molecular Biodiscovery
C33	RSK mediated YB-1 phosphorylation – a key event required for completion of cytokinesis	Sunali Mehta	University Of Otago, Maurice Wilkins Centre of Biodiscovery
C34	Inhibition of EGFR/HER-2 affects growth of ascitic ovarian cancer cells *in vitro* study	Kenny Chitcholtan	University of Otago
C35	Rural urban differences in breast cancer treatment in New Zealand	Lynne Chepulis	University of Waikato
C36	Expression variability is associated with breast tumour subtype	George Wiggins	University Of Otago
C37	Cancer-associated adipocytes alter protein expression profiles of human breast cancer cells *in vitro*	Rebekah Crake	University of Otago

C38	Development of Plasma Genomic Biomarkers for Mutation Detection and Monitoring in New Zealand Cancer Patients	Sandra Fitzgerald	University Of Auckland
C39	Genome-scale CRISPR/Cas9 knockout screens in cell line-derived xenograft models	Tet-Woo Lee	University of Auckland
C40	The potential use of ascorbate as an epigenetic therapeutic in acute myeloid leukaemia	Andrew Das	University of Otago
C41	Evaluating variants in breast cancer associated genes identified by panel sequencing of New Zealand breast cancer patients	Vanessa Lattimore	University Of Otago
C42	Intravital optical window imaging of RhoA-, Rac1- and Akt-FRET biosensor mice monitoring drug treatment response in cancer.	Max Nobis	Garvan Institute of Medical Research
C43	Evidence for a new mechanism of inhibiting an oncogenic PI3K enzyme	Jack Flanagan	University Of Auckland
C44	Personalised medicine approach in pancreatic cancer reveals fine-tuned stromal FAK manipulation improves global response the gemcitabine and Abraxane while sensitising circulating tumour cells to shear stress in transit	Kendelle Murphy	Garvan Institute of Medical Research
C45	Characterisation of growth hormone signal transduction in New Zealand Melanoma (NZM) cell lines	Karla Sousa	University of Auckland
C46	Post-Tumour-Implantation Exercise does not alter Tumour Growth Rate in Mice with Subcutaneous B16-F10 Melanoma	Linda Buss	[1]University of Otago
C47	Engineered Nanoparticles as Potential Therapeutics for Acute Myeloid Leukemia	Tatiana Syrovets	Ulm University
C48	Targeting Long Non-Coding RNAs to Inhibit Colorectal Tumor Progression and Metastasis	Brandon Wright	University Of Otago
C49	CRISPR/Cas9 screening to identify lncRNAS as novel therapeutic targets in triple-negative breast cancer	Megan O'Malley	University Of Otago
C50	Is EBF3 promoter hypermethylation an oncogenic epi-mutation in multiple	Michael Eccles	University Of Otago

	cancer types?		
C51	Quantifying PD-L1 expression in response to oestrogen deprivation in oestrogen receptor positive breast cancer	Conor Mcguinness	University Of Otago
C52	Identifying synthetic lethal vulnerabilities in cohesin-haploinsufficient MCF10A cells	Chue Vin (Alice) Chin	University Of Otago
C53	Investigating the role of DNA methylation, gene expression, long non-coding RNA and transposable elements in constitutive PD-L1 expression in melanoma cell lines	Antonio Ahn	University Of Otago
C54	p53 does not cause growth arrest or death in normal B cells but only affects division rate	Sankalita Ray	University Of Otago
C55	Co-targeting BRAF and VEGF receptors synergistically inhibited the growth of BRAF-mutant and BRAF wild-type melanomas	Khanh Bao Tran	University Of Auckland
C56	Cathepsin D: a protease attracts mesenchymal stem cells for homing to tumor sites	Gowthami Vangala	University Of Otago
C57	Investigating the Clinical Significance of Fibroblast Growth Factor Inducible 14 in Breast Cancer	Joshua Harris	University Of Otago
C58	The role of the p53 isoform Δ133TP53 in treatment resistance.	Ramona Andrea Eiholzer	University Of Otago
C59	Investigation of Mitochondrial Transfer in Human Erythroblasts	Brittany Lewer	Victoria University Of Wellington, Malaghan Institute Of Medical Research
C60	The NMDA receptor is an important component of intracellular calcium homeostasis in megakaryocytic leukaemia cells, and its loss of function promotes cell differentiation	James Iain Hearn	University of Auckland
C61	Antihelminthic benzimidazoles mebendazole and albendazole kill cancer cells via classical apoptosis	Jakeb Petersen	University of Otago

C62	Validation of an Androgen Bioassay for Measurement Breast Cancer Patient Serum.	Rachel Lund	University of Otago
C63	The regulation of the tumour hypoxic response by ascorbate and association with clinicopathological factors and patient outcome in breast cancer	Margreet Vissers	University of Otago
C64	A mitochondria-selective near-infrared-emitting fluorescent dye for cellular imaging studies	Jiney Jose	University of Auckland
C65	The long non-coding RNA MaTAR17 is a new driver of tumour progression.	Debina Sarkar	University of Otago
C66	Withdrawn		
C67	Mechanisms of Active DNA Demethylation in somatic cells	Issam Mayyas	University of Otago
C68	Understanding mitochondrial transfer in therapy - Applied single molecule genotyping to detect and quantify mitochondrial transfer.	Matthew Rowe	Victoria University of Wellington
C69	Investigating p53 isoforms in HPV positive cervical cancers and in the placenta	Ashley Reily-Bell	University of Otago
C70	Genetic identification of bone marrow mitochondrial transfer in a leukaemic murine model	Georgia Carson	Malaghan Institute of Medical Research, University of Wellington
C71	Novel targeting of PI3K/mTOR and ERK in ovarian cancer cell line models is influenced by culture method	Elizabeth Dunn	University of Canterbury
C72	Visualisation of long non-coding RNA *ANRIL* in Breast Cancer	Paulomi Mehta-Mujoo	University Of Otago
C73	Investigating Tumour Evolution in a Single Patient with Disseminated Cancer	Tamsin Robb	University Of Auckland
C74	The hypoxia-activated EGFR/HER2 inhibitor Tarloxotinib is activated by the plasma membrane reductase STEAP4	Shevan Silva	University Of Auckland

C26: Long non-coding RNAs as drivers of tumor progression and metastasis

Sarah D. Diermeier[1]

[1]Department of Biochemistry, University of Otago, Dunedin, NZ

Genome-wide studies revealed that most of the human genome does not encode for proteins and identified thousands of non-coding RNAs. Of these non-coding transcripts, long non-coding RNAs (lncRNAs) represent the largest and most diverse class. They are defined by length (>200 nt), transcribed by RNA polymerase II and commonly originate from intergenic regions. LncRNAs can be capped, spliced and polyadenylated but lack a significant open reading frame. Members of this class have been implicated as regulatory molecules in many cellular functions including epigenetic gene regulation, splicing and translation. Recently, lncRNAs emerge as crucial players in cancer by impacting acquired capabilities of cancer cells such as cell proliferation, angiogenesis or invasion.
We identified 30 potentially oncogenic lncRNAs in breast cancer, termed Mammary Tumor Associated RNAs (MaTARs). The expression of MaTARs correlates with breast cancer subtype and/or hormone receptor status, indicating potential clinical relevance. In addition, MaTARs are expressed in a tumor-specific manner, making them excellent candidates for drug development as systemic therapeutic intervention will only affect cancer cells. To functionally validate the role of MaTARs, we performed antisense knockdown experiments *in vitro* and *in vivo*, resulting in reduced mammary tumor growth and metastasis. Loss-of-function models generated by CRISPR/Cas9 genome editing or CRISPR interference (CRISPRi) further confirmed that MaTARs are driving tumor progression.

Ongoing studies are investigating the molecular mechanism by which MaTARs function. Our results suggest that lncRNAs are likely important drivers of tumor progression and represent promising new therapeutic targets.

C27: Cancer Stem Cell Subpopulations in Colon Adenocarcinoma

Munro, M.J.[1,2], Wickremesekera, S.K.[1,3], Peng, L.[2], Tan, S.T.[1,4], Reginald Marsh[1,5], Itinteang, T.[1]

[1]Gillies McIndoe Research Institute, Wellington, NZ; [2]Centre for Biodiscovery, Victoria University of Wellington, Wellington, NZ; [3]Upper Gastrointestinal, Hepatobiliary & Pancreatic Section, Department of General Surgery, Wellington Regional Hospital, Wellington, NZ; [4]Wellington Regional Plastic, Maxillofacial & Burns Unit, Hutt Hospital, Wellington; [5]University of Auckland, Auckland, NZ

Background The cancer stem cell (CSC) concept proposes that cancer is driven by CSCs which self-renew and produce cells with uncontrolled replication. OCT4, SOX2, NANOG, KLF4 and c-MYC are genes used to produce induced pluripotent stem cells (iPSC). This study investigated iPSC marker expression in primary colon adenocarcinoma (CA), comparing their relative abundance and localisation in low-grade CA (LGCA), high-grade CA (HGCA), and patient-matched 'normal colon' (NC).

Methods DAB IHC was carried out on LGCA (n=9) and HGCA (n=8) samples and their matched NC (n=17) to analyse the expression and localisation of these iPSC markers. Six LGCA and six HGCA samples were selected to confirm transcriptional activation by RT-qPCR.

Results DAB IHC showed differences in the abundance and localisation of iPSC markers. A general trend from nuclear to cytoplasmic localisation was observed when comparing NC to LGCA; however, HGCA commonly displayed both nuclear and cytoplasmic localisation, with increased expression by surrounding stromal cells.

Two CSC subpopulations were identified by IHC: one within the epithelium expressing NANOG (0.01% NC, 9.7% LGCA, 52.4% HGCA) and the other within the stroma expressing OCT4 (0.26% NC, 24.3% LGCA, 30.8% HGCA).

All LGCA and HGCA samples could be successfully graded with 100% accuracy based on just four elements of the data: stromal KLF4 in NC ($p=0.020$) and CA ($p=0.034$), and epithelial OCT4 ($p=0.001$) and NANOG ($p=0.026$) in CA crypts (Discriminant function analysis: canonical correlation = 0.981; wilkes lambda = 0.037).

RT-qPCR confirmed gene expression of all iPSC markers within all tissue samples.

Conclusions These results reveal novel differences in iPSC marker expression between CA and matched NC, revealing CSC subpopulations identifiable by their unique expression pattern. This suggests that certain combinations of iPSC markers could provide a useful diagnostic and prognostic tool for CA, warranting further investigation into the characteristics of these CSCs.

C28: Proteomic analysis of breast cancer brain metastasis microenvironment in-vivo reveals a unique milieu intérieur of metabolic reprogramming

Kalita-de Croft, P.[1,2], Straube, J.[2], Al-Ejeh, F.[1,2], Saunus, J.M.[1,2], Lakhani, S.R.[1,3],
[1]University of Queensland, UQ Centre for Clinical Research, Brisbane, Queensland, Australia.
[2]Queensland Institute for Medical Research (QIMR) Berghofer, Brisbane, QLD, Australia.
[3]Pathology Queensland, Royal Brisbane Women's Hospital, Brisbane, Queensland, Australia.

Brain metastasis (BM) is an unfortunate clinical complication that occurs in about 15-30% of the patients with metastatic-breast cancer[1]. Extremely poor prognosis and neurological impairments of sensory and cognitive functions are salient features of BM, with survival rates varying between 4-12 months post-diagnosis [2, 3]. Therefore, there is an urgent need of unravelling novel mechanisms of BM including treatment-resistance. The tumour-microenvironment (TME) provides both the framework and mechanism for metastatic outgrowth. Despite immense negative selection-pressure tumour-cells colonise using adaptive mechanisms, including: oxidative-stress resistance, repurposing neurotransmitters and mimicking neural traits[4]. These transformations could be clinically targetable and hence we set out to identify novel adaptations in breast cancer-brain metastasis by performing proteomic analysis of the mouse brain compartment of breast cancer brain xenografts.

MDA-MB-231 breast cancer cells were stereotactically injected into NOD/SCID mouse hosts (n=5). Mock-injected (PBS) and matching uninvolved brain (n=4) were used as controls. After three weeks, brain tissues (tumour-associated, mock and normal) were isolated using affinity-based magnetic bead separation (Miltenyi Biotec) and proteomics was performed by Mass Spectrometry (MS)-Swath at Australian Proteome Analysis Facility (APAF).

Unsupervised hierarchical clustering exhibited forty-one differentially expressed proteins. We employed String and IPA analysis to further reveal Gene-Ontology (GO) terms associated with metabolic stress and extracellular vesicle transport such as extracellular exosomes and mitochondrial proteins (FDR <0.05 cut off) to be deregulated. Furthermore, they belonged to mitochondrial dysfunction, sirtuin signalling and apoptosis signalling pathways. These findings suggest that tumour associated brain exhibits metabolic reprogramming, evident from deregulation of exosomal and mitochondrial pathways. This indicates that the bioenergetics demand of the microenvironment has altered the *milieu intérieur* of the brain. Apart from validating these findings on additional mouse brain xenografts and clinical samples, future work will focus on studying these metabolic changes for targeting and therapeutic purposes.

1. Tabouret E, Chinot O, Metellus P, Tallet A, Viens P, Goncalves A: ***Recent trends in epidemiology of brain metastases: an overview***. *Anticancer Res* 2012, **32**(11):4655-4662.
2. Niikura N, Hayashi N, Masuda N, Takashima S, Nakamura R, Watanabe K, Kanbayashi C, Ishida M, Hozumi Y, Tsuneizumi M *et al*: ***Treatment outcomes and prognostic factors for patients with brain metastases from breast cancer of each subtype: a multicenter retrospective analysis***. *Breast Cancer Res Treat* 2014, **147**(1):103-112.
3. Quigley MR, Fukui O, Chew B, Bhatia S, Karlovits S: ***The shifting landscape of metastatic breast cancer to the CNS***. *Neurosurg Rev* 2013, **36**(3):377-382.
4. Quail DF, Joyce JA: ***The Microenvironmental Landscape of Brain Tumors***. *Cancer Cell* 2017, **31**(3):326-341.

C29: Development of a 3D breast cancer-adipocyte model: a tool for studying tumour microenvironment interactions *in vitro*

Wise, J.[1&2], Lim, K.[2], Currie, M.[1], Morrin, H.[1], Woodfield, T.[2], Phillips, E.[1]

[1]Mackenzie Cancer Research Group, University of Otago Christchurch, Christchurch 8011, New Zealand
[2]Christchurch Regenerative Medicine and Tissue Engineering Group, University of Otago Christchurch, Christchurch 8011, New Zealand

The breast cancer tumour microenvironment (TME) contains a large proportion of adipocytes, which cancer cells encounter during early invasion into local tissues[1]. Research is limited by a lack of model systems that effectively replicate TME interactions. In human breast tumour stroma, adipocytes exhibit a decrease in late adipocyte differentiation markers and a more fibroblast-like phenotype, becoming known as 'Cancer-Associated Adipocytes' (CAA). *In vitro* 2D transwell co-culture studies indicate CAA induce a more chemo-resistant and invasive phenotype in breast cancer cells[2]. However, 2D transwell co-culture prevents direct contact between cells. Therefore, the aim of this study is to develop a 3D *in vitro* model system to study interactions between breast cancer cells and adipocytes.

