Outcomes of patients treated with perioperative epirubicin, cisplatin and fluoropyrimidine chemotherapy for gastro-oesophageal cancer in a New Zealand Cancer Centre

Tsai, Wendy1; Barton, Sarah1, Woods, Lisa2; and Clarke, Kate1
1. Wellington Blood and Cancer Centre, Capital and Coast DHB, Wellington, New Zealand
2. School of Mathematics and Statistics, Victoria University of Wellington, New Zealand

Background
In patients with resectable gastric or lower oesophageal adenocarcinomas the perioperative regimen of ECF (epirubicin, cisplatin, fluorouracil) has been proven to down-stage and significantly improve progression-free survival (PFS) and overall survival (OS) compared with surgery alone. This treatment has been applied in our centre since 2007. This is a retrospective audit to review and compare local clinical outcomes and quality to gold standard, taken as the phase III “MAGIC” (Medical Research Council Adjuvant Gastric Infusional Chemotherapy) trial results.

Methods
Patients with potentially resectable oesophageal, oesophagogastric junction, or gastric adenocarcinoma treated with perioperative ECF or ECX (epirubicin, cisplatin, capecitabine) were retrospectively selected from pharmacy records. The aims of our audit were comparison of OS, PFS, clinical data, treatment related toxicity and dose intensity. OS and PFS were assessed using the Kaplan-Meier method.

Results
Between 2007 and June 2015, 52 eligible patients (73% male and 27% female) with median age of 60 were identified. Fewer patients had primary gastric tumours (36.5% vs 74%) in our centre compared to MAGIC. A similar number of patients underwent attempted surgery (90.4% vs 91.6% in MAGIC), and this was considered curative (R0/R1) in 75% compared with 69.3% in MAGIC. 3/52 of our patients received a modified chemotherapy regimen (2 with cisplatin substituted with carboplatin, and 1 with cisplatin substituted with docetaxel). 51/52 (98%) patients completed 3 cycles of neo-adjuvant chemotherapy compared with 90.7% in MAGIC, and 20/52 (38.5%) patients completed the entire 6 cycles of perioperative chemotherapy compared to 49.5% in MAGIC. Overall patients in our centre had more advanced pathological stage than those in MAGIC: ypT4 17.1% vs 4.7%, ypN3 29.1% vs 1.5%. ypCR was achieved in 4/52 (7.7%). The median number of lymph nodes harvested was 21. With median follow-up of 16.7 months, median PFS was 20.8 months (95%CI=14.6-27.0) and median OS was 25.5 months (95%CI=14.9-36.2), compared with median PFS of 16 months and median OS of 26 months in MAGIC. There was one death during chemotherapy, from myocardial infarction, compared with 7 (1.4%) in MAGIC. 18/52 (34.6%) of patients required hospital admission for Grade 3/4 chemotherapy-related toxicity, predominantly for diarrhoea, nausea, vomiting, and neutropenic (1/52 (2%)) and non-neutropenic fever. This was largely similar to MAGIC.

Conclusion
This audit showed that use of the perioperative regimen of ECF/ECX in patients with resectable gastric, GOJ or lower oesophageal adenocarcinomas in our centre achieved comparable efficacy outcomes to MAGIC, with acceptable chemotherapy-related toxicity.
Effect of tamoxifen treatment on expression of pro-inflammatory markers in breast cancer

Bundock, Anna¹, Hazlett, Jody¹ and Dunbier, Anita¹
¹Centre for Translational Cancer Research and Department of Biochemistry, University of Otago, Dunedin, New Zealand

Introduction
Approximately 80% of human breast cancers present as oestrogen receptor-positive (ER+). The dependence of ER+ breast cancers on oestrogen for cell growth and survival renders them susceptible to anti-oestrogen therapy such as tamoxifen or aromatase inhibitors (AI). However, even with treatment, a significant fraction of tumours recur.

Our previous research has revealed that oestrogen deprivation induces upregulation of pro-inflammatory chemokines in MCF-7 cells and AI-treated breast cancers. This chemokine expression is associated with recruitment of immune cells, suggesting a role for these chemokines in attracting immune cells to the tumour microenvironment. It is not known, however, whether the chemokine expression observed in MCF-7 cells is replicated in other ER+ breast cancer cell lines, and whether oestrogen deprivation by tamoxifen treatment elicits the same response as simulated AI treatment.

