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C1: Cancer in four dimensions

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Cancer research over the last three decades has been dominated by advances in molecular genetics, including the sequencing of the human genome. The development of dynamic models of human cancer, facilitated by advances in flow cytometry, imaging techniques and molecular studies, has been less spectacular but nevertheless provides an important framework for understanding the basis cancer therapy. Consideration of a dynamic model of cancer raises a number of questions. For instance, why do human tumour xenografts in mice have doubling times of around 4 days while human tumours from which they are derived have doubling times closer to 4 months? Why do human tumours have such a high content of macrophages and why do these macrophages express the protein Gas6 and its receptor Axl? What is going on during tumour dormancy, where microscopic tumours may persist for years without net growth? Do gene mutations drive proliferation or does proliferation drive the appearance of mutations? One of the goals of our research group over the last 30 years has been to study the transition of cancer cells from their clinical origin to their status as a laboratory tumour cell line. More than 1500 clinical samples have been cultured and over 200 cell lines have been developed, many from melanoma. For some of these lines, proliferation kinetics have been studied with the help of flow cytometry and mathematical modelling. Overall, it appears that the genetic status of a tumour cell changes to only a small extent during the transition from tumour sample to a cell line, but the cytokinetic properties change a great deal. The emerging model emphasises the amazing potential that clinical tumours have repopulate after therapy, as well as to generate new variant populations. Tumour evolution is likely to commence well before a cancer is clinically detectable, continuing even after the isolation and serial passage of a cell line. The processes underlying tumour repopulation and evolution have important implications for cancer treatment.

CB2: How to Improve the Outcome of Breast Cancer A Clinician's Perspective

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A breast cancer clinician wants to know 3 things from the molecular biology revolution - the cause of breast cancer, who needs treatment additional to surgery and which treatment to give for that patient.

While cancer is a disease of genetic instability, most breast cancer are considered sporadic and only about 5% are caused by a recognised inherited genetic susceptibility, though in these cases the risk of breast cancer development is extremely high. This knowledge has not yet led to specific therapy, except prophylactic surgery, but it is to be hoped that directed therapies will soon be developed.

Early detection and surgery, often with radiation, still have the biggest impact on outcome and cure 60- 70% of breast cancer. On a daily basis medical oncologists are faced with two questions - whose cancer has already spread beyond the breast and therefore needs added treatment and which treatment.

Patients presenting with, or later developing metastatic, disease will inevitably die of their disease, but may have their survival extended by systemic therapy, which may include endocrine manipulation, chemotherapy and biological therapy. These same treatments used in the immediate post-operative period (called adjuvant therapy) may prevent subsequent development of metastases in a proportion of those patients whose disease would otherwise recur, but the major challenge if how to identify those who will benefit.

Oestrogen promotes breast cancer growth in most patients and oestrogen deprivation will lead to tumour shrinkage for a while, though resistance is inevitable, with subsequent regrowth. Recent improved understanding of the multiple mechanisms controlling cell growth has led to development of several new agents, blocking additional pathways and reversing this resistance, at least temporarily. Trials are underway to ascertain whether such medicines may increase cure rates if used as adjuvant therapy.

Chemotherapy drugs are various cell poisons, which disrupt DNA synthesis, leading to cell death, but like endocrine therapy provide only temporary control in metastatic disease, in most tumours, because of the development of resistant clones, but will increase the cure rate after surgery by eliminating residual microscopic disease, in a proportion of patients. Some types of breast cancer are less responsive to chemotherapy. Up to 15% of breast cancers have mutation or amplification of the HER2 gene, which gives a more aggressive phenotype. Trastuzuzmab (Herceptin) is a humanised monoclonal antibody to the HER2 receptor which restores chemosensitivity. Combined chemotherapy and trastuzumab has changed HER2 amplified breast cancer from the most

aggressive phenotype to that with the best prognosis. A major search is underway for ''druggable' targets in other subtypes.

The commonest dilemma facing clinicians daily is trying to assess which patients, who appear cured by surgery are harbouring microscopic disease, which will kill them if untreated. Initially the main clinical prognostic factor (axillary node involvement) was used to determine who to treat, but this led to under treatment because not all node negative patients are cured by surgery. Combinations of multiple prognostic factors led to computer programmes predicting prognosis and benefit from treatment, but these have led to overtreatment of many and under treatment of some others. Various genomic tests measuring activity of several genes involved in cell proliferation have recently have now been developed. These are used to predict the likelihood of relapse and help estimate whether the patient will benefit from chemotherapy. Trials have compared their performance with the computer programmes. but results are not yet available. Even they only assess risk of relapse, so are imprecise.

It is hoped that with ever better understanding of the molecular control of cell growth we will be able to differentiate more precisely those most likely to relapse, treat only those patients and apply therapies targeted to the particular abnormal cellular pathway(s), which is/are driving that growth.

CB46: ORAXOL: New Zealand's role in the development of a novel oral chemotherapy agent

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Background: Paclitaxel is a widely used intravenous chemotherapeutic agent with activity in multiple solid tumours. It has poor oral bioavailability due to the active excretion by p-glycoprotein (Pgp) present on intestinal epithelial cells. Oraxol (Kinex) is a combination of oral paclitaxel and HM30181, a novel, orally administered, non-absorbed specific inhibitor of intestinal Pgp. Oral paclitaxel would avoid the need for IV injections, avoid severe anaphylactic reactions and the side-effects of steroid pre-medication, cost savings and greater patient convenience. The phase 1 BA/BE study of Oraxol has been undertaken in Dunedin, and an ongoing phase 2 study is underway at 4 New Zealand sites. We report the results of our absolute bioavailability study and update on New Zealand's leading role in the development of Oraxoll. Methods: We conducted an open label, randomized cross-over pharmacokinetic (PK) study to determine the bioavailability (PK, AUC) and safety of escalating doses of Oraxol over 2 days compared to IV paclitaxel 80mg/m2 over 1 hour in patients with incurable cancer receiving weekly paclitaxel. Results: In the BA/BE study, Oraxol was well tolerated with no grade 3/4 drug toxicities at any of the dose levels. AUC0-∞ and Cmax increased with increasing Oraxol dose from 270mg to 274mg/m2 but not with dose escalation to 313mg/m2. Conclusions: Oraxol 615 mg/m2 over 2 days achieved paclitaxel AUC comparable to IV paclitaxel 80 mg/m2. Oraxol safety profile was acceptable without grade 3-4 toxicities. Oraxol represents an ideal drug candidate to replace IV paclitaxel. Ongoing development in New Zealand is underway.

CB4: Decoding challenging cancers

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It is now well established that a cancer's mutational burden drives tumour formation, influences disease progression and can dictate sensitivity to chemotherapy. Over recent years, many large scale programs have mapped out the mutational landscapes of most common cancers and a wealth of mutation-directed therapies therapies. Australia's contribution to the International Cancer Genome Consortium (ICGC) has sought to resolve the root causes of somatic mutation, the core pathways promoting tumour formation, and identify potential clinical opportunities in many solid cancers (pancreatic adenocarcinoma, Neuroendocrine tumours, melanoma, high-grade serous ovarian and esophageal adenocarcinoma. This presentation will review how these global cancer atlases provide insights into the etiology of into Rare, Recalcitrant, and Therapeutically Refractory cancers and are paving the way towards novel therapies and better patient outcomes.

CB5: Hereditary Diffuse Gastric Cancer: battling the curse

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Hereditary Diffuse Gastric Cancer (HDGC) is a cancer syndrome caused by germline mutations in the gene encoding the cell-to-cell adhesion protein E-cadherin (*CDH1*). HDGC was first described in a Māori family who challenged the traditional belief that the high incidence of cancer in their family was due to mākutu (curse). *CDH1* mutation carriers have a 70% lifetime risk of diffuse type gastric cancer (DGC). Female carriers also have an elevated risk of lobular breast cancer (LBC), estimated to be as high as 40%.

The current international clinical management guidelines for HDGC recommend that mutation carriers undergo prophylactic gastrectomy in their 20s and breast cancer surveillance starting at around 30yrs of age. These guidelines have been very effective in reducing the mortality in HDGC families, but gastrectomy is associated with high (but usually manageable) morbidity and many mutation carriers opt for gastric endoscopic surveillance instead. When conducted by an endoscopist experienced with HDGC, surveillance can be very useful in identifying patients with advancing DGC, but it is imperfect and progressive disease can be missed.

The life history of HDGC involves the early development of multifocal stage T1a gastric signet ring cell carcinomas. These T1a lesions are largely indolent, athough their progression to advanced disease is difficult to predict. We hypothesise that elimination of these lesions would provide the means to prevent the development of advanced disease in mutation carriers. Accordingly, we have been taking a synthetic lethal approach to identify vulnerabilities in E-cadherin-negative cells which can be exploited by drug treatment. Using an isogenic pair of non malignant breast cell lines (MCF10A) with and without E-cadherin, we have identified several drugs and synergistic drug combinations which are more toxic to E-cadherin-negative cells than E-cadherin-expressing cells. The mechanism of synthetic lethality involves the elevated sensitivity of E-cadherin-negative cells to inhibition of cell survival pathways such as PI3K/AKT signalling. To further test these drugs as potential HDGC chemoprevention compounds we are currently developing an engineered mouse model of DGC and LBC that involves the inducible, cre-mediated deletion of *cdh1* from the stem cell compartments of epithelial tissues. Validation of our drug combinations in this mouse model will allow the design of several staged clinical studies which will progressively test whether chemoprevention can be used as an adjunct to surveillance for the better management of HDGC.