Human breast adipocytes were enzymatically isolated from breast adipose tissue collected from patients (EthicsAprrocal12/319). Estrogen receptor positive (ER+; MCF7) and triple negative (ER-, PR-, HER2-; MDA-MB-231) breast cancer cells were encapsulated alone or with mature adipocytes in 5, 7.5 and 10 weight percent (wt%) photopolymerisable gelatin-methacryloyl (Gel-MA) hydrogels (Ø5mm x 1.5mm height). Metabolic activity (AlamarBlue®), cell viability (live/dead) and immunofluorescent staining of lipid (Perilipin) and proliferative (Ki67) markers were assessed.

Breast cancer cells showed high levels of viability, proliferation and metabolic activity throughout the 21 days in 3D culture, with cells in 5wt% Gel-MA displaying the highest cell viability and metabolic activity. Co-cultured mature adipocytes and breast cancer cells showed higher metabolic activity than when cultured alone. Mature adipocytes cultured alone were maintained within the Gel-MA discs throughout the 21 days of 3D culture. However, when co-cultured with MDA-MB-231 cells there was no detectable perilipin expression by day-21, indicating altered adipocyte phenotype in co-culture with cancer cells.

This 3D co-culture system may provide a more physiologically mimetic model for studying cellular interactions within the TME, and screening novel therapeutics.

1. Wang, Y.-Y. *et al. Adipose tissue and breast epithelial cells: A dangerous dynamic duo in breast cancer*. *Cancer Lett.* **324,** 142–151 (2012).
2. Dirat, B. *et al. Cancer-associated adipocytes exhibit an activated phenotype and contribute to breast cancer invasion*. *Cancer Res.* **71,** 2455–2465 (2011).

C30: The Role of Pseudokinase *TRIB1* in Breast Cancer

McMillan, Hamish[1,2], Mace, Peter[1] and Dunbier, Anita[1,2]

[1]University of Otago, Department of Biochemistry, Dunedin, NZ [2]Centre for Translational Cancer Research, Dunedin, NZ

Tribbles homolog 1 (TRB1) is a protein that regulates transcription factors and signalling pathways in healthy cells but can drive malignancy when overexpressed. A member of a pseudokinase family, TRB1 overexpression in bone marrow drives the development of acute myeloid leukaemia (AML) through the degradation of key CCAAT-enhancer binding protein (cEBP) transcription factor family members. TRB1 overexpression in AML is frequently driven by amplification of *TRIB1*, the gene encoding TRB1. *TRIB1* is co-located with the oncogene *MYC* on 8q34 and the two genes are frequently co-amplified. Screening for *MYC* synthetic lethal interaction partners identified *TRIB1*, suggesting that the co-amplification of these genes may be important in *MYC* driven cancers that commonly have a poor outcome[1]. Despite associations with other cancer types the importance and role of *TRIB1* in solid tumours such as breast cancer is poorly defined.

Using siRNA and shRNA mediated knockdown across three different cell lines with varying levels of both *TRIB1* and *MYC* expression, we set out to understand the role of *TRIB1* in breast cancer. Knockdown of *TRIB1* by siRNA provided >50% knockdown of *TRIB1* three days after transfection. Knockdown resulted in slowed MCF7 and HCC1419 cell growth over seven days. There was a 50% decrease in MCF7 cell number three days after *TRIB1* siRNA transfection when compared with cells transfected with a RISC-free siRNA control. Similarly, a 30% decrease in HCC1419 cell number was observed five days after *TRIB1* siRNA transfection when compared with cells transfected with RISC-free siRNA control. Decreased growth with knockdown suggests that TRB1 is involved in the regulation of the cellular proliferation in breast cancer. Given TRB1's previously defined role as an adaptor protein in AML, we aim to understand how TRB1 controls levels of various substrates in breast cancer to elucidate its role in breast cancer proliferation.

1. Toyoshima, M. et al. *Functional genomics identifies therapeutic targets for MYC-driven cancer.* Proc. Natl. Acad. Sci. U.S.A. **109,** 9545–9550 (2012).

C31: p63 promotes proliferation in Melanoma cell lines via regulation of AQP3

Henderson, L.[1], Mehta, S.[1], Fleming, N.[1], Eccles, M.[1], Chatterjee, A.[1], Print, C.[2, 3], Shepherd, P.[2, 3], Braithwaite, A.[1]
[1]Department of Pathology, University of Otago, Dunedin, NZ, [2]Department of Molecular Medicine and Pathology, University of Auckland, Auckland, NZ, [3]Maurice Wilkins Centre for Molecular Biodiscovery, The University of Auckland, Auckland, NZ.

New Zealand has the highest incidence of melanoma in the world with over 4000 people diagnosed and over 300 dying from it every year. It has been shown that there is increased p63 expression in melanoma cell lines and we have shown that knockdown of p63 (using siRNA) in over 20 melanoma cell lines caused growth arrest. To investigate whether this was occurring in a specific phase of the cell cycle, cells stably transfected with the FUCCI plasmid (cell cycle reporter constructs) were used. Knockdown of p63 caused an arrest of cell cycle in early G1 phase. Knockdown of p63 in these cells showed a morphological phenotype defined by small rounded cells that have minimal cytoplasm and appear dehydrated. This phenotype was also observed in 20 melanoma cell lines.

To investigation downstream targets of p63 correlation analyses of the melanoma samples in the TCGA were carried out. This analysis revealed a number of candidates that were membrane transporters; such as aquaporin-3(AQP-3), the gap-junction α and β families, and the Desmocollin family. Interestingly p63-deficient mice lose approximately 30 times more water than wildtype mice due to increased skin permeability and appear to die of dehydration. Additional testing revealed AQP-3 to be an intriguing candidate with its expression linked to p63 expression *in vitro*. Subsequent knockdown of AQP-3 alone (using siRNA) revealed a similar phenotype to p63 knockdown. Currently further testing is underway to examine this and other novel pathways and whether they could be manipulated therapeutically.

C32: The TP53 isoform Δ133p53β in glioblastoma development.

Slatter T.L.[1,2], Kazantseva M.[1,2], Eiholzer R.A.[1], Mehta S.[1,2], Taha A.[3], Bowie S.[1], Roth I.[1], Joruiz S.M.[4], Royds J.A. [1], Zhou J. [1,5], Hung N.A.[1], Braithwaite A.W.[1,2]

[1]Department of Pathology, Dunedin School of Medicine, University of Otago, Dunedin, New Zealand.
[2]Maurice Wilkins Centre for Molecular Biodiscovery, New Zealand.
[3]Department of Neurosurgery, Southern District Heath Board, New Zealand.
[4]Jacqui Wood Cancer Centre, Division of Cancer Research, University of Dundee, United Kingdom.
[5]Department of Radiology, [5]Southern District Health Board, New Zealand.

Tumour protein 53 (p53) isoforms have tumour promoting, migration and inflammatory properties. The Δ133p53β isoform is increased in glioblastomas with a poorer prognosis, an increased tumour associated macrophage content and wild-type p53. These findings suggest Δ133p53β is a factor that is an alternative to mutant p53 in glioblastoma development, and one that also contributes to a more aggressive tumour microenvironment. This study investigated how Δ133p53β isoforms become established in glioblastoma, and once elevated how Δ133p53β contributes to tumour progression using human glioblastomas, *in vitro* based analyses using Δ133p53β expressing cells, and an animal model with p53 isoform expression in the brain. *In situ* based analyses in glioblastomas found *Δ133p53β* expression localised to malignant cells in areas with increased hypoxia, and in cells with the monocyte chemoattractant protein C-C motif chemokine ligand 2 (CCL2) expressed. Δ133p53β positive tumours had increased macrophage colony stimulating factor 1 receptor (CSF1R) and programmed death ligand 1 (PDL1) positive cells. *In vitro* based analyses confirmed that hypoxia increases *Δ133p53β* expression when wild-type p53 was present. Clonal cell lines stabling expressing Δ133p53β had increased PDL1 and epidermal growth factor receptor (EGFR) on the cell surface and were resistance to temozolomide treatment. These findings will be tested in a new animal model. Our findings suggest elevated Δ133p53β is an alternative pathway to TP53 mutation in glioblastoma that aids tumour progression by promoting an immunosuppressive and chemoresistant environment. Adding Δ133p53β to a TP53 signature along with TP53 mutation status will better predict treatment resistance in glioblastoma.

C33: RSK mediated YB-1 phosphorylation – a key event required for completion of cytokinesis

Sunali Mehta[1,2], Michael Algie[1], Tariq Al Jabri[3], Cushla McKinney[1], Chandra Verma[4] Weini Ma[3], Pragathi Massamsetti[3], Luke Henderson[1], Maree Gould[1], Kim Parker[1], Puja Bhatia[1], Rhodri Harfoot[1], Torsten Kleffmann[1], Scott Cohen[3], Megan Chircop[3], Tony Cesare[3], Adele Woolley[1,2] and Antony Braithwaite[1,2,3]

[1] Pathology department, University of Otago, Dunedin, New Zealand
[2] Maurice Wilkins Centre of Biodiscovery, University of Otago, Dunedin New Zealand
[3] Children's Medical Research Institute, Sydney, Australia
[4] A-Star, Singapore

Y-box-binding protein 1 (YB-1) is a well-known multifunctional protein with tumour promoting functions. Elevated YB-1 levels are associated with relapse and poor prognosis in breast and other cancers. It has been well documented that YB-1 regulates cell proliferation and survival, suggesting it has an important role in regulating the cell cycle. However, based on the current literature the cell cycle phase regulated by YB-1 remains ambiguous. Furthermore, phosphorylation of YB-1 at different sites can alter its function. The aim of this study is to determine the phase of the cell cycle regulated by YB-1 and if this is phosphorylation dependent. Using cells stably expressing the FUCCI cell cycle reporters and time lapse live cell imaging, we have shown that YB-1 is essential for promoting cytokinesis by mediating the spatial recruitment of the chromosome passenger complex (CPC) to the cleavage furrow. We have also demonstrated that knockdown of YB-1 increases the G1 phase transit time of the cell cycle. To determine if phosphorylation of YB-1 is important in regulating cytokinesis, we have identified 4 kinases that can phosphorylate Serine/Threonine motif in YB-1. These include AKT, p90 ribosomal S6 kinase (RSK), Casein Kinase 2 (CSK2) and Aurora B kinase (AURKB). We have shown that only inhibition of RSK and AURKB results in failure of cytokinesis similar to reducing YB-1. Finally, we have shown that over-expression of YB-1 can overcome inhibition by RSK but not AURKB suggesting that YB-1 is a substrate of RSK. Overall our data suggest RSK mediated phosphorylation of YB-1 is important in regulating cytokinesis. Thus, inhibiting RSK can prevent YB-1 activation making it a promising therapeutic target. We are currently identifying specific YB-1 phosphorylation sites that can regulate cytokinesis and are substrates of RSK.

C34: Inhibition of EGFR/HER-2 affects growth of ascitic ovarian cancer cells *in vitro* study

Chitcholtan, K., Harker, D., Simcock, B. and Sykes, P.
Gynaecological Cancer Research Group, Department of Obstetrics and Gynaecology, University of Otago, Christchurch, New Zealand

Elevated expression of epidermal growth factor receptor (EGFR) and HER-2 has been shown to correlate with a poor prognosis in ovarian cancers. Gefitinib, a reversible EGFR inhibitor, and canertinib, an irreversible dual EGFR/HER-2 inhibitor showed a modest clinical response. Consequently, these inhibitors are not commonly used in the treatment of ovarian cancer. Ascitic fluid contains heterogeneity of cell populations including small clusters of cancerous ovarian cells that are likely the source of metastasis. Cell clusters grow on the surface of abdominal walls and subsequently exacerbate the progression of the disease. Because there are limited options for treatment of women with advanced disease associated with ascites, the elimination of ascitic cancer cells is a very important strategy for clinical management. The presence of EGFR and HER-2 at the primary and metastatic sites was investigated in the past. However, the role of these proteins in the survival of the ascitic cancer cells has not yet been investigated. Therefore, the therapeutic window for the use of EGFR and HER-2 inhibitors can be exploited in ascitic cancer cell populations.

We hypothesise that clusters of EGFR/HER-2 positive ovarian cancer cells are more susceptible to inhibitors than receptor negative cells. We used ovarian cancer cell lines (OVCAR-5, SKOV-3 and OVCAR-4) and cells from ascitic fluids from 20 ovarian cancer patients. Cells were cultured in a non-adherent surface to encourage the cluster formation. Results showed that canertinib significantly inhibited cell growth and induced apoptosis in EGFR/HER-2 positive SKOV-3 clusters but gefitinib exhibited marginal effects. Cell cycle proteins and associated signaling proteins, pAKT and pERK were reduced in canertinib treated cells. Cells from ascitic fluids showed different levels of EGFR and HER-2 expressions. Both gefitinib and canertinib selectively reduced cellular metabolisms of EGFR positive ascitic cells, but only canertinib selectively showed the reduction of cellular metabolism in EGFR/HER-2 positive cells. There was no correlation between the expressions of protein receptors with drug sensitivity.

In conclusion, the SKOV-3 EGFR/HER-2 positive ovarian cancer cell line was very sensitive to an irreversible inhibitor, canertinib. However, ascitic ovarian cancer cells EGFR and HER-2 positive showed selective sensitivity to inhibitors. This modest response to the inhibitors may be due to compensatory activation by other proteins.

C35: Rural urban differences in breast cancer treatment in New Zealand

Chepulis, L.[1], Lao, C.[1], Lawrenson, R[1], Mark Elwood,[2] Diana Sarfati,[3] Ian Campbell[4]

[1]Waikato Medical Research Centre, University of Waikato, Hamilton, NZ, [2] School of Population Health, The University of Auckland, Auckland, NZ,[3] Department of Public Health, The University of Otago, Wellington, NZ; [4] School of Medicine, The University of Auckland, Auckland, NZ

Rural communities often experience reduced access to health care because of distance from specialist services and fewer general practitioners. This, in turn, can lead to a difference in health outcomes and inequity in certain regions of New Zealand. Approximately 30% of New Zealand's population lives rurally, though this reaches 60% in the Waikato region. In contrast, Auckland is predominantly considered to be an urban area.