This study aimed to examine the chemokine expression associated with tamoxifen treatment in MCF-7 and T47D cells and compare this pattern to the observed expression in oestrogen deprived MCF-7 cells.

Methods
MCF-7 cells were seeded in 6-well plates and treated with either 10μM of tamoxifen or a 0.05% DMSO control for 72 hours. RNA was extracted 24, 48 and 72 hours post seeding. For T47D cells, treatment (5μM tamoxifen or DMSO control) continued until 128 hours post seeding. qPCR was performed using probes for the three chemokines of interest, CCL5, CCL22 and CXCL16, and two reference genes, FKBP15 and PUM1.

Results
In MCF-7 cells, expression of CCL22 was not differentially expressed between tamoxifen-treated and control cells at 24 and 48 hours, but was significantly higher in the treatment group after 72 hours of treatment (p<0.05). No differences in CCL5 and CXCL16 were observed.

In T47D cells, expression of CCL5 remained unchanged between the treatment and control at 24, 48 and 72 hours but was significantly lower in the treatment group at 128 hours. Similarly, expression of CCL22 was found to be significantly lower in the treatment group at 128 hours post seeding (p<0.0005). Conversely, expression of CXCL16 was significantly higher in treatment group compared to the control at both 72 and 128 hours. (p<0.05 and p<0.005 respectively).
Conclusions
The chemokine expression pattern elicited by tamoxifen-treated MCF-7 cells differs from oestrogen-deprived MCF-7 cells. The expression pattern also differs from tamoxifen treated T47D cells. This data suggests that the differing mechanisms of action of the drugs and alterations in the genetic background of the cell lines could affect the inflammatory response to anti-oestrogen therapy.
Using physiological and pharmacological ascorbate to manipulate HIF-1 activity in \textit{Gulo}^{-/} mice

\textbf{Campbell EJ}^{1}, Bozonet S^{1}, Robinson BA^{2,3}, Vissers MCM^{1}, Dachs GU^{1}.

\textit{1 Department of Pathology, University of Otago, Christchurch, 2 Canterbury Regional Cancer and Blood Service, CDHB, Christchurch, 3 Department of Medicine, University of Otago, Christchurch}

Hypoxia-inducible factor-1 (HIF-1) controls the expression of numerous genes involved in tumour growth and spread. Ascorbate acts as a co-factor for the HIF-hydroxylases that control HIF-1 levels and activity. This study investigated the effect of physiological intake and pharmacological administration of ascorbate on the HIF-1 pathway and tumour growth in \textit{Gulo}^{-/} mice. These mice cannot synthesise their own ascorbate, making them a good model of the human ascorbate dependency condition.

C57BL/6 \textit{Gulo}^{-/} mice were supplemented with adequate (330 mg/L) or optimal (3300 mg/L) ascorbate in their drinking water, with these levels of supplementation resulting in below saturation or saturating plasma and tissue levels of ascorbate, respectively. The animals were implanted subcutaneously with syngeneic Lewis Lung carcinoma (LL/2) cells. For pharmacological intervention, tumour-bearing mice on adequate ascorbate were injected with ascorbate (1g/kg ip; HDVC) on a daily basis or every second day. Tumour levels of ascorbate were measured using HPLC, HIF-1α and its target CA-IX were analysed by western blotting, and VEGF by ELISA.

\textit{Gulo}^{-/} mice maintained on optimal ascorbate had increased tumour ascorbate levels (0.60 ± 0.17 µmol/g), compared to mice on adequate ascorbate (0.21 ± 0.07 µmol/g). These elevated levels were similar to those in tumors of mice receiving daily HDVC (0.58 ± 0.11 µmol/g), and higher than those receiving ascorbate every second day (0.34 ± 0.13 µmol/g). Increased tumour ascorbate levels were associated with significantly slower tumour growth and reduced levels of HIF-1, CA-IX and VEGF (p<0.05).

Our data demonstrated that restoration of optimal intracellular ascorbate, either by continuous dietary supplementation or daily pharmacological administration, was associated with a decline in tumour aggression via dampened HIF-1 activity.