CB6: Targeting T cells to cancer antigens

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The capability to rapidly generate molecular profiles of human cancers offers enticing opportunities for precision immunotherapy. Over the next few years, molecular analysis of cancer cells and their microenvironment is likely to enable better targeting of immune modulators such as checkpoint blockers. But ultimately it may prove necessary to stimulate T cell activity against specific tumour antigens in many patients using vaccines or Adoptive Cell Therapy (ACT). Abundant molecular targets for T cells have already been identified with very good specificity for tumours (in stark contrast to the paucity of tumour-specific CAR-T targets). However the variability in tumour antigen expression between patients will necessitate different T cell treatments for different patients - and potentially unique products for each patient in order to establish durable tumour control. We have developed a peptide-based vaccine technology which could be used to generate precision vaccines for individual patients, and the main barriers to rolling out this type of technology are regulatory rather than practical. We have also developed new techniques to grow T cells for ACT that suggest efficient generation of unique T cell cultures targeted at a patient's own oncogenic proteins may be feasible. Although expensive, these two technologies could be used in future for the sub-group of patients who fail to respond to more generic immune therapy due to weak or absent spontaneous T cell responses to their tumours.

CB7: Transient tissue 'priming' via ROCK manipulation uncouples pancreatic cancer progression, chemo-resistance and the onset of the metastatic niche: insights from intravital biosensor imaging

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Tumor development and metastasis occur in complex settings with reciprocal feedback from micro-environmental cues influencing both progression and drug response in multiple cancers. Here, we used intravital imaging to assess how transient manipulation of the tumor tissue, or 'priming', using the Rho-kinase inhibitor Fasudil affects response to chemotherapy. FRET imaging of a CDK1 biosensor to monitor cytotoxic drugs efficacy *in vivo* revealed that 'priming' improves pancreatic cancer response to Gemcitabine/Abraxane at both primary and secondary sites. Transient 'priming' also impaired extravasation efficiency and fibrotic niche remodeling within the liver, while reducing metastases-to-metastases direct communication, an important feature of cancer spread. Last, we provide preclinical rationale that sequential 'priming' in stratified patient-derived settings offers new opportunities in this disease.

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CB8: TAZ/YAP and their partners in the Hippo pathway

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Our lab has identified TAZ as a novel oncoprotein in 2008. TAZ and its paralog YAP are mammalian counterpart of fly Yorkie. TAZ and YAP act as the transcriptional co-activators for transcriptional factors such as TEAD1-4. Interaction with TEADs is essential for oncogenic property of TAZ. We also resolved the crystal structure of YAP-TEAD complex as well as the Vgll-TEAD complex. Amot proteins and Wbp2 were identified as negative and positive regulators of TAZ/YAP, respectively. The Hippo core kinases are the core components of the Hippo pathway to restrict the activity of TAZ and YAP. We have recently identified that Amot proteins are also regulated by the Hippo core kinases. The functional and mechanistic aspects of these proteins in human cancer will be discussed.

CB9: IL-6 signalling mediates tumorigenesis, invasion and metastasis driven by the Δ133p53 isoform

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The *TP53* locus is now known to encode multiple splicing isoforms. The functions of the Δ133p53 isoforms are not well understood but elevated levels have been reported for a number of malignancies. Previously we generated a mouse model of the human Δ133p53 isoform, designated Δ122p53. Δ122p53 mice are tumour prone and display a pro-inflammatory phenotype including an elevation of multiple pro-inflammatory cytokines, notably IL-6. *In vitro* the isoform promotes cell migration, which is in part dependent on IL-6. To determine the effect of the elevated IL-6 on Δ122p53 mediated pathologies we crossed theΔ122p53 mice with IL-6 deficient mice. Loss of IL-6 reduces tumour frequency, and reduces the extent of organ involvement for lymphomas. Human colorectal cancers with elevated Δ133p53 transcript are more invasive and are associated with poorer survival. We also show that the isoform directly elevates IL-6 transcription and activates the JAK/STAT-3 and ROCK signalling pathways that may explain the observed migratory/invasive phenotype.

CB10: Surgical success and response to adjuvant therapy in glioblastoma depends on telomere maintenance associated markers

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Telomere maintenance, telomerase activity or the alternative lengthening of telomeres (ALT), is a hallmark of cancer. Prior to temozolomide use in New Zealand, individuals with ALT positive glioblastoma had an improved outcome compared to those with telomerase positive tumours or tumours where the telomere maintenance mechanism was unknown. We recently used a RNASeq analysis to characterise glioblastomas with no telomere maintenance mechanism. We found these tumours were largely comprised of ALT or telomerase positive tumours with a high content of tumour associated macrophages. Further molecular and clinico-pathological analyses were performed on the four-glioblastoma subgroups (ALT with and without a high-content of macrophages, and telomerase with and without a high-content of macrophages) to better understand the heterogeneous patient outcomes. The molecular analyses revealed differences in immune signatures and mutations amongst the different subgroups. The clinico-pathological analyses revealed each telomere-based subtype was correlated with different radiological features using magnetic resonance imaging (MRI). Telomerase positive tumours without macrophages respond to temozolomide, and 'longer-term' survival (>24 months) is now possible. Telomerase positive tumours with macrophages now have the worst prognosis (overall median survival telomerase with and without macrophages P = 0.013). Telomerase positive tumours with macrophages were also associated with an increased frequency of partial surgical resections (56% *vs* < 24% for all other subtypes, P = 0.014), suggesting these tumours were more invasive. These findings have been validated in a second cohort of 110 glioblastomas. Future analyses are directed at better identifying each telomere subgroup before surgery and how each subgroup could be targeted for treatment.

CB11: Investigating the association of Δ133p53 isoform expression and the inflammatory microenvironment in prostate tumours

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Growing evidence suggest inflammation has an important role in the initiation phase of malignancy as well as influencing tumour progression. The Δ133p53, an isoform of the tumour suppressor protein p53, has a tumour-promoting function by inhibiting full-length p53 activity and has a role in inflammation. Δ133p53 has been upregulated in a number of cancers and has been associated with tumour progression and poor patient outcome. The mechanisms by which Δ133p53 promotes inflammation and cancer are not yet fully understood. This study investigated the association between Δ133p53 isoform expression and the inflammatory microenvironment in prostate tumours. Quantitative real time PCR was used to measure Δ133p53 expression in prostate cancer tissues, non-neoplastic prostate tissues and in prostate cancer cell lines. Immunohistochemical staining were performed for CD20 (B cells) and CD163 (macrophages). High Δ133p53 levels were observed in a subset of prostate tumours but not in non-neoplastic prostate or cultured normal or prostate cancer cell lines. Overall, the Δ133p53 expression has shown significant association with B cell infiltration in prostate tumours. Significantly higher numbers of tumour infiltrating $CD163^{+}$ macrophages were also observed in a subset of prostate tumours with high Δ133p53. Our results point to a role for Δ133p53 in prostate cancer inflammation.

CB12: Changes in cellular uric acid homeostasis facilitated by Glucose Transporter 9 (GLUT9) drive activin sensitivity and prostate cancer cell behaviour

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Elevated serum uric acid (SUA) or urate, causing gout, has being linked to cancers, especially prostate cancer (PCa) (1). Activins, inflammatory cytokines of the TGFβ superfamily, act as negative growth regulators in the prostate, and activin insensitivity is considered a hallmark of PCa progression. However, the underlying molecular mechanisms of activin insensitivity and 'cellular uric acid homeostasis' (CUAH) in PCa are unknown. This study aimed to determine how a disturbance of CUAH counteracts growth inhibitory effects of activins in PCa, and to identify the transporter facilitating this.

ActA expression was increased in low-grade PCa, whereas ActB expression was reduced in high-grade and extra-capsular spread PCa. Intracellular urate levels decreased in all prostate pathologies, while expression of GLUT9 decreased in benign prostatic hyperplasia, prostatitis and high-grade PCa. Activin responsive LNCaP cells had higher intracellular and lower secreted urate levels than activin insensitive PC3 cells. LNCaP and DU145 cells showed a decrease of GLUT9 mRNA consistent with prostate disease tissue and PCa cell protein expression. Normal and high extracellular urate (300 and 500μM) was growth promoting *in vitro*, and it antagonised the growth inhibitory effects of ActA and ActB. This was abolished by the gout medication probenecid and reduced by a GLUT9-kd. [^{14}C]-urate transport in LNCaP cells was inhibited by probenecid and matched by a similar inhibition of LNCaP cell proliferation under high extracellular urate.

Implications: Changes of CUAH facilitated by GLUT9 significantly impact prostate cancer cell growth, and lowering SUA levels in PCa could be of therapeutic benefit (2).

1.Fini MA, Elias A, Johnson RJ, Wright RM. Contribution of uric acid to cancer risk, recurrence, and mortality. Clin Transl Med. 2012;1(1):2001-1326.

2.Sangkop F, Singh G, Rodrigues E, Gold E, Bahn A. Uric acid: a modulator of prostate cells and activin sensitivity. Mol Cell Biochem. 2016;414(1-2):187-99.

CB13: Quantification of spatio-temporal correlations of interacting cells using a new statistical approach - Colorectal cancer cells used as a model system

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Inter-cellular communication represents a fundamental process in the majority of biological disciplines as well as pathological conditions. An important factor is the spatio-temporal interactions of cells. In order to quantify these interactions we have developed a statistical approach that can estimate spatio-temporal correlations between cells based on high-content live-cell imaging data. We have used cancer cells as a model system, representing a pathological disease for which numerous kinds of inter-cellular interactions are reported to affect cancer cell phenotype, including interactions with other cell types in the tumour microenvironment (such as fibroblasts) as well as interactions with other cancer cells. So far we have successfully evaluated the interactions between colorectal cancer cells and normal fibroblasts, classified according to unique fluorescent profiles, during cellular growth. As expected we found that cancer cells had a positive self-reinforcing effect on their own growth over short distances, which became smaller, although still significant, over longer distances, confirming that we were able to detect interactions between different cell types. Fibroblasts also had a positive self-reinforcing effect on their own growth, although less than the cancer cells, but had no impact on growth of cancer cells. We are working on further developing the statistical approach to enable analysis of a larger number of cellular groups using multi-coloured LeGO cells[1] as a model system with the purpose of studying interactions between cancer cell subpopulations. For future perspectives applying the method to chemo-treated cancer cells has the potential to elucidate mechanisms involved in treatment resistance and tumour recurrence. However, potential applications go far beyond cancer, and the model can in theory be used to study any kind of intercellular communication in relation to discrete outcome measures such as cellular growth and phenotype switching.