Prospective data has been collected between 2000 and 2013 from the Waikato and Auckland Breast Cancer Registers, including a total of 12249 women diagnosed with invasive breast cancer. This data was analysed to assess the effect of geographical location (rural vs urban) on the treatment options administered to patients domiciled in these two regions.

Nineteen percent of patients (*n*=2364) lived in a rural area, including 311 Māori patients and 2053 non-Māori patients. A higher proportion of rural patients had no primary surgery (8.2% vs 6.5% for urban patients, p=0.003). This result was greater in Māori (12.2% vs 7.7%, p<0.001). Of those receiving surgery, rural patients were more likely to receive breast conserving surgery (58.7% vs 52.5%) and less likely to receive mastectomy than urban patients (41.3% vs 47.5%, p<0.001).

No significant differences were observed for the proportion of women who received radiotherapy or chemotherapy, though women who were ER/PR positive and living rurally were more likely to receive endocrine therapy (79.6% vs 73.5%, p < 0.001). In contrast, rural women who were HER2 positive were significantly less likely to receive Trastuzumab (48.1% vs 60.7%, p < 0.001).

C36: Expression variability is associated with breast tumour subtype

Wiggins, George[1], Pearson, John [2*], Black, Mik[3], Dunbier, Anita. [3], Walker, Logan.[1*]
[1]*Department of Pathology, University of Otago Christchurch, NZ.*
[2]*Biostatistics and Computational Biology Unit, University of Otago Christchurch, NZ.*
[3]*Department of Biochemistry, University of Otago Dunedin, NZ.*
[*]*Jointly supervised this work*

Mutations in *BRCA1* and *BRCA2* are associated with greater risk of developing breast and ovarian cancers. BRCA1 and BRCA2 proteins have an array of functions in cells, however heterozygous mutations only confer a significant increase of disease risk for breast and ovarian cancers, with minor effects in other tissues (e.g. pancreas). Furthermore, despite BRCA1 and BRCA2 shared function, their associated breast tumours differ in histological and molecular subtype. Approximately, 70% of *BRCA1*-related breast tumours display a basal-like phenotype (e.g. high grade and hormonal receptor negative), whilst *BRCA2*-related breast tumours are often luminal (ER positive). Identifying the molecular mechanism(s) of tissue-specificity for BRCA-associated tumours is key to understanding BRCA function in tumour suppression.

Gene expression studies have extensively interrogated breast cancers, including tumours from *BRCA1/2* mutation carriers, however results from these studies lack consensus. We demonstrate the application of an alternative statistical approach, which is to assess differential variability, thereby discovering genes that have a significant difference in variance between populations. We have interrogated expression variability in three familial breast cancer expression data-sets, and a 2116 sporadic breast cancer meta-cohort. We found that basal tumours and *BRCA1*-related tumours showed 39.9% (95% CI 39.4-40.3%) and 13.2% (95% CI 12.6-13.7%) increased expression variability when compared to non-basal and non-*BRCA1*/2 tumours, respectively. We identified pathways involved in immune response and cell-cell adhesion that are differentially variable between tumours of basal and non-basal subtype. Furthermore, 26 genes were differentially variable ($p < 0.01$) across all studies, including several genes (*ART3*, *EN1* and *PAX6*) that were associated with expression changes relative to ER status. These findings are now being investigated further in cell models of *BRCA1* mutation carriers. Our novel findings suggest cell adhesion and immune response may be more regulated in non-basal and non-*BRCA1* related tumours, and demonstrate the potential utility of measuring gene expression variability.

C37: Cancer-associated adipocytes alter protein expression profiles of human breast cancer cells *in vitro*

Crake, Rebekah[1]; Kleffmann, Torsten[2]; Morrin, Helen[1]; Strother, Matthew[1,3]; Robinson, Bridget[1,3]; Phillips, Elisabeth[1]; Currie, Margaret[1]

[1]University of Otago Christchurch, Christchurch, New Zealand, [2]University of Otago, Dunedin, New Zealand, [3]Canterbury Regional Cancer and Haematology Services CDHB, Christchurch, New Zealand.

Stromal adipocytes are likely the first cell type encountered by breast cancer cells as they metastasize. Adipocytes at the invasive edge of human breast tumours *in vivo* exhibit a modified phenotype, and consequently these adipocytes have been named Cancer-Associated-Adipocytes (CAA). Previous *in vitro* research has shown that co-culture with CAA promotes breast cancer cell resistance to radiation and some chemotherapies, as well as breast cancer cell invasiveness both *in vitro* and *in vivo*. The aim of the current research was to identify cellular proteins that are differentially regulated in human breast cancer cells co-cultured with CAA.

This study collected human breast adipose tissue samples, from which pre-adipocytes were isolated, differentiated into mature adipocytes, and co-cultured with human breast cancer cells (MCF-7 and MDA-MB-231) for 3 days. The proteomes of co-cultured and control breast cancer cells were compared quantitatively using iTRAQ labelling and mass spectrometry. Validation of iTRAQ results was performed by Western blotting.

This study identified and quantified a total of 1,126 and 1,218 proteins expressed in MCF-7 and MDA-MB-231 cells, respectively. On average, 85 proteins in CAA-MCF-7 and 63 proteins in CAA-MDA-MB-231 were differentially regulated by 1.5 fold or greater compared to control cells. Of these proteins, more were downregulated (n=53) than upregulated (n=32) in CAA-MCF-7, whereas more proteins were upregulated (n=51) than downregulated (n=12) in CAA-MDA-MB-231. Three proteins, PGK1 (phosphoglycerate kinase 1), PPGB (lysosomal protective protein) and VPS35 (vacuolar protein sorting-associated protein 35) were differentially regulated by 1.5 fold or greater in co-cultured cells. Western blotting results validated mass spectrometry ratios for three candidate proteins of interest.

Overall, this study is the first to investigate the effect of CAA on protein abundance of breast cancer cells *in vitro*, and provides a comprehensive platform for future research to identify novel protein targets associated with breast cancer cell-CAA crosstalk.

C38: Development of Plasma Genomic Biomarkers for Mutation Detection and Monitoring in New Zealand Cancer Patients

Fitzgerald, S.[1], Lasham, A.[1, 2], Blenkiron, C.[1], Shields, P.[1], Rolfe, G.[3], Stephens, R.[4], Mathy, J.[5], Martin, R.[3], Jackson, C.[6], Rodger, E.[7], Eccles, M.[7], Day, R.[7], Guilford, P.[7], Lawrence, B.[1], Print, C.[1, 2]

[1] University of Auckland, New Zealand, [2] Maurice Wilkins Centre, University of Auckland, [3] Waitemata District Health Board, New Zealand, [4]Cancer and Blood Service, Auckland City Hospital, New Zealand, [5]Counties Manukau Health, New Zealand, [6]Southern District Health Board, New Zealand, [7]University of Otago, New Zealand

Circulating tumour DNA (ctDNA) holds great promise, however despite the huge body of research, many challenges remain before ctDNA assays reach their full clinical and scientific potential. In New Zealand, liquid biopsies are well placed to improve inequities in cancer care observed in our rural, Māori and Pacific communities. In our laboratory, we are investigating methods to improve the sensitivity and accuracy to detect ctDNA associated mutations in plasma as well as optimal implementation in NZ. Three platforms have been assessed - a custom QiaSeq Targeted amplicon sequencing panel (199 amplicons), a custom BioRad droplet digital PCR assay (ddPCR) and Agena UltraSEEK panel. Plasma cfDNA containing a *KRAS* G12A mutation from a metastatic colorectal cancer patient, was spiked into a background of normal plasma to directly compare the sensitivity of mutation detection across three platforms. UltraSEEK and ddPCR platforms showed better sensitivity for detection of the *KRAS* G12A mutation, compared to the QiaSeq Targeted DNA sequencing panel, but the DNA sequencing panel has the advantage that novel and uncommon mutations can be identified in ctDNA from cancer patients. The robustness and potential clinical utility of these liquid biopsy platforms for ctDNA detection in plasma was then assessed for Stage III and Stage IV melanoma patients. We have successfully identified melanoma derived mutations and their changes over time in the ctDNA of several patients, and are continuing to monitor these mutations in the blood as the patients undergo treatment. It is hoped that our ability to analyse ctDNA directly from a blood sample, which will be especially tractable for patients in rural communities, will improve clinical monitoring of cancer patients in New Zealand. This work is supported by the Healthier Lives National Science Challenges – He Oranga Hauora.

C39: Genome-scale CRISPR/Cas9 knockout screens in cell line-derived xenograft models

Lee, T.W.[1,2], Hunter, F.[1,2], Li, D.[1], Tsai, P.[3], Kakadiya, P.[3], Shome, A.[1], Wrightson, E.[1], Lynch, C.[1], Bohlander, S.[2,3], Print, C.[2,3,4], Wilson, W.[1,2], Jamieson, S.[1,2,5]

[1]Auckland Cancer Society Research Centre, University of Auckland, Auckland, NZ. [2]Maurice Wilkins Centre for Molecular Biodiscovery, University of Auckland, Auckland, NZ. [3]Department of Molecular Medicine and Pathology, University of Auckland, Auckland, NZ. [4]Bioinformatics Institute, University of Auckland, Auckland, NZ. [5]Department of Pharmacology and Clinical Pharmacology, University of Auckland, Auckland, NZ.

Although molecular profiling of tumours has the potential to be used to individualise anticancer treatment, the genes that promote sensitivity or resistance to many drugs remain largely unknown. Forward genetic screens provide a means for unbiased and systematic discovery of such genes. We have established the ability to conduct such screens using CRISPR/Cas9 whole genome knockout in cultured human cancer cells. While *in vitro* screens are a powerful approach, the central role of the tumour microenvironment in cancer therapy means that the genes controlling drug sensitivity are not necessarily the same in cell culture and tumours. Therefore, we have developed a tumour xenograft CRISPR/Cas9 whole genome knockout model with high sgRNA representation that can be used for discovery of therapeutic genes *in vivo*. We first screened a panel of 25 head and neck squamous cell carcinoma (HNSCC) cell lines to select candidates suitable for screening *in vivo*. UT-SCC-54C, a pseudo-diploid HNSCC cell line with highly efficient xenograft growth was chosen. UT-SCC-54C cells were transduced with the GeCKOv2 CRISPR/Cas9 knockout library, which contains 123,411 different sgRNAs targeting human genes, microRNAs, and non-targeting controls, to generate a cell population with approximately 100 transduced cells/sgRNA. In xenograft experiments, the UT-SCC-54C GeCKO library was inoculated subcutaneously into NOD scid gamma (NSG) mice at a density of 10^7 cells/site. Deep sequencing of tumours collected after 11-14 days of growth with a mean volume of ~250 mm^3 revealed that 83-95% sgRNAs were detectable in individual replicate tumours, and 98% of sgRNAs detected among all tumours. In tumours allowed to grow for approximately 40 days (mean volume of ~1300 mm^3), ~20-40% of sgRNAs were detected in individual tumours with representation of >70% among all tumours. The high sgRNA representation in our xenograft model makes it suitable for genome-scale forward genetic screens *in vivo*.

C40: The potential use of ascorbate as an epigenetic therapeutic in acute myeloid leukaemia

Das, A.B.[1], Kakadia, P.M.[2], Morison, I.M.[3], Bohlander, S.K.[2], Vissers, M.C.M.[1]

[1]Department of Pathology and Biomedical Science, University of Otago, Christchurch, NZ, [2]Molecular Medicine and Pathology, University of Auckland, Auckland, NZ, [3]Department of Pathology, University of Otago, Dunedin, NZ

Acute myeloid leukaemia (AML) develops when genetic changes in precursor cells alter haematopoietic growth and differentiation, resulting in the accumulation of immature myeloid cells. The advent of next generation sequencing has allowed investigators to probe the genetic and epigenetic heterogeneity of this disease with unprecedented detail. Taking this heterogeneity into account, we aim to provide a rationale for the potential use of ascorbate as an adjunct treatment in selected cases of AML.

In AML, mutations that affect proteins involved in epigentic modification appear to be early drivers of the cancer phenotype and the reversibility of these modifications makes them attractive drug targets. Ascorbate has recently come to light as a potential candidate. It is an essential co-factor for the TET and Jumonji enzymes that demethylate DNA and histones, respectively. Ascorbate has also been shown to successfully restore differentiation in cell models of leukaemia where there is decreased TET activity, either due to loss of one allele, or inhibition by the oncometabolite 2-hydroxyglutarate.

Another line of evidence, and inspiration for our rationale, is the case study of an individual who responded to ascorbate treatment after failure to respond to two rounds of chemotherapy. High dose intravenous ascorbate treatment resulted in complete remission for two years after which relapse occurred. This patient's AML was positive for *NPM1* Type J mutation, which is a missense insertion of CCGG in exon 12. Interestingly, *NPM1* mutations are associated with a high co-occurrence of driver mutations in genes involved with DNA methylation (*DNMT3A* and *TET2*). In addition, methylation analysis of patients with *NPM1* mutations has previously shown four distinct subgroups including one with global hypermethylation. Collectively, these data indicate that further investigation of the effects of ascorbate in AML patients harbouring altered TET activity is warranted.

C41: Evaluating variants in breast cancer associated genes identified by panel sequencing of New Zealand breast cancer patients

Lattimore, V.L[1]; Lehnert, K.[2]; Lintott, C.[3]; Sullivan, J.[3]; Morrin, H.[1,4]; Robinson, B.[1,5] and Walker, L.C.[1]
[1] Mackenzie Cancer Research Group, Department of Pathology and Biomedical Science, University of Otago, Christchurch, New Zealand, [2] School of Biological Sciences, The University of Auckland, New Zealand, [3] Genetic Health Service NZ, South Island Hub, Christchurch Hospital, New Zealand, [4] Cancer Society Tissue bank, Department of Pathology and Biomedical Science, University of Otago Christchurch, New Zealand, [5] Canterbury Regional Cancer and Haematology Service, Canterbury District Health Board, Christchurch Hospital, Christchurch, New Zealand.

Routine diagnostic *BRCA1* and *BRCA2* gene screening is typically performed for individuals thought to have a high likelihood of carrying genetic changes that increase their susceptibility to breast cancer. Informed risk assessment for individuals who undergo genetic screening provides well-defined and actionable implications for disease prevention and treatment, critical for reducing their risk of developing and dying from the disease.
Genetic Health Service NZ offers *BRCA1*/*BRCA2* genetic screening to individuals predicted to be at high risk of breast cancer. While ~5% of these tests identify a clinically important mutation, it is unclear how many high risk mutation carriers fail to be referred for testing under existing guidelines. In addition, a significant proportion of these tests return a variant of unknown significance (VUS). Deciding who should receive genetic testing and interpreting the test results are two major dilemmas for health care professionals.