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Pharmacologic vitamin C improves fatigue and quality of life in cancer patients

Carr, Anitra 1, Vissers, Margreet 1, Cook, John 2
1 Department of Pathology, University of Otago, Christchurch, New Zealand
2 New Brighton Health Care, Christchurch, New Zealand

High dose vitamin C has been administered by health care practitioners for many decades as a complementary and alternative therapy for numerous conditions, including cancer and chronic fatigue. Although the use of high dose vitamin C for cancer is relatively common, its use remains controversial and its efficacy remains untested. Recent clinical studies, however, indicate that high dose vitamin C can dramatically improve the quality of life of cancer patients. We have carried out a number of case studies in patients receiving chemotherapy or undergoing palliative care, using the European Organisation for Research and Treatment of Cancer Quality of Life Questionnaire (EORTC-QLQ), which assesses the common cancer-related symptoms of fatigue, nausea/vomiting, pain, dyspnoea, insomnia, appetite loss, constipation and diarrhoea. This questionnaire also assesses physical, role, emotional, cognitive and social functioning and has a global health status score. Because fatigue is one of the most common and debilitating symptoms reported by cancer patients we also used the Multidimensional Fatigue Symptom Inventory (MFSI) which assesses multiple aspects of fatigue, i.e. general, physical, emotional and mental fatigue, and vigour, and has a total fatigue score. Two patients undergoing chemotherapy for breast cancer received high dose intravenous vitamin C (50 g/session) twice a week, at least 48 hours either side of each chemotherapy session. The quality of life (EORTC) and fatigue (MFSI) questionnaires were administered before and after four weeks of vitamin C intervention. We observed a decrease in the symptoms of fatigue, nausea, pain, insomnia, appetite loss and constipation following vitamin C administration, as well as an increase in physical, role, cognitive and social functioning, and an improvement in overall health status. With respect to the multiple aspects of fatigue, a decrease in general, physical and mental fatigue were observed, as well as an increase in vigour, resulting in a complete decrease in overall fatigue. No adverse side effects were observed by the clinical staff or reported by the patients. Vitamin C has potent antioxidant and anti-inflammatory properties, and has been shown to decrease the toxicity of some chemotherapeutic agents. However, we hypothesise that vitamin C is also likely acting via its enzyme cofactor functions, e.g. biosynthesis of carnitine, neurotransmitters and peptide hormones. Appropriately designed placebo controlled trials to confirm efficacy and to elucidate the in vivo mechanisms of action of vitamin C in cancer- and chemotherapy-related quality of life are now required.
Cisplatin/bolus 5-fluorouracil/folinic acid for advanced oesophageal and gastric cancer

Chang Tina¹, Wojtacha, Anna ², Thompson, Paul ², Findlay, Michael ², Ashley, Amanda², Sasidharan, Rita ², Damianovich, Dragan ², Deva, Sanjeev ², Lawrence, Ben ²
1 Department of General Medicine, Auckland City Hospital, Auckland, New Zealand
2 Department of Medical Oncology, Auckland City Hospital, Auckland, New Zealand

Metastatic oesophageal and gastric adenocarcinoma are aggressive cancers with a poor prognosis. Palliative combination chemotherapy using a platinum and a fluoropyrimidine (infusional 5-FU or oral capecitabine) is the international standard of care and extends survival. However, delivery of infusional 5-FU requires semi-permanent venous access, and delivery of capecitabine requires normal swallowing in the presence of tumor mass. To overcome these limitations, a local regimen was developed at Auckland City Hospital where the infusional 5FU / capecitabine was replaced with 3 days of bolus 5FU. This audit evaluates patient characteristics, response rate, survival, and safety of the Cis/5FU/FA regimen in patients with gastric and oesophageal adenocarcinoma.

Review of pharmacy registry data located 39 consecutive patients who received Cis/5-FU/FA as first-line treatment for advanced oesophageal (n=11), gastroesophageal junction (n=10), or gastric (n=18) cancer in the Medical Oncology Department at Auckland City Hospital, New Zealand, from 1st January 2004 to 31st December 2013. Each 3 week cycle consisted of a 1-hour intravenous infusion of cisplatin (60mg/m²) on day 1, and an intravenous bolus of 5-FU (425mg/m²) and calcium folinate (20mg/m²) on day 1, 2 and 3.