1. Weber, K., Thomaschewski, M., Warlich, M., Volz, T., Cornils, K., Niebuhr, B., Täger, M., Lütgehetmann, M., Pollok, J.M., Stocking, C., Dandri, M., Benten, D., Fehse, B (2011). *RGB marking facilitates multicolor clonal cell tracking.* Nature Medicine. 17(4):504-9.

CB14/DD12: Helping cancer vaccines

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The concept that the immune system can target and eliminate cancer tissue has been validated clinically with the success of therapies that unleash the activities of T cells, such as bone marrow transplantation, immune checkpoint blockade and adoptive T cell therapy. Vaccines that specifically induce antitumour T cell responses have not yet had the same level of clinical impact, but this may change as we learn more about how immune responses are generated, and how to exploit this new knowledge in vaccine design. In this context, a brief overview will be presented on how populations of “innate-like” T cells in the lymphoid tissues can help initiate responses to vaccination, and how this knowledge is being used to develop a new series of powerful cancer vaccines.

CB15/DD13: Bioorthogonal synthesis and antitumour activity of CD1d dependent glycolipid-peptide vaccines

Painter, G.F.,[1] Tang, C.,[2] Anderson, R.J.,[1] Compton, B.J., Osmond, T.,[2] Farrand, K.J.,[2] Authier-Hall, A.,[2] Gasser, O.,[2] Hayman, C.M.,[1] Johnston, K.A.,[1] Larsen, D.S.,[3] Weinkove, R.[2] and Hermans, I.F.[2]

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Despite cancer vaccines having the potential to induce T cell responses with high specificity for defined tumour-associated antigens issues remain around lack of clinical efficacy, manufacturing and cost.[1] Synthetic vaccines based on antigenic peptides are highly defined and easily manufactured, however, in order to generate a robust anti-tumour response it is important not only that peptide fragments are acquired by antigen presenting cells (APCs) and presented to T cells in the context of MHC molecules, but also that the APCs are in the correct activation state.[2] This can be achieved by using adjuvants that target pattern-recognition receptors on APCs, such as the Toll-like receptors (TLRs). A less explored approach to activating APCs is to specifically stimulate innate-like T cells in the local environment. The most studied innate-like T cells are the invariant natural killer T (NKT) cells, which recognise glycolipid antigens that bind to the lipid antigen-presenting molecule CD1d.[3] In this paper we will describe the bioorthogonal synthesis of CD1d dependent glycolipid-peptide conjugate vaccines and their antitumor activity in preclinical models. The synthetic strategy includes the use of strain-promoted azide alkyne cycloaddition (SPAAC) and self-immolative linker technologies.

1. (a) Hailemichael, Y.; Dai, Z.; Jaffarzad, N.; Ye, Y.; Medina, M. A.; Huang, X. F.; Dorta-Estremera, S. M.; Greeley, N. R.; Nitti, G.; Peng, W.; Liu, C.; Lou, Y.; Wang, Z.; Ma, W.; Rabinovich, B.; Sowell, R. T.; Schluns, K. S.; Davis, R. E.; Hwu, P.; Overwijk, W. W., *Persistent antigen at vaccination sites induces tumor-specific CD8(+) T cell sequestration, dysfunction and deletion*. *Nature medicine* **2013,** *19* (4), 465-72; (b) Rosenberg, S. A.; Yang, J. C.; Restifo, N. P., *Cancer immunotherapy: moving beyond current vaccines*. *Nature medicine* **2004,** *10* (9), 909-15.
2. Reed, S. G.; Orr, M. T.; Fox, C. B., *Key roles of adjuvants in modern vaccines*. *Nature medicine* **2013,** *19* (12), 1597-608.
3. Bendelac, A.; Savage, P. B.; Teyton, L., *The biology of NKT cells*. *Annual review of immunology* **2007,** *25*, 297-336.

CB16/DD14: Synthesis and Activity of 6″-Deoxy-6″-Thio-α-GalCer and Peptide Conjugates

Larsen, D. S.,[1] Compton, B. J.,[2] Tang, C-W., [3] Johnston, K.A.,[2], Osmond T. L.,[3] Hayman C.M.,[2] Hermans, I.F.,[3] Painter, G.F.[2]

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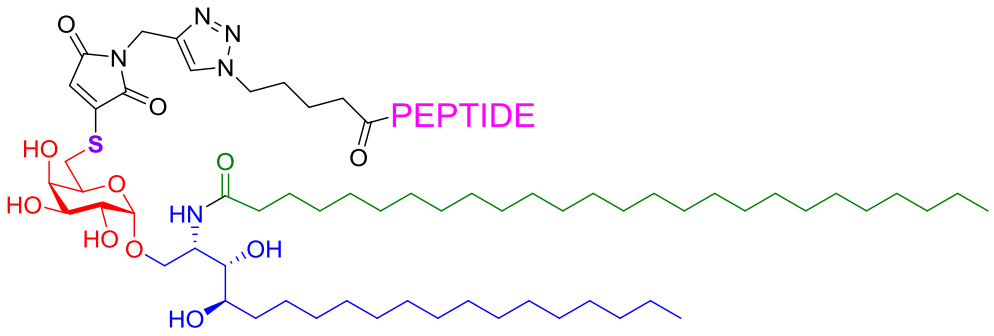
A major challenge in the development of highly defined synthetic vaccines is the codelivery of vaccine components (i.e., antigen and adjuvant) to secondary lymphoid tissue to induce optimal immune responses. This problem can be addressed by synthesizing vaccines that comprise peptide antigens covalently attached to glycolipid adjuvants through biologically cleavable linkers. Toward this, a strategy utilizing previously unreported 6″-deoxy-6″-thio analogues of α-GalCer that can undergo chemoselective conjugation with peptide antigens is described in this presentation.[1] Administration of these conjugate vaccines leads to enhanced priming of antigen specific T cells. This simple vaccine design is broadly applicable to multiple disease indications such as cancer and infectious disease.



6-thio-α-GalCer-Peptide conjugate

1. Compton, B. J.; Tang, C.-w.; Johnston, K. A.; Osmond, T. L.; Hayman, C. M.; Larsen, D. S.; Hermans, I. F.; Painter, G. F., *Synthesis and Activity of 6''-Deoxy-6''-thio-α-GalCer and Peptide Conjugates*. *Org. Lett. 17* (24), 5954-5957 (**2015**)

CB17: Enhancing immune therapies for cancer

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Virus-like particles (VLP) are empty viral protein shells formed from capsid proteins of some viruses. They are a proven platform for immune stimulation and the delivery of subunit vaccines. The current vaccines against Hepatitis B virus and Human Papilloma Virus (HPV) are VLPs, for example. We have generated VLP from *Rabbit hemorrhagic disease virus (RHDV).* We have shown that unlike most VLP, which induce antibody production, RHDV VLP are particularly effective at promoting anti-tumour immunity. We have used these VLP to treat several tumours, including melanoma and colorectal cancer. We have also enhanced the immunogenicity of the VLP by linking immune adjuvants to the particle surface.

Our laboratory is also developing adoptive cell therapies (ACT) for cancer treatment. The majority of the current ACT are focused on using CD8 T cells, however some studies have shown that CD4 T cells can also be effective in tumour rejection, but the methodology of expanding these cells has not been well characterised. We have used the antigen-specificity of CD4 T cells from OTII transgenic mice in order to optimize the methodology for antigen-specific expansion these T cells in tissue culture. These T cells displayed an effector phenotype associated with an anti-tumour response. When these cells were adoptively transferred they demonstrated direct lysis of antigen-specific target cells *in vivo*. However in a mouse model of melanoma, CD4 T cells did not control tumour growth as well as CD8 T cells but a combination therapy of both CD4 and CD8 T cells induced an enhanced level tumour regression, leading to 85% tumour-free survival. Finally we have assessed the ability of the VLP to boost adoptively transferred T cells and shown that the vaccine can act to enhance T cell responses.

CB18/DD16 Trehalose Glycolipids: 'Upping the Ante'

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Vaccines have made a major contribution to the control and eradication of disease, yet there are still many pathogens or ailments for which effective vaccines are not available. There are two general classes of vaccine: prophylactic vaccines, which provide protective immunity against pathogens; and therapeutic vaccines, which assist in treating an existing disease. Both classes of vaccine require an antigen and an adjuvant to be effective, whereby the role of the adjuvant is to enhance the antigen-specific immune response.[1]

To address the need for new and enhanced vaccines there has been much interest in harnessing the potential of bacterial glycolipids as adjuvants. In particular, we are interested in the role of trehalose glycolipids as vaccine adjuvants.[2] This family of glycolipids was first isolated from the cell wall of *M. tuberculosis* and has since been found to activate the innate immune response via binding to the macrophage inducible C-type lectin (Mincle).[3] Using a combination of chemical synthesis and innate immunology, we have been exploring how trehalose glycolipids interact with macrophages so as to determine how the glycolipids may best be optimised for use as vaccine adjuvants.

1. Leroux-Roels G. *'Unmet needs in modern vaccinology: Adjuvants to improve the immune response.' Vaccine*, **2010**, 28S:C25-C36.
2. Stocker BL, Timmer MSM. *'Trehalose Diesters, Lipoteichoic Acids and α-GalCer: Using Chemistry to Understand Immunology.' Carbohydr. Res.* **2014**, *389*, 3-11.
3. Ishikawa E, Ishikawa T, Morita YS, Toyonaga K, Yamada H, Takeuchi O, Kinoshita T, Akira S, Yoshikai Y, Yamasaki S. *'Direct recognition of the mycobacterial glycolipid, trehalose dimycolate, by C-type lectin Mincle'*, *J. Exp. Med.* **2009***,* 206:2879-2888.