Here we have undertaken detailed genetic testing of 370 female breast cancer patients recruited by the Cancer Society Tissue Bank, together with nine VUS carriers and eight high risk women (with no known *BRCA1*/*BRCA2* mutations) from the New Zealand Familial Breast Cancer Study. Our study targeted the coding and non-coding regions of *BRCA1* and *BRCA2,* and the coding regions of *CDH1*, *PALB2*, *PTEN* and *TP53.*

1,685 variants were detected across the targeted genes in the cohort, including 81 VUS (MAF <0.05) in 108 individuals. Eleven known high risk variants were detected in 12 individuals (2 *BRCA1*, 10 *BRCA2* carriers), while 21 VUS (3 *BRCA1*, 11 *BRCA2*, 3 *PALB2*, 1 *CDH1*, 2 *TP53* and 1 *PTEN*) were prioritised for clinical classification through our involvement with the international expert panel ENIGMA.

These variant data will facilitate the development of novel technologies that improve current variant classification systems and therefore more informed clinical decision making for familial breast cancer patients and family members.

C42: Intravital optical window imaging of RhoA-, Rac1- and Akt-FRET biosensor mice monitoring drug treatment response in cancer.

Nobis M.[1], Warren, S.C.[1], Herrmann, D.[1], Conway, J.R.W.[1], Melenec, P.[1], Stoehr J.[1], McCulloch, A.T.[1], Welch, H.C.E.[3], Haigh, J.[4], Strathdee, D.[2], Blyth, K.[2], Sansom, O.J.[2], Morton, J.P.[2], Pajic, M.[1], Anderson, K.I.[5], Timpson, P.[1]

[1]Garvan Institute of Medical Research, The Kinghorn Cancer Centre, St Vincent's Clinical School, Faculty of Medicine, Sydney, NSW, Australia, [2]Cancer Research UK Beatson Institute, Glasgow, Lanarkshire, UK, [3]Signalling Programme, Babraham Institute, Cambridge, Cambridgeshire, UK, [4]Australian Centre for Blood Diseases, Monash University, Melbourne, Victoria, Australia, [5]Centre for Cancer Biology, SA Pathology and University of South Australia School of Medicine, University of Adelaide, Adelaide, South Australia, Australia, [5]Francis Crick Institute, London, UK

Small GTPases such as Rac1 and RhoA enable cells to migrate during development as well as metastasize during cancer progression by actively remodelling the cytoskeleton of cells. Co-option of this activity has been demonstrated both in mammary and pancreatic cancer. Furthermore, in pancreatic cancer the PI3K pathway is aberrantly regulated in ~21% of cases. More specific, time-resolved monitoring of these key drivers ranging from an *in vitro* to *in vivo* settings can be achieved by the use of FRET-biosensors and genetically engineered mice expressing these biosensors to track protein activity and the effect of therapeutic intervention.

Here, we describe the generation and characterization of these FRET-biosensor mice to examine RhoA[1], Rac1[2] and Akt kinase activity in an *in vivo* setting in a variety of cell types in homeostasis as well as in mouse models of cancer. Time-correlated single photon counting (TCSPC) fluorescent lifetime imaging (FLIM) on a multiphoton system of these signalling biosensors in live mice was achieved by the application of optical windows[3]. Elevated levels of Rac1 and RhoA activity were observed in models of invasive mammary and pancreatic cancer such as the polyoma-middle-T-antigen (PyMT) model and the KPC ($KRas^{G12D/+}$ and $p53^{R172H/+}$ driven) model. There, spatially defined to the invasive borders, high small GTPase activity was observed, absent in non-invasive mouse models such as the KC ($KRas^{G12D/+}$ alone) and KPflC ($KRas^{G12D/+}$ and p53 KO). Finally, spatiotemporally resolved imaging of the inhibition of RhoA, Rac1 and Akt activity live *in vivo* was achieved by employing optical windows implanted on top of developed tumours. Treatment was monitored for a period of up to 24h and the therapeutic response further correlated live to the local extra-cellular matrix and to the local vasculature. This allowed for unprecedented insight into treatment dynamics and the strong potential for further tailoring of targeted therapeutics in *in vivo* settings.

In conclusion, the development and use of the FRET biosensor mice represents a strong resource in understanding tissue context specific signalling events during cancer progression and drug target validation *in vivo*.

1. Nobis, M., Herrmann, D., Warren, S.C., *et al*. (2017) *A RhoA-FRET Biosensor Mouse for Intravital Imaging in Normal Tissue Homeostasis and Disease Contexts*. Cell Reports. 21, 274–288

2. Johnsson, A.-K.E., Dai, Y., Nobis, M., *et al*. (2014) *The Rac-FRET mouse reveals tight spatiotemporal control of Rac activity in primary cells and tissues*. Cell Reports. 6, 1153–1164

3. Ritsma, L., Steller, E.J.A., Ellenbroek, S.I.J., *et al*. (2013) *Surgical implantation of an abdominal imaging window for intravital microscopy*. Nature Protocols. 8, 583–594

C43: Evidence for a new mechanism of inhibiting an oncogenic PI3K enzyme

Flanagan, J.U..[1,2,3], Gong, G.Q.[3,4], Lee, W-J.[4], Dickson J.M.J.[3,5], Buchanan, C.M.[3,4], Rewcastle, G.W.[1,3], Gamage, S. [1,3], Denny, W.A.[1,3], Shepherd, P.R.[1,3,4]

[1]Auckland Cancer Society Research Centre, University of Auckland, Auckland, NZ, [2]Department of Pharmacology, University of Auckland, NZ, [3]Maurice Wilkins Centre for Biodiscovery, University of Auckland, Auckland, NZ, [4]Molecular Medicine and Pathology, University of Auckland, Auckland, NZ, [5]School of Biological Sciences, University of Auckland, Auckland, NZ.

The class IA phosphatidylinositol-3 kinase PI3K, phosphorylates the membrane embedded phospholipid phosphatidylinositol-4,5-phosphate (PI(4,5)P_2) making PI(3,4,5)P_3, which is a secondary messenger that promotes membrane localisation and activation of downstream kinases. To perform its reaction, PI3K binds ATP, interfaces with the cells plasma membrane to find PI(4,5)P_2, and binds other proteins including growth factor receptors and members of the Ras superfamily of proteins. The gene encoding PI3K is one of the most mutated in cancer, and many mutations create hyper-activated enzymes. Based on this, there is a huge effort to find inhibitors that are selective for PI3K and its oncogenic mutants. While these inhibitors are in clinical trial, there is little progress in discovering molecules that selectively block the oncogenic forms and only found in cancer.

We used computer aided drug design and chemical synthesis to make new molecules that were tested in biochemical assays of enzyme activity and in PI3K cell signalling assays. Pathway activity was detected by western blotting for phosphorylated AKT and measuring PIP_3 levels.

We identified one molecule that did not inhibit our biochemical assays, but blocked PI3K activity in cells in response to insulin stimulation. When tested it against cell lines harbouring PI3K wild type or oncogenic forms, and the compound selectively inhibited only one type of PI3K oncoprotein. PIP_3 levels were reduced by drug treatment, indicating an effect on PI3K activity. This was supported by little effect on any kinase that affects the levels of phospho-AKT or the kinases that synthesise PIP_2.

We hypothesise that our new compound inhibits formation of the PI3K signalling complex that is only formed by the oncogenic protein. These new data provide evidence that we need to rethink the drug discovery methods normally applied to this kinase and improve our knowledge about oncogenic signalosomes to find PI3K oncogene selective blockers.

C44: Personalised medicine approach in pancreatic cancer reveals fine-tuned stromal FAK manipulation improves global response the gemcitabine and Abraxane while sensitising circulating tumour cells to shear stress in transit

Kendelle J. Murphy[1], Morghan C. Lucas[1], Claire Vennin[1], James R.W. Conway[1], Sean C. Warren[1], Joanna N Skhinas[1], Romain Bidanel[1], Astrid Mangneau[1], Thomas R. Cox[1,2], Lisa Horvath[1], Yingxiao Wang[2], Jennifer P. Morton[4], Owen Sansom[4], Marina Pajic[1,2], David Herrmann[1,2*] and Paul Timpson[1,2*].

[1]Garvan Institute of Medical Research & The Kinghorn Cancer Centre, Cancer Division, Sydney, NSW 2010, Australia[2]St Vincent's Clinical School, Faculty of Medicine, University of NSW, Sydney, NSW 2010, Australia[3]Institute of Engineering in Medicine, University of California, San Diego, La Joll, CA, USA[4]Cancer Research UK, Beatson Institute, Glasgow, UK

Pancreatic ductal adenocarcinoma (PDAC) is predicted to be the second leading cause of cancer mortality by 2030. Current standard-of care gemcitabine/Abraxane is only marginally improved over previous monotherapy regimes, which are generally ineffective in this aggressive treatment-refractory disease. PDAC development occurs in a complex microenvironment, where extensive stromal desmoplasia alters mechanical tumour-stromal interactions promoting tumour progression and metastatic spread. In highly metastatic mouse models of PDAC, we observe enhanced extracellular matrix (ECM) deposition and remodelling throughout disease progression, which occurs in parallel with increased Focal Adhesion Kinase (FAK) expression and activity.

Stratified patient samples suggest a subset of patients with high FAK activity are likely to respond to FAK priming regimes, where fine-tuned ECM manipulation prior to chemotherapy may improve patient outcome. Consequently, we aim to fine-tune FAK inhibition (FAKi) of both the tumour and stromal compartments in primary, transient and secondary sites, while improving global response to standard-of-care. Intravital imaging of the FUCCI cell cycle reporter at secondary sites, post intrasplenic injection, was used to systematically demonstrate that FAKi modulates the ECM whilst also sensitising cells to shear stress prior to standard-of-care therapy, enhancing treatment efficacy whilst reducing metastatic spread. To assess the response of cells to FAKi, we measured real time treatment response *in vivo* with a Förster resonance energy transfer (FRET) biosensor for FAK activity. Here, parallel imaging of collagen by Second Harmonic Generation facilitated dynamic monitoring of tumour cell response to FAKi inhibition in the context of ECM organisation. This subtype-specific fine-tuned stromal manipulation may allow us to maximise gemcitabine/Abraxane therapy whilst reducing drug toxicity and potentially reducing further metastatic spread in patients.

C45: Characterisation of growth hormone signal transduction in New Zealand Melanoma (NZM) cell lines.

Sousa, K.S.[1], Perry, J.P.[2], Tran, K.B.[1], Kolekar, S.[1], Jain, L.[2], Lu, M.[2], Wang, Y.[2], Shepherd, P.R.[1,3]

[1]Molecular Medicine and Pathology, University of Auckland, Auckland, NZ, [2]Liggins Institute, University of Auckland, Auckland, NZ, [3]Auckland Cancer Society Research Centre.

Growth hormone (GH) and insulin-like growth factor 1 (IGF-1) promote important pleiotropic effects on growth and development. The GH signalling cascade is initiated by binding to a cell surface GH receptor (GHR) resulting in activation of JAK2, which in turn, promotes the phosphorylation of STAT5, PI3K/AKT and MAPK. Previous studies have reported high levels of GHR expression in melanoma cell lines.

This study aimed to identify NZ melanoma (NZM) cell lines sensitive to exogenous GH, prolactin (PRL), and the GHR inhibitor (B2036), and to delineate signalling pathway components. Twenty-four NZM cell lines from the Auckland Cancer Society Research Centre were analysed. Cells were treated with GH, PRL, or B2036. Phosphorylation of STAT5, ERK and AKT was assessed by western blotting or AlphaScreen assay. Cell viability was determined using resazurin. Total RNA was isolated, and mRNA expression assessed using a Nanostring PlexSet panel.

14 out of 24 cell lines responded to GH stimulation, and the majority of these responded to GHR inhibition. Activation of STAT5 in response to PRL was observed in one cell line. Analysis of mRNA expression demonstrated that the IGF1 receptor was highly expressed across all cell lines. *GHR* mRNA was mostly detected in GH-responsive cell lines. *GH* mRNA was not detected. GH increased cell proliferation in a subset of cell lines.

In conclusion, the majority of melanoma cell lines tested responded to GH treatment and GHR antagonism suggesting the blocking the actions of growth hormone might be useful to treat melanoma. Ongoing studies will characterise additional components of GHR signal transduction and will determine the efficacy of GHR antagonism in xenograft studies.

C46: Post-Tumour-Implantation Exercise does not alter Tumour Growth Rate in Mice with Subcutaneous B16-F10 Melanoma

Buss, Linda[1], Currie, Margaret[1], Ang, Abel[1], Hock, Barry[2] and Dachs, Gabi[1]

[1]Mackenzie Cancer Research Group and [2]Haematology Research Group, Department of Pathology and Biomedical Science, University of Otago, Christchurch, NZ

Exercise improves survival in breast and colorectal cancer patients, but it is unclear whether this extends to other cancer types, including melanoma. Mechanistically, preclinical rodent studies using orthotopic breast and prostate tumour models have shown increased tumour perfusion, blood vessel maturation and reduced hypoxia with exercise. Regular exercise increases mitochondrial biogenesis in muscle tissue, leading to an increase in mitochondrial proteins such as cytochrome C oxidase subunit 4 (COX-IV). However, it is unknown whether COX-IV expression increases with short-term exercise and whether this is proportional to exercise level.

We aimed to investigate the effect of exercise on tumour growth and the tumour microenvironment in subcutaneous melanoma, and to validate muscular COX-IV expression as a biomarker of exercise. We hypothesise that exercise normalises the tumour microenvironment, resulting in increased perfusion and reduced hypoxia.

Female C57BL/6 mice were randomly assigned to exercise or sedentary control groups following subcutaneous implantation of B16-F10 melanoma into the right flank. Exercising mice were provided with wheels to allow for voluntary running. When tumours reached maximum size, mice were injected with the hypoxia marker pimonidazole and the perfusion marker Hoechst 33342. Mice were then euthanised and the serum, left quadriceps femoris muscle and tumours removed for analysis.

We observed no difference in tumour growth in exercising compared with sedentary mice. There was no difference in perfusion or hypoxia in tumours from exercising vs sedentary mice. COX-IV expression was significantly increased in the muscle of exercising mice ($p=0.04$), but this did not correlate with either the average or cumulative distance run.

Our data show that short-term, post-tumour-implantation exercise does not significantly alter tumour growth rate, perfusion or hypoxia in subcutaneous melanoma, suggesting that the effects of exercise on cancer may be tumour type and/or location-dependent. In addition, short-term exercise increases muscular COX-IV expression in a non-dose-dependent manner.

C47: Engineered Nanoparticles as Potential Therapeutics for Acute Myeloid Leukemia

T. SYROVETS, C. LOOS, A.K. GAISER, S. HAFNER, M. SCHMIECH, T. SIMMET

Institute of Pharmacology of Natural Products & Clinical Pharmacology, Ulm University, Ulm, Germany

Acute myeloid leukemia (AML) is a malignancy associated with an unfavorable prognosis. Particularly, older patients suffer massively from the standard chemotherapy and have a 5-year survival of only 4%. Hence, new therapeutic agents with higher specificity and lower general cytotoxicity are urgently required.

We have previously discovered that amino-functionalized polystyrene nanoparticles (PS-NH_2) inhibit mTOR in leukemia cells. Accordingly, PS-NH_2 inhibit proliferation and induce G_2 cell-cycle arrest in three myeloid leukemia cell lines. Besides, PS-NH_2 trigger apoptosis in leukemia xenografts *in vivo*. At the molecular level, PS-NH_2 also inhibit downstream targets of mTOR, such as Akt and p70 ribosomal S6 kinase 1, followed by overexpression of the cell-cycle regulator $p21^{Cip1/Waf1}$ and degradation of cyclin B1. In leukemia cells, PS-NH_2 elicit autophagy followed by activation of caspase 3 and subsequent induction of apoptosis. By contrast, primary macrophages did not exhibit activated mTOR signaling and proved to be relatively resistant to the PS-NH_2-induced toxicity.

Similar to polystyrene particles, amino-functionalized gold nanoparticles (Au-NH_2) proved to be highly cytotoxic towards AML cell lines. Likewise, Au-NH_2 particles induce cell death in primary human leukemia cells derived from AML patients and reduce their colony-forming potential, whereas normal hematopoietic cells remain unaffected by the treatment with Au-NH_2. In agreement with the *in vitro* data, Au-NH_2 exhibited antileukemic efficacy against primary human AML xenografted into mice. Of note, systemic Au-NH_2 treatment was not associated with any detectable adverse events in treated mice. Thus, this engineered material holds great promise as a novel nanotherapeutic for treatment of acute myeloid leukemia independent of its cytogenetic profile.

C48: Targeting Long Non-Coding RNAs to Inhibit Colorectal Tumor Progression and Metastasis

Wright, B.M.[1], Diermeier, S.D.[1], O'Malley, M.[1]

[1]Department of Biochemistry, University of Otago, Dunedin, NZ.

Long non-coding RNAs (lncRNAs) are ribonucleic acid transcripts longer than 200 nucleotides in length that do not code for a protein. It is currently thought that around 60,000 lncRNA genes exist in the human genome [1] however the function of many of these genes are yet unknown. It has been found that lncRNAs play a role in disease development and cancer, thus illustrating their potential as drug targets.

Here, we investigate the role of one lncRNA in cancer using CRISPR/Cas9 genome editing and CRISPR interference (CRISPRi). CRISPRi is a method of repressing specific genes in cells by using catalytically inactive, "dead" Cas9 fused to a transcriptional repressor that sterically blocks RNA polymerase from transcribing the gene[4].

1. Iyer, Matthew K., Yashar S. Niknafs, Rohit Malik, Udit Singhal, Anirban Sahu, Yasuyuki Hosono, Terrence R. Barrette, et al. 2015. "*The Landscape of Long Noncoding RNAs in the Human Transcriptome*." Nature Genetics 47 (3): 199–208.

2. Yan, Xiaohui, Zhongyi Hu, Yi Feng, Xiaowen Hu, Jiao Yuan, Sihai D. Zhao, Youyou Zhang, et al. 2015. "*Comprehensive Genomic Characterization of Long Non-Coding RNAs across Human Cancers*." Cancer Cell 28 (4): 529–40.

3. Arun, Gayatri, Sarah D. Diermeier, and David L. Spector. 2018. "*Therapeutic Targeting of Long Non-Coding RNAs in Cancer.*" Trends in Molecular Medicine 24 (3): 257–77.

4. Qi, Lei S., Matthew H. Larson, Luke A. Gilbert, Jennifer A. Doudna, Jonathan S. Weissman, Adam P. Arkin, and Wendell A. Lim. 2013. "*Repurposing CRISPR as an RNA-Guided Platform for Sequence-Specific Control of Gene Expression*." Cell 152 (5): 1173–83.

C49: CRISPR/Cas9 screening to identify lncRNAS as novel therapeutic targets in triple-negative breast cancer

O'Malley, M.[1], Xiong, H.[1], Diermeier, S.[1]
[1]Department of Biochemistry, University of Otago, Dunedin, NZ.

Triple-negative breast cancer (TNBC) is a subtype of breast cancer classified by a lack of clinically significant levels of estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor 2 (HER2) in the patient's tumours. Absence of these typical markers of breast cancer mean that most TNBC patients cannot be treated by targeted therapies.

In recent years, long non-coding RNAs (lncRNAs) have emerged as a potential therapeutic target in cancer treatment. LncRNAs are a subtype of non-coding RNAs classified by a length of > 200 nt. By definition, they lack a significant open reading frame which means they are not translated into proteins. Although they do not code for proteins, lncRNAs are biochemically analogous to mRNA. Hundreds of lncRNAs have recently been discovered as key players in at least one of the hallmarks of cancer.

This project aims to assess the biological importance of lncRNAs in TNBC progression and metastasis. The lncRNAs of interest were chosen based on their overexpression in TNBC patients using data found in The Cancer Genome Atlas (TCGA). CRISPR interference (CRISPRi), a CRISPR/Cas9 system which uses a catalytically inactive Cas9 protein, is being employed to create lncRNA knockdown mutants of an epithelial human breast cancer cell line representing metastatic TNBC. We confirmed successful integration of the CRISPRi system into the TNBC cells and validated that the system was effective at silencing both protein-coding and lncRNA genes *in vitro.* We performed a loss-of-function screen using sequence specific single guide RNAs (sgRNAs) to assess the impact of lncRNAs on TNBC cell growth. This study aims to identify lncRNAs that are promising new therapeutic targets for TNBC.

C50: Is EBF3 promoter hypermethylation an oncogenic epi-mutation in multiple cancer types?

Dr. Michael Eccles[1], Dr Euan Rodger[1], Dr Aniruddha Chatterjee[1], Dr Peter Stockwell[1]
[1]*University Of Otago, Dunedin, New Zealand*

Recently we reported the identification of 75 candidate "driver" epimutations, which we hypothesize play a role in melanoma metastasis. For one of these epimutations in *EBF3*, we reported hypermethylation of the *EBF3* promoter and hypomethylation of the *EBF3* gene body in metastatic versus primary melanomas. There was no defining framework by which epimutations can generally be classified as "drivers" of metastasis. Therefore, in our report we proposed a definition whereby epigenetic drivers of metastasis should exhibit epigenetic alterations of large magnitude, and occur significantly more frequently in metastatic versus primary tumours. In addition, we proposed that epigenetic drivers should induce a functional change in the tumour cell phenotype. The approach we used of comparing paired metastasis and primary tumour samples from the same patient to identify epigenetic drivers is relatively new. As a consequence, we decided to investigate *EBF3* promoter and gene body methylation in a further series of both unpaired as well as paired metastatic/primary cancers of other types. We report here promoter hypermethylation and gene body hypomethylation of *EBF3* occurred in a colorectal cancer primary/metastasis pair. Furthermore, in several additional unpaired primary and metastatic tumour samples, promoter hypermethylation and gene body hypomethylation of *EBF3* was common, whereas the *EBF3* promoter was completely hypomethylated and the gene was body hypermethylated in 5/5 normal tissues examined. Therefore, in summary, in an additional primary/metastasis cancer pair we confirmed that characteristic metastasis-associated promoter hypermethylation occurs in *EBF3*. However, unexpectedly we observed that *EBF3* promoter hypermethylation was also frequently observed in cancer compared with normal tissues where it was generally hypomethylated, suggesting it has a more general oncogenic driver role.

C51: Quantifying PD-L1 expression in response to oestrogen deprivation in oestrogen receptor positive breast cancer

McGuinness, Conor[1] and Dunbier, Anita[1]

[1]Centre for Translational Cancer Research and Department of Biochemistry, University of Otago, Dunedin, New Zealand

Up to 50% of ER+ breast cancer patients do not respond to endocrine therapy, and many who do respond ultimately develop resistance to therapy. Our research has identified an immune-related signature that correlates with poor response to antioestrogen treatments (Dunbier *et al.*, 2013). Programmed cell death ligand 1 (PD-L1), encoded by the gene *CD274,* is normally expressed to inhibit autoimmune responses by binding to programmed death 1 (PD-1), expressed on T-cells. Cancer cells express PD-L1 in order to evade the immune system. Inhibitors of the PD-1/PD-L1 axis, a class of immune checkpoint blockade (ICB) drugs, have been approved for treatment of melanoma and advanced non-small cell lung carcinoma.

The aim of this project is to determine whether ICB could be used in combination with anti-oestrogen therapy to improve response rates in breast cancer patients. We hypothesised that *in vitro* oestrogen deprivation, which mimics treatment with an anti-oestrogen treatment, would induce cultured ER+ breast cancer cells to express PD-L1. We deprived MCF-7 cells of oestrogen and harvested RNA and protein samples at 0, 24, 72 and 120 hours after oestrogen deprivation. We compared expression levels of *Cd274* and PD-L1 to oestrogen treated cells using qPCR and Western blots.

At all time points after 0 hours, relative expression of *CD274* was higher in oestrogen deprived cells compared to oestrogen treated cells, however a statistically significant difference was only observed at 120 hours (Sidak's multiple comparisons test, $p<0.001$).

These results demonstrate that PD-L1 expression increases in response to oestrogen deprivation *in vitro*. This suggests that antioestrogen therapy has the potential to alter the way in which ER+ tumour cells interact with the immune system. This work justifies further research into the potential of ICB in overcoming resistance to anti-oestrogen therapy.

Dunbier, A.K., Ghazoui, Z., Anderson, H., Salter, J., Nerurkar, A., Osin, P., et al. (2013) *Molecular Profiling of Aromatase Inhibitor–Treated Postmenopausal Breast Tumors Identifies Immune-Related Correlates of Resistance.* Clinical Cancer Research. ***19****: 2775–2786.*

C52: Identifying synthetic lethal vulnerabilities in cohesin-haploinsufficient MCF10A cells

Chue Vin, Chin[1], Jisha Antony[1], Jinshu He[2], Kate M. Parsons[2], Amee J. George[2,3], Ross D. Hannan[2] Julia Horsfield[1]

[1]Department of Pathology, University of Otago, Dunedin, NZ
[2]ANU Centre for Therapeutic Discovery, John Curtin School of Medical Research, Australian National University, Acton, Australia
[3]ACRF Department of Cancer Biology and Therapeutics, John Curtin School of Medical Research, Australian National University, Acton, Australia

Cohesin is a multi-subunit protein complex that coordinates sister chromatid segregation during cell division. Cohesin complex is also important for DNA damage repair, ribosomal biogenesis and spatial organization of the genome. Recent cancer genome sequencing efforts have identified recurrent mutations in genes encoding cohesin complex subunits across multiple cancer types. Cohesin mutations promote cancer development by blocking cell differentiation and promoting self-renewal, but because cohesin is essential to cell viability, cohesin mutations also expose cells to vulnerabilities that can be targeted in cancer cells. Finding genes or pathways whose inactivation would kill only cohesin mutated cells would therefore lead to new therapies for cancer patients with cohesin mutations.

Synthetic lethality occurs between two genes when mutation of either gene alone enables cell viability but simultaneous perturbation of two genes results in cell death. To identify synthetic lethal vulnerabilities in cancer harbouring mutations in cohesin complex genes, we generated isogenic MCF10A cell lines with cohesin haploinsufficiency using CRISPR-Cas 9 gene editing. We provided evidence that haploinsufficiency of cohesin complex genes in MCF10A cells is associated with several cancer phenotypes such as increased DNA damage, defective mitotic checkpoints and disrupted nucleolar formation.

We are currently optimising a high throughput synthetic lethal screen of approximately 3000 chemical compounds, including FDA-approved drug, kinase inhibitors and epigenetic modifiers, using a panel of MCF10A isogenic cell lines with a heterozygous cohesin deletion. We will first evaluate differential drug activities by calculating a ratio of normalized percent viability in MCF10A isogenic cell lines and analysing dose-response parameters. A selection of synthetic lethal hits will then be further evaluated and validated in a secondary screen to monitor phenotypic responses to compounds such as DNA damage, nucleolar and nuclear phenotypic changes. These cellular phenotypes can serve as a basis to understand the pathway underlying the mechanism of cohesin mutations in cancer.

C53: Investigating the role of DNA methylation, gene expression, long non-coding RNA and transposable elements in constitutive PD-L1 expression in melanoma cell lines

Ahn, A.[1], Rodger, E.J.[1, 2], Stockwell P.A.[1], Parry M.,[3] Motwani J.[1], Gallagher S.J.[4], Shklovskaya E.[4], Tiffen J.[4], Hersey P.[4], Chatterjee, A.[1,2] † Eccles, M.R.[1,2] †

[1]Department of Pathology, University of Otago, Dunedin, NZ. [2]Maurice Wilkins Centre, NZ. [3]Department of Mathematics & Statistics, University of Otago. [4]The Centenary Institute, University of Sydney

† Joint senior authorship

Many cancers suppress the anti-tumor immune response by expressing the PD-L1 immune checkpoint protein. Several mechanisms that regulate PD-L1 levels have been uncovered, although whether DNA methylation plays a role in PD-L1 regulation remains unclear. In this research we have shown that marked loss of global DNA methylation, particularly in intergenic regions and repeat elements, is associated with constitutive expression of PD-L1 in melanoma cell lines compared to cell lines without PD-L1 expression. Additionally, genome-wide transcription levels, including immune response genes, were significantly upregulated in constitutive PD-L1 expressing cell lines, as well as in lymphocyte negative, PD-L1 positive melanomas (equivalent to PD-L1 constitutive), versus PDL1 negative melanomas in The Cancer Genome Atlas database (TCGA). Finally, decitabine-mediated inhibition of global methylation in melanoma cells led to increased PD-L1 and immune response gene expression. Therefore, we identified that global DNA demethylation influences PD-L1 expression in melanoma, and hence the ability of melanoma cells to evade anti-tumor immune responses. To explore this further, we investigated the role of long non-coding RNA (lncRNA) and transposable elements in regulating PD-L1 using RNA-seq data. We found upregulation of many intergenic lncRNA's, which are in the genomic vicinity of many immune related protein-coding genes. Moreover, we identified candidate endogenous retrovirus elements that may play a role in PD-L1 expression. Overall, these results have implications for combining epigenetic therapy with immunotherapy.