CB19: Characterisation of circular RNA in melanoma cell lines

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New Zealand has the highest incidence of melanoma in the world. *ANRIL*, a large non coding RNA is involved in regulating the expression of a number of tumor suppressor proteins in the *CDKN2A/B* locus in human melanoma. This locus houses *p16INK4A* which encodes p16, a potent cell cycle regulator, whose function is lost in nearly 50% of human cancers. *ANRIL* is suggested to interact with the *p16INK4A* locus although the exact nature of their association remains poorly understood. Recent studies have discovered the existence of circular isoforms of *ANRIL* (*circANRIL*) in addition to its linear counterparts. We aimed to investigate the functional mechanism of *ANRIL* by characterising the multiple isoforms of *circANRIL* in melanoma cell lines.

The *circANRIL* were systematically amplified using outward-facing primers designed against each of the exons of *ANRIL*. The resulting PCR products were cloned, sequenced and mapped against the ANRIL genome in order to identify various *circANRIL*. We then localised and quantified *circANRIL* within each sub-cellular fraction by cell fractionation and quantitative-PCR respectively.

Using the results of the sequence analysis we were able to construct a library of all *circANRIL* isoforms identified in melanoma cell lines. The cell fractionation experiments confirmed the cytoplasmic localisation of *circANRIL* which is distinct to the nuclear localisation of its linear counterpart.

The identification of numerous *circANRIL* in melanoma cell lines demonstrates the complexity of the *CDKN2A/B* locus and suggests a complex regulatory network for *p16INK4A* expression. Characterisation of these isoforms enable us to manipulate their expression of further functional analysis. The localisation of *circANRIL* in the cytoplasm, together with previous data, suggests that *circANRIL* may be associating with supramolecular complexes in the cytoplasm and proposes that *circANRIL* may exert its functions as a post-transcriptional regulator of *p16*.

CB20 / DDxx - TBA

CB21: Structural adaptations of Class III PI3K and a PIKK to regulate cellular homeostasis

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Many anti-cancer therapeutics induce cellular stress, and induction of autophagy is a pro-survival response to this stress. An early event in macroautophagy is production of phosphatidylinositol 3-phosphate (PtdIns3P) at the site of autophagosome induction. Our structural work has focused on two enzymes important for regulating autophagy, Vps34, the class III phosphoinositide 3-kinase that synthesizes PtdIns3P to promote autophagy and TOR, a S/T protein kinase that inhibits autophagy. The kinase domains of Vps34 and mTOR share a common ancestor and are present in all eukaryotic cells. When cells are starved of amino acids, Vps34 becomes upregulated and macroautophagy is initiated. In contrast, when amino acids are abundant in lysosomes, mTOR can become activated on the lysosomal membrane and it suppresses Vps34 activity. Both mTOR and Vps34 are present in large multi-subunit complexes whose structures we have determined.

Vps34 associates with a Vps15 regulatory S/T protein kinase, and this heterodimer can associate with another heterodimer, consisting of either Vps30 (Beclin1) with Vps38 (UVRAG) or Vps30 (Beclin1) with Atg14 (Atg14L). The complex containing Atg14 is known as complex I and activated in autophagy, while the Vps38 (UVRAG)-containing complex is known as complex II, which is involved in endocytic sorting.

Our crystal structure of the 385 kDa endosomal complex II (PIK3C3-CII) shows that the subunits form a Y-shaped complex, centered on the Vps34 C2 domain. The catalytic arm of the Y is made up of Vps34 and Vps15 where the Vps15 kinase domain binds to the activation loop of Vps34. Vps30 and Vps38 form the adaptor arm of the Y. Hydrogen-Deuterium Exchange Mass Spectrometry (HDX-MS) suggests that the adaptor arm of the complex interacts with membranes. Complex II is active on low-curvature membranes made of brain lipids, while complex I prefers highly curved membranes. Activities of complex I and II are exquisitely sensitive to the membrane packing density. HDX-MS shows that a fifth subunit known as Atg38/NRBF2 binds the Vps30/Atg14 arm of complex I, using its N-terminal MIT domain to bridge the coiled-coil I regions of Atg14 and Vps30 in the base of the Y of complex I. The Atg38 C-terminal domain is important for localization to the pre-autophagosomal structure (PAS) and homodimerization.

TOR regulates a range of cellular processes, including inhibiting Vps34 complex I. Our cryo-EM structure of the core of the two types of TOR complexes in cells shows a symmetric dimer. As with Vps34, the C-terminal kinase domain of Tor is preceded by a helical solenoid region, however, this region is greatly expanded in Tor and other PIKKs. The N-terminal residues of Tor form an α-helical solenoid with a complex topology. Our experimentally determined topology traces the course of the polypeptide for most of the 1300 residues of the α-helical solenoid in a direction that is opposite to what was recently proposed for mTORC1. Our topology implies that in mTORC1,

RAPTOR binds to the dimer interface of mTOR, consistent with the ability of RAPTOR to stabilise the complex.

CB22: DNA replication stress induces mitotic cell death through the telomere DNA damage response and cohesion failure

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Telomeres are the protective nucleoprotein structures that regulate DNA damage response (DDR) activation by the naturally occurring chromosome ends. Progressive telomere erosion during cellular ageing regulates both replicative senescence and telomere crisis, through activation of the telomere DDR[1,2]. Our research team recently identified an additional and novel telomere length-independent mechanism, where the telomere DDR is activated by the Aurora B and ATM kinase as a result of mitotic arrest[3]. Further, we identified mitotic telomere DDR activation as a contributing factor to cell death at telomere crisis during ageing, and in response to chemotherapeutic mitotic poisons[2]. The broad implication of these discoveries is that any cellular process leading to mitotic arrest may signal cell death via the telomere DDR. Because general DNA replication stress has been implicated in mitotic chromosome segregation errors[4], we investigated whether replication stress may also be leading to mitotic arrest and mitotic cell death, signalled by telomere DDR-activation.
Using molecular and cell biology approaches, based primarily on live- and fixed cell imaging, we have identified that pharmacologically-induced DNA replication stress results in mitotic arrest and mitotic cell death. Surprisingly, over 80% of cell death events in response to replication stress occurred during mitosis. We determined that replication stress-dependent mitotic arrest was Spindle Assembly Checkpoint (SAC)-dependent, and that maintenance of prolonged SAC activation, due to replication stress, led to two prominent mitotic chromosomal phenotypes: 1) an Aurora B and ATM-dependent telomere DDR, regulated by the telomere protein TRF2, and 2) cohesion failure initiated at the centromeres. Exacerbating or suppressing the mitotic telomere DDR, by knock-down or over-expression of TRF2, sensitized or suppressed mitotic cell death, respectively, without impacting cohesion failure. While suppressing cohesion failure by depletion of the cohesion against Wapl, also suppressed the mitotic cell death phenotype, but did not affect the telomere DDR. Together these data indicate that the telomere DDR and mitotic cohesion failure are independent drivers of cell death in response to DNA replication stress. We have therefore identified a novel mechanism, where general DNA replication stress leads to cell death, primarily during mitosis. Moreover, we have mechanistically identified that mitotic cell death due to replication stress is signalled through independent but parallel pathways, dependent upon cohesion loss and telomere DDR activation.

1.Cesare, AJ & Karlseder, J. A three-state model of telomere control over human proliferative boundaries. *Curr Opin Cell Biol* **24**, 731-8 (2012).
2.Hayashi, MT*, et al.* Cell death during crisis is mediated by mitotic telomere deprotection. *Nature* **522**, 492-6 (2015).
3.Hayashi, MT*, et al.* A telomere-dependent DNA damage checkpoint induced by prolonged mitotic arrest. *Nat Struct Mol Biol* **19**, 387-94 (2012).
4.Burrell, RA*, et al.* Replication stress links structural and numerical cancer chromosomal instability. *Nature* **494**, 492-6 (2013).

CB23: Role of YB-1 in cell cycle

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YB-1 (Y-box-binding protein 1) is known to facilitate many of the hallmarks of cancer. Increasing evidence suggests that it is an excellent molecular marker of cancer progression. Several studies have demonstrated knocking down YB-1 inhibits proliferation and tumour growth. This suggests an important role of YB-1 in regulating cell cycle. Furthermore, However, phosphorylation of YB-1 at different sites can alter its function. The aim of this study is to identify key phosphorylation sites on YB-1 important in regulating cell cycle checkpoints that can be therapeutically targeted.

We have identified common phosphorylation sites by mass spectrometry in breast and lung cancer cells. Using site directed mutagenesis, mutant YB-1 defective for phosphorylation at the identified sites were generated. We are currently screening the ability of YB-1 and these mutants in regulating different phases of the cell cycle using A549 cells stably transfected with the FUCCI plasmid.

Preliminary results suggest that YB-1 plays an important role in M-G1 phase of the human cancer cell cycle. Also we have been able to identify key phosphorylation sites on YB-1 that are important in regulation of the cell cycle.

Our results suggest that one or more of the identified phosphorylation sites on YB-1 can be targeted therapeutically. We hope in the future that this intervention in turn will reduce the tumour burden in cancer patients leading to improved outcomes.