C54: p53 does not cause growth arrest or death in normal B cells but only affects division rate

Ray S.[1], McKinney C. [1], Roth L.[1], Heinzel S.[3], Hodgkin P.[3], Mehta S.[1,2], Slatter T.L.[1,2], Braithwaite A.W.[1,2]

[1]Department of Pathology, Dunedin School of Medicine, University of Otago, Dunedin, New Zealand.
[2]Maurice Wilkins Centre for Molecular Biodiscovery, New Zealand.
[3] Walter and Eliza Hall Institute of Medical Research, Melbourne.

The tumour suppressor p53 is a well-known transcription factor that is necessary to prevent tumours. It is thought to function by cell cycle arrest, senescence or apoptosis to prevent clonal expansion of mutated cells. Cancer cells are mostly studied to understand the role of p53. To investigate p53's functions in normal cells, we studied murine splenic primary B cells. We compared cell growth and survival parameters in p53 knock out cells (p53-/-) with p53 wild type cells (p53+/+) with and without treatment with a p53 agonist (nutlin). The results showed a reduced viable cell number in nutlin treated cells as expected. However, mathematical modelling (Hawkins et al., 2007) showed p53 was not inducing growth arrest or death, as this was happening at the same time as cells without p53. The only observed difference was the rate of division with increased p53 activity associated with a reduced division rate. These results suggest that p53 only regulates the rate of division, but not the time to arrest and die which are instead imprinted intrinsic cellular mechanisms. To determine how p53 affects the rate of cell division, current work is investigating possible candidate pathways used by p53.

C55: Co-targeting BRAF and VEGF receptors synergistically inhibited the growth of BRAF-mutant and BRAF wild-type melanomas

Tran, K.B.[1,2,3], Jamieson, S.[1,3], Print, C.[1,3], Shih, J.H.[1,2], Wang, Q.[1,2], Tsai, P.[2], Blenkiron, C.[2], Kolekar, S.[1], Hunter, F. [1,3], Jabed, A.J.[2], Seyfoddin, V[1,2], Buchanan, C.[2,3], Baguley, B.[1,3], and Shepherd, P.R. [1,2,3]

[1]Auckland Cancer Society Research Centre, University of Auckland, New Zealand
[2]Department of Molecular Medicine and Pathology, University of Auckland, New Zealand
[3]Maurice Wilkins Centre, University of Auckland, New Zealand

BRAF inhibitors such as vemurafenib (VEM) are only effective as single agent mealnoma therapy in BRAF-mutant melanomas and resistance to the treatment develops within 6 to 12 months. We investigated whether targeting VEGF receptors could increase the efficacy of the BRAF inhibition therapy. We measured levels of VEGF-A secretion from our unique NZM panel of melanoma cell lines. Variations of the VEGF pathways in these cells were analysed by exome sequencing, RNASeq and western blotting. Xenograft and syngeneic models were used to investigate efficacy and safety of a combination of VEM and the VEGFR2 inhibitor axitinib (AXI) *in vivo*. Species specific tumour RNA sequencing was performed to identify pathways uniquely affected by the drug combination in tumour cells and host stroma. RNAscope and immunohistochemistry were used to further analyse effects of the drugs in the tumours.

V600E-mutant melanoma cell lines secreted VEGF at significantly higher levels compared to lines with RAS mutations or nonBRAF/nonRAS lines. VEM downregulated VEGF secretion in V600E-mutant cell lines but not in RAS-mutant or nonBRAF/nonRAS cell lines. We found that the VEM + AXI combination synergistically inhibited the tumor growth. Interestingly, the combination also inhibited the growth of BRAF-wildtype xenografts and syngeneic B16 tumors. When AXI was replaced with our in-house VEGFR2 inhibitor SN35332, the combination also provided synergistic effect, suggesting the combined effects were likely pathway specific. Pathway-related synthetic lethality was identified in EMT, p53, TGF-beta, and angiogenesis hallmark pathways. Finally, we developed a cell line resistant to vemurafenib and showed that the combination of VEM + AXI resensitized the tumors to BRAF inhibition therapy.

Together, this study provides an important link between the VEGF axis and BRAF signalling in melanoma biology and co-targeting those two axes could enhance the efficacy of BRAF inhibition therapy not only in BRAF-mutant but also in BRAF-wild type tumours.

C56: Cathepsin D: a protease attracts mesenchymal stem cells for homing to tumor sites

Gowthami vangala[1], Andrew Crdige[2], Sarah baird[1]

[1]Department of Pharmacology and Toxicology, University of Otago, [2]Departemnt of Biochemistry, University of Otago, NZ

Bone marrow derived mesenchymal stem cells (MSCs) have been identified at the sites of tumor, where they can act as pro or anti-tumorigenic. The specific signalling molecules and proteases secreted by the tumor cells that activate MSCs to mobilize to the sites of tumors are still unclear. Here, we described the role of one of the proteases, cathepsin D in homing the MSCs to the tumor sites through *in vitro* studies.

MDA-MB-231 and HT29 cells or their conditioned media were co-cultured in lower wells of Transwell insert with upper wells consisting of MSCs with or without protease inhibitors to analyse the migration (collagen I coated) and invasion (through matrigel) assays.

Here, we investigated the migration and invasion of MSCs induced by the cancer cells and their 3 day conditioned media were found to be increased. Increased levels of proteases could play a major role in regulation of the tumors, so we focussed on proteases by which MSCs get attracted to the tumor cells. We noticed that migrations were decreased with a wide range of protease inhibitors ($P<0.05$, ANOVA, n=3 with pepstatin A and GM6001 in both the cell lines). Pepstatin A is a potent inhibitor of cathepsin D (cath D), consistently decreased the migrations of MSCs. Furthermore, cath D expression was strongly detected in tumor cells when compared to the MSCs. To confirm the effects of cath D, it is inhibited by siRNA which also showed decrease in migration (231 cells, $P<0.001$, n=3 and HT29 cells $p<0.001$, n=3) and invasion (231 cells $P<0.001$, n=3 and HT29 cells $P<0.0001$, n=3) of MSCs. The signalling pathway MAPK/ERK was found to be activated and addition of pepstatin A reversed this activation to a significant level.

These findings provide the first evidence that cathepsin D is an important protease that plays a role in attracting the MSCs. We demonstrated for the first time that cancer cells secreted cathepsin D which increases migration and invasion of MSCs and ERK signalling pathway may be involved in this MSCs migration by cancer cells.

C57: Investigating the Clinical Significance of Fibroblast Growth Factor Inducible 14 in Breast Cancer

Harris, J.J.[1], Black, M.A.[1], Miller, L.D.[2], Cunliffe, H.E.[1]

[1]Department of Pathology, University of Otago, Dunedin, NZ, [2]Wake Forest School of Medicine, Winston-Salem, USA.

Breast cancer accounts for over 600 deaths per year in New Zealand women, primarily due to metastatic disease progression. Fibroblast Growth Factor Inducible 14 (Fn14), is a cell surface receptor overexpressed in the majority of invasive breast cancers and not in normal breast epithelium. Functional studies in-vitro show that Fn14 is an active promoter of breast cancer cell invasion. While overexpression of Fn14 correlates with clinical markers of breast cancer progression, the clinical significance of Fn14 as a prognostic biomarker has not yet been explored. This study aims to define the clinical utility of Fn14 expression in breast cancer by using gene expression profiles from a combined cohort of 2116 breast cancer patients curated from 14 independent studies.

Fn14 expression was significantly associated with tumours defined immunohistochemically as ER-negative ($p < 0.0001$), HER2-positive ($p < 0.001$) or Triple-Negative (TNBC) ($p < 0.031$). Further associations were observed for tumours intrinsically defined as Basal-like ($p < 0.0001$) or HER2-enriched ($p < 0.0001$). No association was observed for Luminal A or B (ER-positive) tumours. Multi-variable Cox proportional hazards modelling revealed that patients in the upper tertile of Fn14 expression, compared to the lower tertile, had an increased risk of a metastatic event when all other clinical factors were held constant (HR 1.83 [95% CI 1.49-2.25]; $p = 0.004$). Survival analysis revealed that Fn14 expression improved the prognostic accuracy of Distant Metastasis-Free Survival (DMFS) in hormone-receptor negative (ER $p = 0.022$, PR $p < 0.005$), HER2-positive ($p = 0.007$), and TNBC ($p = 0.025$) patients by significantly stratifying them into further risk groups, with higher Fn14 expression tertiles correlating with poorer DMFS. These findings indicate that Fn14 is a significant independent prognostic biomarker of DMFS that warrants further investigation in patients with ER-negative, HER2-positive, and TNBC disease.

C58: The role of the p53 isoform Δ133TP53 in treatment resistance.

Eiholzer R.A.[1], Kazantseva M.[1,2], Mehta S.[1,2], Roth I.[1], Taha A.[3], Hung N.A.[1], Slatter T.L.[1,2], Braithwaite A.W. [1,2].

[1]Department of Pathology, Dunedin School of Medicine, University of Otago, Dunedin, New Zealand.
[2]Maurice Wilkins Centre for Molecular Biodiscovery, New Zealand.
[3]Department of Neurosurgery, Southern District Health Board, New Zealand.

Tumour protein 53 (TP53) is a tumour suppressor responsible for triggering cell cycle arrest, DNA repair or apoptosis in response to cellular stress and DNA damage. TP53 has many isoforms including the Δ133TP53 isoform, which has been associated with inflammation, angiogenesis, migration, and invasion. Recent work has shown an association between increased *Δ133TP53* expression and a poorer prognosis for those with glioblastoma.

To investigate the possible association between Δ133TP53 isoform expression and treatment resistance in glioblastoma, *in vitro* experiments using a mouse embryonic fibroblast cell line that was engineered to express either the mouse mimic of the Δ133TP53 isoform, known as Δ122TP53, or an empty control vector (p53 null) were done. Cells were treated with temozolomide, a chemotherapeutic drug used to treat glioblastoma and *tert*-butyl hydrogen peroxide (tBHP), a compound that causes oxidative stress. Results from these experiments showed that cells that express Δ122TP53 showed improved cell survival in response to both temozolomide and oxidative stress when compared to p53 null cells suggesting resistance to some forms of DNA damage and cellular stress. Temozolomide causes single stranded DNA damage suggesting a possible involvement of Δ122TP53 in repairing this damage. Current *in vitro* work involves using a variety of other therapeutic drugs that work through different DNA damage mechanisms to gain a better understanding of the spectrum of treatment resistance associated with Δ133TP53. Future directions of this project will test for treatment resistance *in vivo* using a new mouse glioblastoma model that expresses Δ122TP53 only in glial cells. This model will also allow us to investigate and develop treatment regimens to target Δ133TP53 tumours.

C59: Investigation of Mitochondrial Transfer in Human Erythroblasts

Lewer, B.[1,2], Weinkove, R.[2,3], Berridge, M.[2], Rowe, M.[1], McConnell, M.[1,2]
[1]Centre for Biodiscovery, School of Biological Sciences, Victoria University of Wellington, NZ,
[2]Malaghan Institute of Medical Research, Wellington, NZ, [3]Wellington Blood and Cancer Centre, Capital and Coast District Health Board, Wellington, NZ

Mitochondria are the main energy producers of mammalian cells. Popular belief suggests that mitochondria are confined to the single cell they were derived in, however there is an increasing body of knowledge and evidence to suggest that mitochondria can be transferred between different cell types. Research is primarily conducted in cancers and injured cell models. Surprisingly, there is minimal data exploring mitochondrial transfer between normal human cells. We will explore mitochondrial transfer in primary human cells *ex vivo* using erythroblasts generated from whole peripheral blood of healthy donors. Initial studies have been performed with human erythroblast-like cancer cell lines – HL-60, HEL and K562, which each have mitochondrial DNA polymorphisms that allow cell-line specific detection of mitochondria.

HL-60□0 are HL-60 cells depleted of mitochondrial DNA using low-dose ethidium bromide, which depend on pyruvate and uridine supplementation for survival in culture. To determine whether HL-60□0 cells would take up mitochondrial DNA under metabolic stress, they were cultured for 96 hours in the presence or absence of pyruvate and uridine either alone, co-cultured with HEL cells, or in filtered conditioned media derived from HEL cells. Viability was assessed via after 24, 48 and 96 hours using flow cytometry. We found that HL-60□0 survival and proliferation increased when in conditioned media, and more so when in direct co-culture with another cell. To determine whether this was associated with uptake of mitochondrial DNA by HL-60□0 cells, specific mitochondrial polymorphisms were analysed from HL-60□0 cells cultured in HEL-conditioned media using allele-specific qPCR. Presence of HEL mitochondrial DNA was not detected in HL-60□0 populations using this technique. This indicates that physical contact may be required for transfer of mitochondrial DNA in this context, or cells may require more time to establish the polymorphism within the population.

C60: The NMDA receptor is an important component of intracellular calcium homeostasis in megakaryocytic leukaemia cells, and its loss of function promotes cell differentiation

Hearn J.I. [1], Green T.N. [1], Nursalim Y.N. [1], Ladvanszky L. [1], Chopra M. [1], Poulsen R.C. [2], Blenkiron C. [1], Bohlander S.K. [1], Kalev M.L. [1]

[1]Department of Molecular Medicine and Pathology, University of Auckland, Auckland, NZ,
[2]Department of Medicine, University of Auckland, Auckland, NZ

The biology of megakaryocytes (bone marrow platelet progenitors) and the cancers arising from them are poorly understood. With no specific treatments available, patient outcomes are poor. Calcium homeostasis is where intracellular calcium concentration is maintained at a steady state. On this homeostatic background, calcium signalling mediates processes key to cancer pathogenesis (including cell proliferation, differentiation, and death) by oscillating calcium concentration. The discovery of mutations in *CALR*, a gene involved in calcium homeostasis, has implicated abnormal calcium pathways in megakaryocytic malignancies.

We hypothesise deregulation of calcium homeostasis leads to widespread changes in the calcium signalosome that promote malignancy. We have previously identified the NMDA receptor (NMDAR) as a novel pathway for calcium entry into megakaryocytes; NMDAR activity promotes proliferation of megakaryocytic leukaemia cell lines, implying NMDAR calcium entry supports leukaemia growth[1]. The aim of this study was to determine the mechanism through which NMDAR-mediated calcium entry supports malignancy.