CB24: Insights into the origins and pathogenesis of embryonal rhabdomyosarcoma

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Rhabdomyosarcoma (RMS) is the most common pediatric soft tissue sarcoma. Despite aggressive therapy, survival for RMS has not improved for three decades, emphasizing the need to uncover the molecular underpinnings of the disease. RMS includes two histolopathologic subtypes: alveolar RMS, driven by the fusion protein PAX3/7-FOXO1, and embryonal RMS (ERMS), which is genetically heterogeneous. RMS has been presumed to originate from derailed muscle progenitors based on the histologic appearance and gene expression pattern of the tumors. However, an origin restricted to skeletal muscle does not explain RMS occurring in tissues devoid of skeletal muscle such as the prostate, bladder, biliary tree and the omentum. Previously, we showed that activation of Sonic Hedgehog signaling through expression of a conditional, constitutively active Smoothened allele, *SmoM2*, under control of an adipocyte-restricted adipose protein 2 (aP2)-Cre recombinase transgene in mice gives rise to aggressive skeletal muscle tumors that display the histologic and molecular characteristics of human ERMS. We illustrated the transcriptome of the aP2-Cre;SmoM2 tumors recapitulates both other mouse ERMS models as well as human ERMS. With the short latency and anatomic restricted tumor location, we sought to leverage this model to explore the cell of origin. Lineage tracing the aP2-Cre with reporter mice illustrated aP2-Cre expression in both brown and white adipose tissue as well as a discrete population of cells lying between skeletal muscle fibers. These aP2-lineage cells are distinct from Pax7-positive skeletal muscle stem cells or satellite cells. The aP2-lineage cells do not contribute to myotube formation. When compared to *aP2-Cre;R26-Tom* mice, the addition of oncogenic SmoM2 (*aP2-Cre;R26-Tom;SmoM2*) results in embryonic expansion of the aP2-lineage interstitial muscle cells. Our findings suggest that non-skeletal muscle progenitors are a potential cell of origin for Sonic Hedgehog-driven ERMS.

CB25: Targeting cohesin mutant myeloid leukaemias

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Cohesin is a multisubunit protein complex, known for its role in cell division and gene regulation[1]. Mutations in the subunits of cohesin or disruption in its function have been identified in a range of cancers[2]. In leukaemia 12-13% have recurrent mutations in cohesin genes and an additional 15% of leukaemias without cohesin mutations have low expression of cohesin genes[3].

In leukaemias, mutations in the cohesin subunit are mutually exclusive, generally heterozygous, and predicted to result in reduced cohesin function[3]; there cannot be a complete loss of function, because a cell completely lacking cohesin would not be able to divide. ***This implies that pathways to malignancy arise from a modest reduction in dose or function of cohesin.***

Cohesin also has roles in DNA repair and ribosome biogenesis pathways[4], which are important for cell growth and proliferation. In MCF7 breast cancer cells, we found that cohesin depletion disrupts expression of genes involved in cell proliferation and ribosome biogenesis pathways, mTOR and PI3K[5]. The vital DNA repair and cell growth functions of cohesin suggest that cohesin mutations might render cells vulnerable to drugs that target these pathways. Poly (ADP)-ribose polymerase (PARP) inhibitors that target the DNA damage repair pathway have been shown to exhibit synthetic lethality with cohesin mutation[6]. We have used the CRISPR-CAS9 gene editing system to engineer chronic myelogenous leukaemia cell line, K562, to contain leukaemia associated nonsense mutation either in the RAD21 (E212*) or SMC3 (R254*) subunit of cohesin. K562 cells with RAD21 mutation have reduced levels of RAD21 protein and show a slight reduction in growth. We are currently characterizing the cohesin mutant cell lines for alteration in morphological and molecular characteristics and testing their sensitivity to PI3K/mTOR and PARP inhibitors.

References

1.Dorsett, D. & Strom, L. *The ancient and evolving roles of cohesin in gene expression and DNA repair*. Curr Biol **22**, R240-50 (2012).

2.Leiserson, M.D.M. et al. *Pan-cancer network analysis identifies combinations of rare somatic mutations across pathways and protein complexes*. Nat Genet **47**, 106-114 (2015).

3.Thota, S. & Viny, A.D. *Genetic alterations of the cohesin complex genes in myeloid malignancies*. **124**, 1790-8 (2014).

4.Solomon, D.A., Kim, J.-S. & Waldman, T. *Cohesin gene mutations in tumorigenesis: from discovery to clinical significance*. BMB Reports **47**, 299-310 (2014).

5.Antony, J. et al. *Cohesin modulates transcription of estrogen-responsive genes*. Biochim Biophys Acta **24**, 00311-3 (2014).

6.McLellan, J.L. et al. *Synthetic lethality of cohesins with PARPs and replication fork mediators*. PLoS genetics **8**, e1002574 (2012)

CB26: Characterization of CALM-AF10 induced leukaemia in a murine bone marrow transplantation model

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Acute leukaemia (AL) is characterised by a block in differentiation and an increase in proliferation of haematopoietic cells resulting in an accumulation of immature blast cells. AL is the most aggressive form of leukaemia and, in the absence of treatment, can be fatal within a few days of diagnosis. AL is caused by somatic genetic aberrations. These include chromosomal aberrations and mutations. A rare but recurrent translocation observed in acute myeloid and lymphoblastic leukaemia is the t(10;11)(p13;q14) which leads to the *CALM-AF10* fusion gene. We used the CALM-AF10 minimal fusion protein (CA-MF), which contains the regions of CALM and AF10 required for leukaemogenesis, in a murine retroviral transduction bone marrow transplantation model (MBMTM) to induce leukaemia in mice. 12 mice transplanted with CA-MF expressing bone marrow have developed AL with a median latency of 131 days. None of the mice (n=14) transplanted with bone marrow cells transduced with a GFP expressing retrovirus have developed leukaemia up to 270 days post transplant. Interestingly, immunophenotypic analysis of the leukaemias shows two distinct phenotypes: myeloid and biphenotypic leukaemia. We hypothesise that this is due to different secondary mutations that are acquired during the latency period. We are in the process of identifying these secondary mutations using murine whole exome sequencing. Ours is the first report that CALM-AF10 or CA-MF can induce two distinct phenotypes in the MBMTM. Furthermore, this first syngeneic leukaemia model in immunocompetent mice established in New Zealand will be a unique resource to characterise leukaemic stem cells and to develop and test novel immunotherapy strategies.

CB27: Predicting cancer spread before it happens

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E-cadherin-mediated cell-cell junctions play a prominent role in maintaining epithelial architecture. Their disruption or deregulation in cancer can lead to the collapse of tumor epithelia that precedes invasion and metastasis.

Here, we have generated an E-cadherin-GFP mouse, which enables intravital photobleaching (FRAP) and quantification of E-cadherin mobility in live tissue, without affecting normal biology. We assessed E-cadherin mobility in native pancreatic tissue or upon genetic manipulation involving Kras and p53, both of which are commonly mutated in pancreatic cancer patients. We reveal a mobilization of E-cadherin specifically in invasive pancreatic tumours that can be targeted with anti-invasive treatment[1]. Our E-cadherin-GFP mouse may thus be a valuable tool to fundamentally expand our understanding of E-cadherin-mediated events in native microenvironments.

Future intravital FRAP imaging through titanium imaging windows in one and the same mouse over the course of tumour progression will allow us to identify and map hotspots of tumour dissolution events to predict when and where cancer will spread.

1.Erami, Z., Herrmann, D., et al. (2016). *Intravital FRAP Imaging using an E-cadherin-GFP Mouse Reveals Disease- and Drug-Dependent Dynamic Regulation of Cell-Cell Junctions in Live Tissue*. Cell reports. 14(1): 152-67.

CB28: Patient-derived and cell line xenograft models of head and neck cancer for preclinical testing of hypoxia activated prodrugs

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Head and neck squamous cell carcinoma (HNSCC) is the 6th most common malignancy worldwide. It is widely treated with chemoradiotherapy, but there is compelling clinical evidence that hypoxia is a negative prognostic factor for treatment outcome in this setting. We have established a large number of patient-derived xenograft (PDX) and cell line xenograft (CLX) models of HNSCC that we can use for testing the anticancer efficacy of novel hypoxia-activated prodrugs (HAPs). PDX models were generated by engrafting tumour fragments from patients undergoing surgery at Auckland City Hospital into highly immunodeficient Nod Scid Gamma mice and CLX models by inoculation of early passage cell lines from the University of Turku. To date, we have evaluated the tumour growth of 33 different HNSCC tumour models (6 PDXs and 27 CLXs) and have characterised the most favourable models for their tumour hypoxia status. A prototype compound from a recent class of HAPs (nitroCBIs or nitrochloromethylbenzindolines) that we are progressing towards clinical evaluation has shown considerable activity at inhibiting and in some cases curing tumour growth in a CLX model and is currently undergoing efficacy testing in PDX models. We expect that antitumour efficacy testing in these CLX and PDX tumour models will support the clinical development of nitroCBIs in HNSCC.

CB29: 5-fluorouracil uptake in human gastrointestinal cells

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5-fluorouracil (5-FU) is a widely used and effective drug for the treatment of gastrointestinal and metastatic breast cancers, however severe and sometimes life-threatening side effects occur in a substantial number of patients receiving this drug. These often affect the normal cells lining the gastro-intestinal tract and can result in significant, debilitating, and sometimes life-threatening toxicities such as mucositis and diarrhoea. While variation in the activity of the metabolic enzyme dihydropyrimidine dehydrogenase (DPD) is responsible for 5-FU toxicity in a minority of patients, the underlying cause of adverse events is unknown in the majority of individuals. Our research group has hypothesised that some individuals have aberrantly high uptake of 5-FU into mucosal epithelial cells.

Although 5-FU has been in use for more than 50 years there is little information about the mechanism of cellular uptake and the identity of the transmembrane transporters involved. We have developed a functional assay to characterise 5-FU uptake into human gastrointestinal cell lines (CaCo2, HCT116) and *ex vivo* buccal mucosal cells collected from healthy volunteers, and have determined the kinetics of 5-FU uptake into these cells. The sodium (Na^+) and temperature dependence of this uptake have also been assessed.