We used CRISPR-Cas9 to interrupt expression of the obligatory NMDAR subunit, GluN1, in Meg-01 cells. NMDAR loss-of-function was confirmed by reduced binding of radiolabelled NMDAR ligand and loss of NMDAR-mediated calcium entry. Cells showed increased myeloid differentiation as demonstrated by flow cytometry. Calcium flux after thapsigargin stimulation demonstrated endoplasmic reticulum (ER) calcium stores were depleted. Increased expression of ER stress genes and increased levels of lipidated LC3 indicated induction of ER stress and autophagy. We observed reduced BrdU incorporation and elevated lactate dehydrogenase release. RNA microarray analysis showed differential expression of calcium homeostasis genes. Our results indicate NMDARs regulate intracellular calcium homeostasis in megakaryocytes and that calcium pathways are important in leukaemia proliferation, suggesting a novel pathway for leukaemia modulation.

1. Kamal, T. *et al. N*-methyl-d-aspartate receptor mediated calcium influx supports in vitro differentiation of normal mouse megakaryocytes but proliferation of leukemic cell lines. *Res. Pract. Thromb. Haemost.* **2,** 125–138 (2018).

C61: Antihelminthic benzimidazoles mebendazole and albendazole kill cancer cells via classical apoptosis

Petersen, J.S.S.M, Baird, S.K.
Department of Pharmacology & Toxicology, University of Otago, Dunedin, N.Z.

Antihelminthic benzimidazole drugs mebendazole and albendazole are commonly used to treat a variety of worm infestations in humans. Their mechanism of action against helminths is well-established and involves the inhibition of microtubule formation. Mebendazole has recently shown promising results in pre-clinical *in vitro* and *in vivo* cancer studies and is currently in Phase I trials for treatment of glioma. However, the way in which it causes cell death in cancer cells has not been fully explored.

Here, the *in vitro* analysis of the anticancer mechanism of action of mebendazole and a structural analogue albendazole was undertaken. The two drugs were screened for cytotoxicity in three cancer cell lines (MDA-MB-231, MCF-7, HT-29) and one non-transformed mesenchymal stem-cell line (RCB2157), using the MTT assay. Their effects on the cell cycle and cell death mechanisms were analysed using flow cytometry and fluorescent microscopy and spectroscopy.

Mebendazole and albendazole were found to selectively kill cancer cells, being most potent in the colorectal cancer cell line HT-29, with IC_{50} values of 1.3 ± 0.1 µM and 1.4 ± 0.1 µM, respectively. Both mebendazole and albendazole induced caspase-3 activation 3-4-fold. Phosphatidylserine exposure, mitochondrial and lysosomal membrane permeability and reactive oxygen species production were all significantly increased compared to control and peaked at 24 hours, with DNA fragmentation increasing in a time-dependent manner peaking at 48 hours. Using Hoechst 33342 staining, nuclear features of apoptosis such as chromatin condensation were found following treatment with both drugs. Cell cycle arrest in the G2/M phase was found, and tubulin structures were significantly altered. Mebendazole and albendazole appear to cause cancer cell death via a mechanism of classical apoptosis and cell cycle arrest, which may originate from the destabilisation of microtubules.

C62: Validation of an Androgen Bioassay for Measurement Breast Cancer Patient Serum.

Lund, R.A.[1] and Heather, A.K.[1]

[1]Department of Physiology, University of Otago, Dunedin, New Zealand.

Menopause is a high-risk factor for estrogen receptor positive (ER+) breast cancer. Adrenal-derived androgens may underpin this risk however little is known about underlying mechanisms. While analytical chemistry techniques measure the concentration of known androgens, net androgenicity of breast cancer patient serum may provide a more informative assessment of breast cancer risk since known and unknown androgens can be measured. Before such studies can be conducted, androgen receptor (AR) bioassays need to be validated as a tool to measure serum bioactivity. The aim of this study was to evaluate a yeast cell- and human embryonic kidney (HEK293) cell AR bioassay for the reliable measurement of serum AR bioactivity. AR bioassays comprise a cell stably co-transfected with an AR expression plasmid and an androgen sensitive reporter plasmid. Proteins such as albumin and steroid hormone binding globulin bind 98% of androgens and upon heat denaturation of the proteins, bound androgens are released. In the HEK293-AR bioassay, heating testosterone-spiked serum consistently increased AR bioactivity by up to 137%, whereas the yeast-AR bioassay produced inconsistent results. Expected sex differences in AR bioactivity of male and female serum were observed (average of >73,000 units in male serum versus <1000 units in female serum, $p<0.001$). Heating male and female serum augmented AR bioactivity up to 145% and 145,000%, respectively. Taken together, these results show that the HEK293-AR bioassay reliably outperforms the yeast-AR bioassay for measuring serum AR bioactivity and assay performance is further improved by heat treating serum. This study provides a platform for future endeavours to measure AR bioactivity in the serum of patients at risk of ER+ breast cancer relapse.

C63: The regulation of the tumour hypoxic response by ascorbate and association with clinicopathological factors and patient outcome in breast cancer

Elisabeth Campbell[1], Gabi U Dachs[1], Helen R Morrin[1,2], Valerie Davey[3], Bridget A Robinson[1,4], Margreet CM Vissers[5]

[1]Mackenzie Cancer Research Group, Department of Pathology, University of Otago, Christchurch, New Zealand; [2]Cancer Society Tissue Bank, Christchurch, New Zealand; [3]Christchurch Breast Cancer Patient Register, Christchurch Hospital, Christchurch 8011, New Zealand; [4]Canterbury Regional Cancer and Haematology Service, Canterbury District Health Board, Christchurch, NZ; [5]Centre for Free Radical Research, Department of Pathology, University of Otago, Christchurch, New Zealand

High expression of the global transcription factor HIF-1 allows cellular adaptation to the hypoxic tumour microenvironment, regulating tumour growth and spread. We have determined the HIF-1 pathway activity in banked tumour tissue from a cohort of 52 women with invasive ductal carcinoma by analysing HIF-1α protein and downstream gene targets (CAIX, BNIP-3 and VEGF). HIF-1α protein and HIF-1 activity score were elevated in higher grade tumours and in necrotic or large tumours, and HIF-1 activity was found to be strongly associated with ten-year progression-free and overall patient survival.

HIF-1 activity is controlled by post-translational hydroxylation of specific proline and asparagine residues on the HIF-1α protein subunit. The hydroxylases responsible for this reaction require oxygen and 2-oxoglutarate as substrates, and iron and ascorbate as cofactors. Our previous analyses of human endometrial and colorectal cancer samples, and mouse tumour models, have demonstrated a strong inverse association between tumour ascorbate levels and HIF-1 pathway activity.

Tumour ascorbate levels were measured in the cohort of 52 breast cancer patients and the association between ascorbate content, HIF-1 activity and clinicopathological data was determined. Lower tumour ascorbate levels were associated with younger patient age, more high-grade tumours, increased tumour size and more necrotic tumours. HIF-1α protein levels and HIF-1 pathway activity were lower in tumours with increased ascorbate, with the highest levels having a highly significant impact.

Data from this study indicate that optimum intracellular ascorbate can dampen the hypoxic response, and this may be of clinical importance. We hypothesise that ascorbate levels above a given threshold are able to moderate the hypoxic response and could reduce HIF-driven tumour aggression. The potential regulation of HIF-1 by modulation of tumour ascorbate content is recommended for future prospective clinical studies.

C64: A mitochondria-selective near-infrared-emitting fluorescent dye for cellular imaging studies

Peter Choi[a], Katsuya Noguchi[b], Munetaka Ishiyama[b], William A. Denny[a], Jiney Jose[a]
[a]Auckland Cancer Society Research Centre, School of Medical Sciences, University of Auckland, Private Bag 92019, Auckland 1142, New Zealand
[b]Dojindo Laboratories Co., Ltd, Techno-Research Park Tabaru 2025-5 Mashiki-machi, Kamimashiki-gun, 861-2202, Japan

Organelle selective fluorescent probes are useful tools for delineating functional and morphological changes of various organelles, especially in a diseased state. There are number of such probes reported in the literature, exemplifying the detailed synthetic efforts various groups have put in over the years to make them available to the scientific community. These probes are biocompatible, nontoxic and mostly selective towards their target. Although much work has been accomplished with molecules emitting in the 400 – 600 nm range, there still exists an opportunity to fine tune their emission towards the near infrared region (700 -1000 nm) wherein there is minimal back ground fluorescence due to absorbing cellular entities and much greater tissue penetration.

An ongoing research project in our group focuses on developing organelle selective fluorescent probes for studying disease initiation at a cellular level. Mitochondrial malfunction is associated with many diseases such as cancer, arthritis and heart disease. Fluorescent dyes have been used to develop fluorescently tagged drug molecules in order to study the interaction of drugs with various receptor targets at the cellular level. One advantage of such a strategy is the ability to delineate the specific or nonspecific interaction of a drug with various receptors which could then be used to improve the efficacy of the drug. To the best of our knowledge there are no literature reports of a near infrared dye attached to Amoxapine, a tricyclic dibenzoxapine antidepressant widely used in the treatment of MDD to study its mitochondrial selectivity. We therefore synthesized the Amoxapine-IR-786 dye and were pleased to find that it was indeed selective towards mitochondria in HeLa cells with emission at 787 nm.[1] This work paves way for future exploration of similar synergistic drug-dye combinations with improved photophysical properties and organelle selectivity.

1. Choi, P.; Noguchi, K.; Ishiyama, M.; Denny, W. A.; Jose, J., *Bioorg Med Chem Lett* **2018,** accepted.

C65: The long non-coding RNA MaTAR17 is a new driver of tumour progression.

Sarkar, D.[1], Wright, B.[1], Diermeier, S.D.[1]

[1]Department of Biochemistry, University of Otago, Dunedin, NZ.

Genome-wide studies revealed that majority of the human transcriptome consists of non-coding RNAs, but their function and mode of action remains elusive. Diermeier et al., identified and characterised 30 Mammary Tumour Associated RNAs (*MaTAR*s) that are overexpressed in breast cancer compared to normal tissue. *MaTARs* have the potential to be used as therapeutic tools owing to their tumour-specific expression. Unlike other lncRNAs, upregulated expression of *MaTAR17* is not limited to breast cancer and is overexpressed in colorectal, lung and head and neck cancer. Overexpression of *MaTAR17* in several cancer types indicates a more general role of this lncRNA in tumour progression. Knockout of *MaTAR17* using the CRISPR/Cas9 system resulted in reduced cell proliferation *in vitro* and *in vivo*. Analysis using RISE (a database of RNA interactome from sequencing experiments), revealed possible RNA-RNA interaction between Microtubule Actin Filament 1 (*MACF1*) mRNA and *MaTAR17*. Furthermore, Adenomatous Polyposis Coli (*APC*) was identified as a protein interaction partner of *MaTAR17*.

Aberrant *MACF1* expression is linked to initiation of tumour cell proliferation, migration and metastasis in breast, colon and lung cancer. Additionally, *APC*, a well-characterized tumour suppressor gene contributes to development of colon and breast cancer. Investigation of probable interactions between *MaTAR17*, *MACF1* mRNA and APC will therefore provide an insight into the function of *MaTAR17* in tumour progression.

Preliminary gene expression studies using quantitative RT-PCR revealed reduced expression of *MACF1* and *APC* in *MaTAR17* knockout cells. *In vitro* localisation studies using RNA-FISH will validate RNA-RNA interaction between *MaTAR17* and *MACF1* mRNA. Ongoing studies using Chromatin Isolation by RNA Purification (ChIRP) assays will help identify possible binding sites of APC and *MACF1* to *MaTAR17*. ChIRP will also enable identification of other additional binding partners (RNA/DNA/protein) of *MaTAR17*. Investigating the molecular mechanism via which *MaTAR17* acts in the cell will aid us to explore the therapeutic potential of the lncRNA in the future.

C66: Pathogenesis of granulosa cell tumours of the ovary

Arora, S.[1], Blenkiron, C.[2], Shelling A.N.[1], Muthukaruppan, A. [1]

[1]Department of Obstetrics and Gynaecology, University of Auckland, Auckland, NZ, [2]Department of Molecular Medicine and Pathology, University of Auckland, NZ.

Granulosa cell tumours (GCTs), a rare type of ovarian cancer, have adult and juvenile subtypes. 97% of adult GCTs have a specific FOXL2 mutation and 50% of these tumours also harbour a mutation in the hTERT promoter. The link between the acquisition of hTERT promoter mutations and GCT recurrence, with existing FOXL2 mutations is unclear. However, work from our laboratory suggests that many genes regulated by mutant FOXL2 are significantly enriched for the TGF-β signalling pathway.

We hypothesise that the TGF-β pathway may be involved in progression of GCTs downstream of FOXL2 that may by implicated in the acquisition of hTERT promoter mutations and recurrence. Phenotype changes in KGN granulosa cancer cell line (representative of adult GCTs harbouring the FOXL2 mutation), were assessed by manipulation of the TGF-β pathway using inhibitory drugs and siRNA. Gene expression changes in the TGF-β pathway and hTERT were also assessed using RT-qPCR. Preliminary data suggests that TGF-β inhibitor, Pirfenidone has an inhibitory effect on cancer cell proliferation up to 72 hours after treatment. Experiments to confirm if expression of hTERT, FOXL2 and selected downstream genes are affected using RT-qPCR, are underway. Determining if FOXL2 and/or hTERT is involved in GCT development and progression via the TGF-β pathway is important as it may be used to predict recurrence in GCT patients and opens avenues to the development of GCT specific therapeutics.

C67: Mechanisms of Active DNA Demethylation in somatic cells

Issam Mayyas[1], Rob Weeks[1], Mark Hampton[2], Tim Hore[3], Ian Morison[1].

[1]Department of Pathology, University of Otago, Dunedin School of Medicine, NZ, [2]Department of Pathology, University of Otago, Christchurch, NZ. [3]Department of Anatomy, University of Otago, NZ

While, every cell in an organism is genetically identical there are marked phenotypic differences between tissues and organs that are controlled by epigenetic modifications. The most stable epigenetic modification is methylation of cytosine. Many cancers show significant global loss of methylation. Our research investigates the mechanism of changes in DNA methylation as they occur during human life using a *barcoded hairpin-bisulphite sequencing technique*. We have observed rapid demethylation in cultured Jurkat cells (T cell leukaemia) implicating novel mechanisms of active demethylation that have not yet been recognised by researchers in the field. It is likely that the demethylation pathways that we are studying operate during the onset of cancer and the existence of molecules (ascorbate or transition metals) that alter TET activity may have implications for modification of this process. While there is a substantial amount to be done before making therapeutic or dietary recommendations, our results might provide a rationale for long-term intervention to alter an individual's epigenetic risk.

C68: Understanding mitochondrial transfer in therapy - Applied single molecule genotyping to detect and quantify mitochondrial transfer.

Rowe, M.[1,2], Senanayake, D.[1], McConnell, M.J.[1,2].
[1]Centre for Biodiscovery, Victoria University of Wellington, New Zealand, [2]Malaghan Institute of Medical Research, Wellington, New Zealand.

The transfer of mitochondria between mammalian cells is a physiologically relevant phenomenon, however the signals driving this process are not yet clear. We hypothesise that therapeutics which impact mitochondrial function may enhance the rate of intercellular mitochondrial transfer, and this may serve as a component of resistance to therapy in many cancers. Using genetic methodologies, we have overcome serious limitations on techniques for study in this field, and may now accurately quantify the effect of therapies on the rate of mitochondrial transfer in vitro.
In order to both detect and quantify intercellular mitochondrial transfer, a novel strategy for *in situ* molecular genotyping has been targeted toward single nucleotide polymorphisms (SNP) within the mitochondrial genome (mtDNA). This strategy exploits a highly selective ligase, that when combined with padlock oligonucleotides, generate a template for target-primed rolling circle amplification. This molecular technique allows amplification of individual mtDNA molecules carrying a target SNP *in situ*. Exogenous mtDNA which has transferred into 'recipient' cells is then detected by high throughput confocal imaging.

These Images are analysed via an integrated machine-learning pipeline focused on reduction of false discovery rate, and allows us to accurately trace and quantify the transfer of mitochondrial genomes between cells *in vitro*. Using these tools, a robust, quantitative method to support microscopic observation of this phenomenon has been developed, which has enabled insights into the effect of cancer therapeutics on mitochondrial transfer.

C69: Investigating p53 isoforms in HPV positive cervical cancers and in the placenta

A. Reily-Bell [1], A. Fisher[1], C.J. Devenish[2], N.A. Hung[1], A.W. Braithwaite[1], T.L Slatter[1]
[1]Department of Pathology, University of Otago, Dunedin School of Medicine, Dunedin, New Zealand; [2]Department of Women's and Children's Health, University of Otago, Dunedin School of Medicine, Dunedin, New Zealand

Human papillomavirus (HPV) is well known to be the leading cause of cervical cancer worldwide. It has also been found at an increased frequency in the placentas of women with birth complications such as preeclampsia, pre-term birth and fetal growth restriction (1). Isoforms of the tumour protein 53 (p53) are associated with cancer promoting properties and those that would also affect placental function such as proliferation, invasion and angiogenesis. The physiological function of p53 isoforms is unknown; however, recent studies suggest p53 isoforms are involved in pathogen related responses. Of interest is to determine if HPV could increase p53 isoform expression, and if so could HPV further add to complications by upregulating p53 isoform function. This study aimed to determine if p53 isoforms were increased in tissues with HPV DNA. P53 isoform expression and HPV E6 and E7 were investigated using *in situ* hybridisation (RNAscope) in cervical cancer cases and placentas. All cervical cases had *E6* and *E7* expression. Forty percent of placenta cases had HPV *E6* and *E7* expression, providing the first *in situ* based evidence that HPV genes are expressed in the placenta. P53 isoform expression was found in all cervical cancer cases, but was not detected in placental cases. These preliminary findings suggest p53 isoforms are increased in cervical cancer, but further work is required to determine if p53 isoforms are increased with HPV directly.

1. Slatter TL, Hung NG, Clow WM, Royds JA, Devenish CJ, Hung NA. *A clinicopathological study of episomal papillomavirus infection of the human placenta and pregnancy complication*s. Mod Pathol. 2015;28(10):1369-82.

C70: Genetic identification of bone marrow mitochondrial transfer in a leukaemic murine model

Carson, G.M.[1,2], Berridge, M.V.[1], McConnell, M.J.[2,1]

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

Mitochondria are organelles found in nearly all eukaryotic species, and play a key role in cell metabolism, survival and death. Although it is accepted that mitochondria arose from a bacterial symbiont, the idea that mitochondria have potential independence from their host cell remains niche. Yet evidence has arisen in the past decade that mitochondria move between eukaryotic cells. Multiple in vitro and in vivo studies have appeared to show this. However, the literature presents anecdotal data with sub-optimal methods for identifying mitochondria and little attempt at robust or genetic quantification.

To address the prevalence of intercellular mitochondrial transfer in murine bone marrow using genetic techniques, an in vivo acute myeloid leukaemia (AML)-like murine cancer model has been established. The C1498 cell line has mitochondrial polymorphisms distinct from the host C57BL/6 mouse. Transplantation of C1498 cells followed by homing to the bone marrow niche leads to the formation of chimeric bone marrow. After different periods of “in vivo co-culture” the cancer cells were reproducibly separated to 100% purity from host bone marrow using flow cytometry. Detection of C57BL/6-specific mitochondrial DNA in the C1498 cells, and vice versa, was carried out using allele-specific PCR and demonstrated that the basal level of transfer was very low, beyond the point of detection. To increase sensitivity, an amplicon deep-sequencing approach has been developed. To induce mitochondrial injury, C1498 cells were transfected with a mitochondrial-targeted mutant uracil N-glycosylase to permanently deplete mitochondrial DNA. These cells, with no mitochondrial DNA, displayed decreased respiration and viability without metabolic support, and will be used in the in vivo model to assess the effect of injury on transfer.

C71: Novel targeting of PI3K/mTOR and ERK in ovarian cancer cell line models is influenced by culture method

Dunn, E.[1], Garrill, A[1]

[1]School of Biological Sciences, University of Canterbury, Christchurch, NZ

Ovarian cancer is a deadly gynaecological malignancy with a 5-year survival rate of approximately 50%. It is a highly heterogeneous disease, making treatment difficult as resistance frequently occurs and relapse is extremely common. To overcome this heterogeneity finding a wide range of effective targeted treatments is imperative, as are accurate predictions of treatment efficacy. Drug sensitivity experiments conducted in cell line models are used to determine predictive biomarkers, which are genetic changes influencing sensitivity to a given targeted drug.

The PI3K/mTOR and Ras/Raf kinase signalling pathways display frequent aberrations in ovarian cancer. These pathways have similar functions in the cell, promoting cell proliferation and survival. Mutations to these pathways in ovarian cancer will promote growth of the cancer and the evasion of cell death signals. Therefore, these pathways present important potential drug targets for the treatment of ovarian cancer. Several inhibitors to these pathways have been developed, but unfortunately, these have underperformed in clinical trials. There is significant cross activation and cross regulation between these two pathways, and as they preform similar functions in the cells, it is thought that the cell may bypass an inhibitor through increased activation in the adjacent pathway. Therefore, we are investigating the potential of combination therapies to overcome this. Combining PI3K and Ras/Raf pathway inhibitors results in a significant decrease in metabolic activity and growth in several ovarian cancer cell lines. However, in certain cell lines this response is dependent on the culture method used. Some are sensitive to the inhibitors in monolayer culture but resistant in spheroidal cultures. This has wide implications as spheroidal cultures are thought to more accurately represent potential tumour responses, but most research is still conducted on monolayer cultures. This may explain some discrepancy in the translation of results from cell line models to clinical trials.

C72: Visualisation of long non-coding RNA *ANRIL* in Breast Cancer

Paulomi Mehta-Mujoo,1 Michael Landmann,2 Anna P. Pilbrow,3 Margaret J. Currie,4 Helen R. Morrin,4 Noelyn A. Hung,1 and Tania L. Slatter1
1Department of Pathology, Dunedin School of Medicine, University of Otago, Dunedin, New Zealand 2Department of Surgery, Dunedin School of Medicine, University of Otago, Dunedin New Zealand 3Department of Medicine, University of Otago, Christchurch, New Zealand 4Department of Pathology, University of Otago, Christchurch, New Zealand

Background Breast cancer progression is associated with poor prognosis and understanding the underlying biochemistry may aid targeted management and treatment of patients. Recent progress has demonstrated that expression of long non-coding RNAs (lncRNA) may aid in development of biomarkers for cancer progression. *ANRIL* is one such lncRNA found at chromosome position 9p21 which has been implicated in many cancers with poor prognosis. *Aim* The study aimed to obtain a wider understanding of *ANRIL* by investigating expression in normal associated tissue (NAT) adjacent to breast tumour and matched tumour tissue. *Methods* This study looked at *ANRIL* expression quantitatively (RT-qPCR) and semi-quantitatively using RNAscope *in-situ hybridisation,* in formalin fixed paraffin embedded 31 primary breast cancer cases. *Results*: *ANRIL* expression levels in tumour tissue was significantly higher than NAT tissue (p=0.029). This was further confirmed by using RNAscope. *Conclusion.* Difference in expression levels between the two tissue types is suggestive of the tumour promoting properties of *ANRIL* which was also validated through in-situ hybridisation. Future studies would need to validate these results on a larger sample size to determine the prognostic value of increased *ANRIL* in breast cancer.

C73: Investigating Tumour Evolution in a Single Patient with Disseminated Cancer

Robb, T.[1], Blenkiron, C.[1], Tsai, P.[1], Parker, K.[1], Drummond, A.[2], Black, M.[3], Gavryushkin, A.[4], Woodhouse, B.[1], Houseman, P.[1], Coats, E.[1], Shields, P.[1], Fitzgerald, S.[1], Wright, D.[5], Tse, R.[5], Kramer, N.[5], Barker, C.[5], Triggs, Y.[5], Stables, S.[5], Modahl, L.[5], Reeve, J.[5], Shepherd, P.[1], Lawrence, B.[1]*, Print, C.[1]*

[1]Faculty of Medical and Health Sciences, The University of Auckland, NZ, [2]Faculty of Science, The University of Auckland, NZ, [3]Department of Biochemistry, University of Otago, NZ, [4]Department of Computer Science, University of Otago, NZ, [5]Auckland District Health Board, Auckland, NZ. * Cris Print and Ben Lawrence have contributed equally to the leadership of this study

For cancer biologists, understanding the evolution and genomic heterogeneity of multiple tumours in a single patient is an important goal. For oncologists, genomic maps of multiple tumours in each patient, could facilitate improved precision oncology; enabling selection of therapeutic targets universal to a patient's tumours, private resistance mechanisms found in individual metastases, and metastasis-specific ctDNA biomarkers for better disease monitoring.

A patient with a primary lung neuroendocrine tumour and 88 metastases requested and consented to rapid autopsy, providing a rare opportunity to study tumour evolution and heterogeneity in a single patient. This multi-layered genomic investigation, augmenting clinical notes and imaging, produces a personalised evolutionary model of disease progression. Following comprehensive ethical consultation the patient and her extended family provided informed consent to collection of tumours through rapid autopsy, whereby 89 lesions were sampled extensively. From an initial 30 FFPE tumour samples, representing a broad spatial distribution of the patient's total tumour burden, DNA whole exome sequencing (WES), transcriptome mRNA sequencing and RNA microarray analysis was completed, to provide a bank of genomic information to guide evolutionary investigations. We have built statistical evolutionary phylogenetic models from differences observed across the genomic data to produce a broad evolutionary picture encompassing single nucleotide variants (SNVs), indels, copy number variants (CNVs), and RNA expression.

Overall, the tumours were generally homogenous in terms of SNVs, indels and CNVs, mirroring histopathological homogeneity. However, key differences in SNVs that may have driven metastatic events were noted and used to infer tumour lineages and evolutionary progression; indicating that multiple ancestral lineages within the primary tumour may have seeded different metastatic tumours.

This patient's generous donation represents an invaluable opportunity to draw on well-annotated clinical history alongside multi-layered genomic data to investigate biological drivers of tumour evolution, important to cancer biologists and oncologists alike.

C74: The hypoxia-activated EGFR/HER2 inhibitor Tarloxotinib is activated by the plasma membrane reductase STEAP4

Silva, Shevan[1,2], Guise, Christopher[1,2,3], Bull, Matthew[1,2], Print, Cristin[2,3,4], Smaill, Jeff[1,2], and Patterson, Adam[1,2].

[1] Translational Therapeutics Team, Auckland Cancer Society Research Centre, School of Medical Sciences, University of Auckland, Auckland, New Zealand;
[2] Maurice Wilkins Centre for Molecular Biodiscovery, School of Biological Sciences, University of Auckland, Auckland, New Zealand
[3] Department of Molecular Medicine and Pathology, School of Medical Sciences, University of Auckland, Auckland, New Zealand
[4] Bioinformatics Institute, University of Auckland, Auckland, New Zealand

Background: Tarloxotinib is a clinical-stage prodrug that releases a potent, irreversible EGFR/HER2 inhibitor (Tarlox-TKI) selectively in severely hypoxic regions of tumours (< 0.1% O_2). Mechanistic studies show that tarloxotinib is cell-excluded due to the positive-charge of the quaternary ammonium cation to which the 4-nitroimidazole trigger is appended. One-electron reduction leads to loss of the trigger moiety under oxygen-deficient conditions, releasing the neutral, diffusible 'warhead' tarlox-TKI. Characterisation of oxidoreductases that facilitate one-electron reduction of tarloxotinib is critical for its clinical development.
Material and methods: The rate of anoxic tarloxotinib metabolism (tarlox-TKI formation) was monitored by LC-MS/MS and compared with RNA transcript abundance (Affymetrix Primeview gene arrays) across a panel of human neoplastic cell lines. Plasmid-based cDNA expression and siRNA knockdown studies were employed to evaluate the role of putative oxidoreductases in tarloxotinib metabolism. CRISPR/cas9 mediated gene knockout was used to further interrogate the role of the predominant tarloxotinib reductase *in vitro* and *in vivo*.
Results: Amongst 36,000 transcripts and variants, the most highly correlated relationships to anoxic tarloxotinib metabolism were associated with *STEAP4* (Six-Transmembrane Epithelial Antigen of Prostate, family member 4). Gene overexpression in two low STEAP4 expressing cell lines (C33A and H1299) increased anoxic tarloxotinib metabolism by 28 – 59-fold relative to parental cells. siRNA knockdown of *STEAP4* suppressed tarloxotinib metabolism by >70% while knockdown of other putative oxidoreductases led to no changes in tarloxotinib metabolism. Gene disruption of *STEAP4* using a CRISPR/cas9 system indicated STEAP4 was the dominant tarloxotinib reductase *in vitro* and *in vivo*. STEAP4 is an NADPH-dependent, FAD and heme containing metalloreductase required for reduction of extracellular Fe3+ and Cu2+ to facilitate plasma membrane transport. Analysis of 163 databases from the Cancer Genome Atlas (TCGA) covering over 22,000 individual cancers indicates *STEAP4* mRNA is highly expressed in certain cancers including prostate, non-small cell lung, breast, esophageal, gastric and Head/Neck.
Conclusions: Identifying individual patients with elevated STEAP4 activity in their cancer may be important to guide patient selection during the clinical development of tarloxotinib. Tarloxotinib is currently under exclusive license to Rain Therapeutics Inc., Fremont, USA, and development is anticipated in various STEAP4-positive cancers.