This work has demonstrated rapid, temperature dependent, and saturable uptake of 5-FU in both the cell lines and the primary buccal mucosa. The temperature dependency suggests that this process involves either a facilitative transporter or an energy-dependent active transporter, although further work with metabolic inhibitors is required to confirm these findings. Additionally a substantial decrease in uptake was observed when Na^+ was absent from the incubations, indicating a sodium-dependent component contributes to 5-FU uptake in these cells. Further biochemical and genomic characterisation of 5-FU uptake by these transporters is underway.

CB30: Using CRISPR/Cas9 methodology to identify and validate novel cell death regulators for cancer therapy.

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CRISPR/Cas9 mediated genome engineering provides an easy and rapid way to edit genes *in vitro* and *in vivo*. Initial experimental strategies utilised proved to have a low efficiency and were not broadly applicable to all cell types. In order to overcome this hurdle and to allow for efficient modification of genes in the haematopoietic system, we have developed a novel drug-inducible lentiviral system to deliver the CRISPR/Cas9 platform to cells permitting efficient genome engineering *in vitro* and *in vivo*. Additionally we have recently also implemented the CRISPR/Cas9 technology to produce genetically modified mice and to perform whole genome screens.

CB31: Sleeping Beauty uncovers cooperating driver genes in a preclinical mouse model of myeloid leukemia

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Myeloid leukemia is associated with few predominant mutations and translocations in humans. We have developed an aggressive, fully-penetrant mouse model of myeloid leukemia using Sleeping Beauty (SB) insertional mutagenesis (SB|ML) and have identified more than 400 statistically defined candidate cancer genes (CCGs), 87 of which have orthologs mutated in human AML. Pathway enrichment analysis using human orthologs of SB|ML CCGs confirmed that MAP-Kinase and JAK-STAT signaling play an important role in the disease. Among the top driver genes was a suite of transcription factors that act downstream of MAP-Kinase signaling, including Ets-family member genes *Erg*, *Ets1* and *Fli1*. To uncover cooperating relationships among driver genes, we have developed a quantitative capture-hybridization methodology, termed SBCapSeq, to sequence SB insertions from single tumor cells. Using this approach, we have harnessed SB insertion data to characterize the intra-tumor heterogeneity of a myeloid tumor and identified two distinct subclonal populations anchored by different cooperating driver genes, *Erg* and *Ghr*, or *Ets1* and *Notch1*. RNA-seq analysis confirmed that SB insertions drive expression of chimeric transcripts for these four driver genes, and demonstrates that SB drives the mis-expression of these oncoproteins to initiate and sustain myeloid leukemia *in vivo*. SB-driven myeloid leukemia is a robust, transplantable genetic model that offers a unique platform for preclinical testing. SB|ML tumors can be stratified for targeted therapies based on identified interacting driver genes using single cell sequencing. Finally, SB can be harnessed to uncover loci that confer therapy response or resistance.

CB32: Development of automated high-content microscopy-assisted assays for telomere biology

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Telomeres are nucleoprotein structures at chromosome termini that help maintain genome stability and regulate cellular ageing. A major focus of telomere research is understanding how cellular processes, such as the DNA damage response (DDR), or nuclear structures, such as promyelocytic leukemia nuclear bodies (PML-Nbs), interact with the chromosome ends. Because telomeres are composed of many kilobasepairs of a tandem six-nucleotide repeat, they are highly amenable to visualization with fluorescence in situ hybridization (FISH). Combining telomere FISH with immunofluorescence microscopy enables visualization and quantification of telomere specific events. However, to date this has remained a qualitative or low-throughout experiment due to the time required to manually capture and analyse images.

We therefore set out to develop multiple automated microscopy-based high content screening (HCS) algorithms to expedite research on telomere biology. Automated HCS have major advantages due to their ability to screen many features simultaneously at the single cell level and apply strict objective measures. We utilized a Zeiss automated multi-slide scanning platform equipped with Metasystems software and developed an informatics workflow which enabled the quantitation of multiple fluorescent markers from specific subnuclear regions of individual cells. Key to this workflow are customised algorithms which automatically distinguish individual interphase cells, segment cells into defined regions of interest, measure fluorescent marker intensities and compute marker co-localisations specific to these regions. In this presentation I will discuss two algorithms: one that assays telomere interactions with DDR markers as measure of telomere health, and a second that quantifies PML-NBs with and without telomere DNA. The latter method being critical to identify the mechanism of HSV-1^{ICP0null} synthetic lethality in cancer cells. These multi-parametric cellular assays are unbiased, fully automated and quantitative, and resulted in at least a 20-fold increase in data acquisition. In the future we anticipate applying these algorithms to a wide range of research questions related to telomere biology with potential to be applied to the drug discovery and development pipeline.

CB33: Materials Matter: Authenticating cell lines for cancer research

Amanda Capes-Davis

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"All models are wrong but some are useful." This quote from mathematician George Box reminds us that there is no perfect model system for research. Cancer researchers have used cell lines as model systems for more than sixty years, but we continue to debate whether they truly represent the tumours from which they were originally established. Cell lines are living materials and so it is understandable that they will change over time. Culture conditions, selection of clonal populations, genetic drift and contamination can all influence a cell line's behaviour. We need to think critically about the materials we use and the risks to our research that may result.

Cell line misidentification is an important risk when using any cell line as a model system. A misidentified cell line no longer corresponds to the original donor and may originate from a different species, cell type or disease state. Although misidentification has a number of causes, it commonly arises through cross-contamination – the accidental introduction of cells from another culture. More than 400 misidentified cell lines now exist with no known authentic stocks, as compiled in ICLAC's list of cross-contaminated or otherwise misidentified cell lines (http://iclac.org/databases/cross-contaminations/). Misidentified cell lines continue to be widely used in many research fields, including cancer research.

Misidentified cell lines can be detected using authentication testing. Authentication testing relies on comparison through genotypic analysis, examining material held by one laboratory and comparing it to samples held elsewhere. In 2001, short tandem repeat (STR) profiling was proposed as an international reference standard for human cell lines. Ten years later scientists in the field of authentication testing came together to write a consensus Standard for human cell line authentication, recommending STR profiling for interlaboratory comparison. Short nucleotide polymorphism (SNP) analysis is another effective test method, particularly for genome-based applications and rapid internal testing where an STR profile has already confirmed authenticity.

Fifty years of work on misidentified cell lines has shown that inappropriate cell line models are widely used. The problem will not be remediated unless we develop consensus-based benchmarks and guidelines for all work that uses cell lines. An increasing number of journals and funding bodies are moving to set requirements for cell line authentication. Recently, the National Institutes of Health (NIH) established principles and guidelines for reporting of preclinical research and now require authentication of key resources to be incorporated into funding applications. These initiatives will decrease the use of misidentified cell lines. However, misidentification is one of many risks when using cell lines as model systems. We must improve the way in which we approach materials, based on an objective assessment of risk, to ensure that they are effective tools for cancer research.

CB34: Simultaneous visualisation of RhoA and Rac1 activation using multi-spectral FLIM

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The prototypical RhoGTPases Rac1 and RhoA are temporally and spatially synchronized during cell protrusion; this tight control and dynamic regulation of activity is necessary for coordinated cell migration in vitro. Aberrant regulation of Rac1 and RhoA is often observed in cancer can lead to loss of epithelial polarity, local invasion and metastasis. Genetically expressed Förster resonance energy transfer (FRET) biosensors provide a powerful tool to study the activation of these GTPases individually in a live cell and *in-vivo* setting.

In this work, we demonstrate an approach to simultaneous imaging of Rac1 and RhoA biosensors in live tissue to study the spatiotemporal relationship between these two molecules. We crossed a recently published Rac1 FRET biosensor reporter mouse[1] with a RhoA FRET mouse to produce a dual reporter mouse. Using a custom-built multichannel fluorescence lifetime imaging (FLIM) system we are able to resolve the activation level of Rac and Rho on a subcellular level. We employ a spectral-lifetime fitting approach to overcome the spectral overlap between the biosensors by exploiting the difference in lifetimes of the biosensor fluorophores.

We demonstrate that we are able to readout the activation of Rac1 and RhoA in cancer cell lines and primary fibroblasts and neutrophils derived from the dual biosensor mouse. We then show that we can visualise the activation of the two proteins in freshly excised pancreas samples from the biosensor mouse treated with Rac1 and RhoA activators and inhibitors by accounting for tissue autofluorescence.

We believe that the ability to visualise the spatio-temporal dynamics of the Rho-Rac signalling network *in-vivo* will provide insights into the role of this pathway in cancer progression and may provide a useful platform to read out the effects of therapeutics targeting this network and its upstream regulators.

1. Johnsson, AK et al (2016). *The Rac-FRET mouse reveals tight spatiotemporal control of Rac activity in primary cells and tissues.* Cell Reports*. 6(6):1153-64.*

CB35: Targeting chemoresistance within the hypoxic microenvironment of pancreatic cancer

Conway, J.R.W.[1], Warren, S.C.[1], Shearer, R.F.[1], Herrmann, D.[1], Magenau, A.[1], Croucher, D.R.[1], Sansom, O.J.[2], Pajic, M.[1], Morton, J.P.[2], Haigh, J.J.[3], Timpson, P.[1]

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By applying novel intravital imaging techniques to dynamically monitor pathway activity upon therapeutic inhibition, we are assessing chemoresistance within the hypoxic microenvironment of pancreatic ductal adenocarcinoma (PDAC). Hypoxia is a negative prognostic factor in PDAC, known to increase radioresistance, chemoresistance, angiogenesis and metastasis. Here we demonstrate pronounced chemoresistance in hypoxia to three clinically relevant inhibitors of the PI3K pathway; Rapamycin (mTORC1 inhibitor), NVP-BEZ235 (dual PI3K/mTORC1 inhibitor) and AZD2014 (mTORC1/2 inhibitor). Further to the clinical problem, we have mapped tumour hypoxia by both immunofluorescence and phosphorescence lifetime (PLIM) imaging techniques and here have shown that these hypoxic regions move sproadically around the tumour. Using advanced Akt Förster Resonance Energy Transfer (FRET) biosensors and state-of-the-art multiphoton imaging, we will demonstrate improved efficacy for dual PI3K/mTOR inhibitors against this dynamic hypoxia-induced chemoresistance. To this goal, we are utilising well-established mouse models of PDAC, in which we have expressed an optimised Akt-FRET biosensor. To date, *in vitro* modeling has involved three-dimensional invasion/proliferation assessment using organotypic assays and assessment of drug kinetics through live cell fluorescence lifetime (FLIM)-FRET imaging and standard molecular techniques. Progressing to *in vivo* intravital imaging, we are dynamically monitoring the therapeutic effect of targeting the PI3K–Akt–mTOR signaling axis within the hypoxic microenvironment of PDAC. Here we are able to assess novel combination therapies using hypoxia-activated prodrugs (ie. TH-302) and angiogenics, with our dual PI3K pathway inhibitors, to improve treatment efficacy. Further to our *in vivo* fidelity, we have developed a novel Akt-FRET biosensor mouse for real time analysis of drug kinetics in the native disease state.

CB36: Dissecting ERBB2 signalling complexes in breast cancer by Bimolecular Complementation Affinity Purification (BiCAP).

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The dynamic assembly of multi-protein complexes is a central mechanism of many cell signalling pathways. This process is key to maintaining the spatiotemporal specificity required for an accurate, yet adaptive response to rapidly changing cellular conditions. Accordingly, many oncogenes can promote tumourigenesis through the formation of aberrant multi-protein complexes. However, there is still a lack of experimental techniques that can facilitate the specific and sensitive deconvolution of these multi-molecular signalling complexes.

Here we describe a novel approach that overcomes many of these existing limitations and allows the specific isolation and downstream proteomic characterisation of any two interacting proteins, to the exclusion of their individual moieties and competing binding partners (Croucher *et al.*, Science Signaling, *In Press*). This novel isolation technique is achieved through the combination of a protein-fragment complementation assay and affinity purification with a conformation specific nanobody, which we have termed Bimolecular Complementation Affinity Purification (BiCAP). We also demonstrate the utility of this approach through characterisation of the specific interactome of the breast cancer oncogene ERBB2, in the form of a homodimer or a heterodimer with either EGFR or ERBB3. Through this analysis we have observed dimer-specific interaction patterns for key adaptor proteins that direct the differential signalling capacity of these dimers, and also identified a number of novel interacting partners. Functional analysis for one novel interaction has also led to the identification of a non-canonical mechanism of ERK activation that is specific to the ERBB2:ERBB3 heterodimer, which acts through the adaptor protein FAM59A to induce ERK activation in breast cancer cells.

CB37: Mapping enhancer networks by chromatin conformation capture

Timothy Sadlon[2], Veronika Bandara[1], Cheryl Brown[1], Marc Beyer[3], Joachim Schultze[3], Stephen Bent[1], Alastair Forrest[4] Simon C Barry[1-2]

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It is now evident that coordinated gene regulation occurs in a cell specific manner to bring together regulatory elements and coding regions, and this is conformation dependant. While bioinformatics can predict targets of transcription factors with some accuracy, and genomics datasets can now identify functional motifs in chromatin, such as super enhancers and lncRNAs, the targets of these regions cannot be predicted by linear annotation models. Chromatin Conformation capture can be used to determine which non coding elements interact with specific genes, and we can superimpose on this our FOXP3 binding site data (FOXP3 Chip). This analysis reveals the conformation dependant transcriptional regulation of key genes, and will also allow for the first time annotation of SNPs from disease linkage analysis (GWAS) to functional targets. This approach will shed light on the 80% of SNPs that do not disrupt a coding region, and whose targets are currently unknown. As proof of principle we have used SATB1, a key FOXP3 repressed gene in Treg (Beyer *et al* Nature Immunology 2011) and breast cancer (Mc Innes *et al* Oncogene 2011) as a conformation capture target. Using 4Cseq we have identified a FOXP3 responsive super enhancer over 300Kb upstream, and this region includes 5 enhancer elements. We now confirm that this enhancer is repressed by FOXP3, and overlaps a number of IBD/Colitis SNPs from GWAS datasets. The functional impact of disease linked SNPs that map to this region on SATB1 expression is now under investigation.

CB38: Bioinformatic analysis of insertional mutagenesis data from Sleeping Beauty mouse models of cancer

Mik Black[1]

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The Sleeping Beauty (SB) insertional mutagenesis system allows the creation of inducible and tissue-specific mouse models of human cancer. The data generated by these models comprises a genome-wide list of transposon insertion sites, which can be analysed across a collection of tumours to identify "candidate cancer genes", that are involved in tumour formation, and also to determine biological pathways that are potentially being disrupted via insertional mutagenesis.

An important part of this analysis is to determine which components of the information derived from the SB mouse models are applicable to the study of human tumours. In this talk I will describe the approaches we have developed for identifying pathway dysregulation on a per tumour basis, in both mouse and human tumours. I will also present methods for connecting the SB mouse data with genomic and clinical data from human tumours, including tools that we have developed for visualisation and exploratory analysis of these data sets. Examples presented will utilise data generated by collaborators at the Houston Methodist Research Institute (Houston, TX, USA), along with publicly accessible human tumour data from The Cancer Genome Atlas (TCGA) and the International Cancer Genome Consortium (ICGC) projects.

CB39: A framework and example for identifying epigenetic drivers of cancer metastasis

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Remarkable progress has been made in the last decade to document genetic events that cause primary cancers. However, a big unsolved question is how a primary cancer cell becomes metastatic and what are the molecular events that underpin this process. Extensive sequencing efforts indicate that gene mutations may not be a causal factor for primary to metastatic transition. On the other hand, epigenetic changes (e.g., DNA methylation) are dynamic in nature and therefore are likely to play an important role in deciphering the metastatic phenotypes and this area of research has just started to be appreciated. However, unlike driver genetic mutations, there is no established framework for defining and identifying driver and passenger epigenetic events for cancer metastasis.

In this talk, I will discuss some key concepts and approaches for identifying DNA methylation drivers involved in the metastasis cascade. I will also present results from our recent work on paired primary and metastatic melanoma cell lines (i.e., derived from the same patient) where we have identified extensive DNA methylation changes in metastatic cell lines compared to normal melanocyte or its corresponding primary cell line. Finally, I will discuss the exciting opportunities in this field and my future research directions.

CB40: TBA

Ben Lawrence[1]

1. Department of Molecular Medicine and Pathology, University of Auckland.

CB41: A common variation within the *IL6* promoter may alter its control by the p53 protein family and influence cancer progression

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Emerging data suggests that the tumour suppressor p53 suppresses cancer associated inflammation, including the expression of IL-6. IL-6 is known to modulate the propensity of cancers to metastasise, and recently it was shown to directly regulate cancer cell morphology, migration and invasion. p53 has two homologues called p63 and p73 that have been implicated in the progression of cancers, but it is not known if they influence IL-6 expression or whether their actions are altered by polymorphisms within the *IL6* promoter.

Methods: Here we examined this using a combination of luciferase reporter assays, CRISPR/cas9 modified cells and analyses of appropriate genomic data.

Results: p73 regulated *IL6* in a similar manner to p53, but in contrast, p63 was activating. Introduction of common single nucleotide polymorphism (SNP) into the promoter altered this regulation and changed the way the proteins collaborated with concurrent proinflammatory signalling. Blocking of p53-specific control of the promoter, as would occur in many cancers, increased its activation by p63 and enhanced the effect of the SNP. Editing of the SNP in cancer cells altered *IL6* expression and the propensity of cells to migrate. Finally, an examination of the SNP in human cancers indicated an association with increased invasion and metastasis.

Significance: These data therefore, point to a new prognostic marker for cancers and may explain its mechanism of action.

CB42: Bacterial dysbiosis and the development of colorectal cancer

R. Purcell[1], J. Pearson[2], J. Keenan[1], S. Schmeier[3], F. Frizelle[1]

1) Department of Surgery, University of Otago, New Zealand, Christchurch, Canterbury, New Zealand; 2) Department of Biostatistics and Computational Biology Unit, University of Otago, New Zealand, Christchurch, Canterbury, New Zealand; 3) Institute of Natural and Mathematical Sciences, Massey University, North Shore City, New Zealand

Colorectal cancer (CRC) is the second most commonly diagnosed cancer in New Zealand with almost 3000 new cases diagnosed annually, and New Zealand has one of the highest reported rates globally, at 37/100,000 people. Most CRCs are sporadic, and follow a pattern one would expect from a yet unidentified environmental source. Recent work from our and other groups, has implicated enterotoxigenic *Bacteroides fragilis* (ETBF) as a "keystone" pathogen in the development of CRC[1, 2]. We investigated the colonic mucosal colonization with ETBF in a large patient cohort, and our data suggest that while ETBF may be present in CRC patients, its predominant association is with early-stage carcinogenic lesions: tubular adenomas and low-grade dysplasia. The significant association between these lesions and, not only the presence of ETBF, but also the relative abundance of ETBF underlines the importance of this microbe in the development of CRC.

ETBF is not linked with all cases of CRC, however, and we hypothesize that different molecular subtypes of CRC may be linked to different patterns of microbial dysbiosis in the gut. We have carried out RNA-sequencing of tumour tissue to determine the CRC molecular subtype[3] and metagenomic analysis of corresponding tumour tissue in order to identify changes in the bacterial community composition related to different subtypes of CRC. We believe that this will reveal further "keystone" bacteria or bacterial virulence factors involved in the development of a particular CRC subtype. This would represent an important step in understanding the interplay between microbial diversity in the gut and the molecular mechanisms involved in the development of CRC.

1.Wu, S.; Rhee, K. J.; Albesiano, E et al, A human colonic commensal promotes colon tumorigenesis via activation of T helper type 17 T cell responses. *Nat Med* **2009,** *15* (9), 1016-22.
2.Ahn, J.; Sinha, R.; Pei, Z. et al, Human gut microbiome and risk for colorectal cancer. *J Natl Cancer Inst* **2013,** *105* (24), 1907-11.
3.Guinney, J.; Dienstmann, R.; Wang, X. et al,The consensus molecular subtypes of colorectal cancer. *Nat Med* **2015,** *21* (11), 1350-6.

CB43: Gene expression variability in breast tumours

Wiggins, G. A. R.[1], Pearson, J. [2], Dunbier, A. [3], Black, M.[3], Walker, L[1].

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Inherited and *de novo* mutations in *BRCA1* and *BRCA2* predisposes women to breast and ovarian cancer: which are believed to contribute to ~5% of all breast cancers and 5-15% of all ovarian cancers. *BRCA1* and *BRCA2* are involved in an array of functions including transcription regulation, cell cycle regulation and DNA repair. In particular, defective repair of double stranded DNA (dsDNA) breaks via homologous recombination is often assumed to be the major cause of *BRCA*-related cancer risk. However, studies of *BRCA1/2* carriers have failed to reveal why many tissues are not associated with significantly elevated cancer risk, or the mechanism by which *BRCA1* and *BRCA2* tumours develop different tumour subtypes. Gene expression studies have been used extensively to interrogate breast cancer, including tumours from *BRCA1/2* mutation carriers, however, these studies have typically focused on differentially expressed genes by comparing the mean of each group and determining statistical significance. We propose that genes key to *BRCA1/2*-related tumours may gain or lose regulation thus altering the variability of expression across a population. Using breast tumour expression data from published studies of patients with known *BRCA1/2* mutation status, we have identified that expression variation can occur while mean expression remain similar between groups. These new data have enabled us to investigate key pathways critical to *BRCA1/2*-related tumour development. Interestingly, *ERBB2* was identified as the most statistically variable gene ($p = 1.7 \times 10^{-5}$) when comparing carriers of *BRCA1/2* mutations versus BRCAx individuals (high-risk patients who have tested negative for BRCA1/2 mutations). Furthermore, we have been able to explore this variability at the single cell level using RNA *in situ* hybridisation analysis of ~300 breast tumours tissue microarrays from high-risk patients who have been tested for *BRCA1/2* mutations.

CB44: Characterising the adaptive response to androgen targeted therapies highlights mechanisms of resistance and opportunities for novel therapeutics approaches

Colleen C Nelson, Brett G Hollier, Jennifer Gunter, Phoebe Sarkar, Katrina Sweeney, Melanie Lehman

Australian Prostate Cancer Research Centre- Queensland, Translational Research Institute, Princess Alexandra Hospital, Institute of Health and Biomedical Innovation, Queensland University of Technology.

The prostate and prostate cancer is inherently dependent on androgens for differentiation, proliferation, and cell survival. Therefore the androgen receptor (AR) remains the central target in advanced prostate cancer (PCa), with a growing pipeline of new drugs targeting the androgen axis. While the intent of androgen/AR targeted therapies (ATTs) is for therapeutic benefit, physiologically ATTs systemically reprogram the equilibrium of interlinked endocrine pathways with induction of features of metabolic syndrome, including hyperinsulinaemia and other metabolic dysfunction. In PCa tumours, ATTs activate androgen-repressed gene networks, promote dedifferentiation and tumour cell plasticity, which, in turn, may facilitate tumour resistance and progression and metastatic potential. We have generated a prostate cancer RNAseq repository of transcriptional response, focussed on ATTs in combination with the investigations of manipulation of the downstream adaptive pathways activated by ATTs. Our aim has been to target the activated adaptive pathways in PCa models with either well-established drugs repurposed for this context, such as anti-diabetic and other metabolic drugs, as well as novel agents for improved cancer control. During these studies we have found complex adaptive interlinkages between tumour cell plasticity and endocrine stimuli and metabolic pathways which underpin treatment resistance and prostate cancer progression that may offer new therapeutic approaches.

CB45: Clinical development of TargomiRs, a microRNA mimic-based treatment for patients with malignant pleural mesothelioma

Glen Reid[1]

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MicroRNAs are responsible for post-transcriptional control of gene expression, and are frequently downregulated in cancer. It is well established that restoring microRNA levels can inhibit tumour growth, and many studies have demonstrated this activity in preclinical models. This has led to the first clinical trials of microRNA replacement therapy for cancer. Our previous work has identified microRNAs with tumour suppressor activity in malignant pleural mesothelioma, an asbestos-related cancer. This talk will describe the development of TargomiRs – microRNA mimics delivered by targeted bacterial minicells – and preliminary results from a phase I trial of this therapy in mesothelioma patients.

CB46: New Zealand's first GMO release: Pexa-Vec, for liver cancer therapy clinical trials

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[1]Environmental Protection Authority, Wellington, NZ, [2]Current address: Medsafe, Wellington, NZ.

Viruses have been genetically modified (GM) for a variety of therapeutic purposes. Gene therapy uses GM viral vectors to deliver genes into cells to treat congenital disorders, live-attenuated GM vaccines have been developed to protect against infectious disease, and oncolytic GM viruses have been developed to enhance selectivity and potency against cancer.

In New Zealand, all live genetically modified organisms (GMOs) require approval under the Hazardous Substances and New Organisms Act 1996 (the HSNO Act). The EPA has the power to approve medicines that contain a live GMO as a "qualifying organism" under section 38I of the HSNO Act. Until recently, the only approval given under this pathway was for a live vaccine for equine influenza. However, this was never released, since an outbreak of the disease never occurred in New Zealand.

In 2015, the EPA received an application for the release of Pexa-Vec – a GM live-attenuated vaccinia virus, which has been or is currently being evaluated against various types of cancer in clinical trials in multiple countries. In October 2015, the EPA approved the conditional release of Pexa-Vec for use in a Phase 3 clinical trial for patients with hepatocellular carcinoma. After assessing the potential risks to the health and safety of the public, valued species, natural habitats and the environment, the EPA made the decision to approve the importation of Pexa-Vec for release, subject to seven controls. In this approval process, the EPA did not assesses the efficacy and safety of the GMO medicine on the treated/vaccinated individual. This part of the process is the responsibility of Medsafe, who approved Pexa-Vec for clinical trial under section 30 of the Medicines Act 1981.

CB47: Looking into the future: how far could genomics and bioinformatics go to help cancer patients?

Cristin Print[1]

[1]Department of Molecular Medicine and Pathology, University of Auckland, New Zealand

Genomics and bioinformatics are rapidly penetrating into clinical oncology. Internationally, cancer bioinformaticians are gaining experience in the "n=1 analysis" techniques required to provide clinically useful information for individual cancer patients. In addition, a flood of observational studies and clinical trials into the use of genomics in cancer patient care are now being published. So what is the next step? In this talk I will argue that the most exciting contribution of cancer genomics is still to come, once we can move beyond lists of drug response-associated mutations and gene expression profiles, and instead undertake a much broader multi-modal genomic analysis of each patient's tumour(s). The talk will explore several points for discussion about how we achieve this. I will show examples of the exciting levels of tumour complexity that new genomic methods can now identify. Would we gain specificity, or just add noise, by clinically using combinations of mutation, gene expression, allelic imbalance, methylation, heterogeneity, 3D DNA structure and histopathology? Will liquid biopsies (plasma genomics) really revolutionise screening, diagnosis, treatment stratification and monitoring for relapse? How can genomics improve our understanding of tumour-immune interactions and of responses to immunotherapies? Can mathematical modelling of the future evolution of each tumour, based on the tumour's current genomic state, allow us to beat tumours at their own game? How will we distill from each patient's genomic data the small fraction of information that has real clinical utility? Will genomic testing for cancer patients become personalised, analogous to personalised drug therapy, with specific multi-modal genomic tests for each patient group? It is a truly exciting time for translational cancer biology research in New Zealand, however the next steps may perhaps require many more scientist-clinician collaborations and more involvement of New Zealand in international patient-focused translational studies.

Summary of Abstracts for the Poster Session Template

No.	Title	Presenter	Institutions
CB48	Is there a role for ascorbate in regulating hypoxia factors in renal cell carcinoma?	Christina Wohlrab	University of Otago, New Zealand
CB49	Identifying associations between patient BMI and tumour molecular characteristics in endometrial cancer	Van Cao	University of Otago, New Zealand
CB50	FOXP3 and miR-155 cooperate to down regulate ZEB2 independently of ZEB1 to help maintain normal breast epithelial homeostasis..	Cheryl Brown	University of Adelaide, Australia
CB51	Statins as novel therapeutic agents in melanoma	Khanh Tran	University of Auckland, New Zealand
CB52	VEGF-A Release is Higher in Melanoma Cells Harbouring V600E BRaf Mutations	Khanh Tran	University of Auckland, New Zealand
CB53	Putting the brakes on breast cancer tumor growth	Anower Jabed	University of Auckland, New Zealand
CB54	Synergistic effect of Class II and Class III PI3K signalling in melanoma cell survival	Qian Wang	University of Auckland, New Zealand
CB55	miR-150: a vicious culprit or an innocent bystander?	Venkatesh Vaidyanathan	University of Auckland, New Zealand
CB56	Multi-omic analysis of non-invasive and invasive melanoma cell lines	Jyoti Motwani	University of Otago, New Zealand
CB57	Melanoma and the regulation of the p53 pathway	Luke Henderson	University of Otago, New Zealand
CB58	Who would have thought that ATP competitive inhibitors could affect protein-membrane interactions?	Grace Gong	University of Auckland, New Zealand
CB59	Surgical success and response to adjuvant therapy in glioblastoma depends on telomere maintenance	Ramona Eiholzer	University of Otago, New Zealand

	associated markers.		
CB60	Characterisation of circular RNA in melanoma cell lines	Kaveesha Bodiyabadu	University of Auckland, New Zealand