Patients treated with this regimen had advanced malignancy (e.g. 90% Stage IV, 49% had ≥2 sites of metastatic disease) and poor pre-treatment performance status (e.g. 52% recorded ECOG were 2-3). Despite these adverse prognostic features, the median overall survival with Cis/5FU/FA therapy was 7.07 months, with median progression free survival of 4.82 months. Patients with gastric cancer showed median OS of 8.82 months and median PFS of 5.63 months. A clinical response was seen in 59%, and radiological response rate meeting the RECIST criteria in 20%. This regimen was fairly non-toxic and although two-thirds of patients experienced a hospital admission during treatment, only 26% of these admissions were treatment-related. Five patients choose to stop treatment due to toxicity.

In conclusion, this novel Cis/5FU/FA regimen (the Thompson Protocol) was easy to deliver and provided effective palliation with comparable survival to other published regimens for palliative management of advanced oesophageal and gastric cancer.
5 Years of Non-Small Cell Lung Cancer Treatment Outcomes

Chen, Kevin¹ and Smith, Catherine²

¹Oncology Department, Canterbury District Health Board, Christchurch, New Zealand

Background

Non-small cell lung cancer (NSCLC) accounts for approximately 80% of primary lung cancer diagnoses. The lung cancer multidisciplinary meeting (MDM) at Christchurch Hospital was established in 2009 to review cases and offer a recommendation regarding management. Our aim is to evaluate the survival outcomes of NSCLC patients undertaking treatment with curative intent and compare with the available international data. We will assess outcomes for all patients undertaking curative intent treatment, with a further analysis on the subgroup of patients who underwent non-surgical treatment with radiotherapy +/- chemotherapy. The majority of these patients were excluded from surgery due to co-morbidities and operative risk.

Methods

A retrospective analysis of all patients with newly diagnosed NSCLC undertaking curative intent treatment between June 2009 to June 2014 was undertaken. The demographics, staging, treatment modality and date of death were then obtained for each patient from electronic records. The data collected was stratified according to staging and subject to statistical analysis, 5-year survival rates calculated and Kaplan-Meier plots generated. The results will then be compared to outcomes published in the International Association for the Study of Lung Cancer (IASLC) Lung Cancer Staging Project, which was used to validate the 7th version of the TNM system.

Results

272 cases were identified, with 146 males (54%) and 126 (46%) females. Mean age was 69 years for both genders. Females showed a predominance for adenocarcinoma compared to males (73.8% vs. 46.6%), with less squamous cell compared to males (25.4% vs 47.9%). Staging at diagnosis was equally distributed between genders, with 55%, 21% and 22% for stages I, II and III respectively. Carcinoma in situ accounted for 2%.

Overall 5-year survival was 56%, 57% and 16% for stages I, II and III respectively. 39% of males and 45% of females underwent nonsurgical treatment. Their 5-year survival was 40%, 35% and 18% for stages I, II and III respectively.

Conclusions

This provided valuable local data on NSCLC treatment outcomes, and would serve as a basis of future reviews. When comparing the 5-year survival for all patients undergoing curative treatment, the Christchurch MDM offers similar, if not superior outcomes compared to the IASLC cohort. There are still insufficient patient numbers to meaningfully compare the outcomes of the non-surgical treatment group, who have poorer outcomes due to medical co-morbidities.

Overall the results support the current practices of NSCLC management by the Christchurch lung cancer MDM.
Inhibition of EGFR/Her-2 affects growth of ascitic ovarian cancer cells in vitro study

Chitcholtan, Kenny, Harker, Dianne, Simcock, Bryony, Evans, John, and Sykes, Peter
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 in women advanced ovarian cancer. Consequently, these inhibitors are not commonly used in the treatment of ovarian cancer. Advanced ovarian cancer can cause an accumulation of body fluid in the abdominal cavity, a medical term known as ascites.

Ascitic fluid contains heterogeneity of cell populations including small clusters of cancerous ovarian cells. These cell clusters are likely the source of metastasis. Cell clusters spread and grow on the surface of abdominal walls and internal organs 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 hypothesize that clusters of EGFR/Her-2 positive ovarian cancer cells are more susceptible to inhibitors than receptor negative cells, and canertinib is more potent than gefitinib in receptor positive cells.

We used ovarian cancer cell lines (OVCAR-5, SKOV-3 and OVCAR-4) and isolated cells from ascitic fluids from 20 patients with advanced ovarian cancer. 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.
Exploring Molecular Links between Obesity and Breast Cancer

Crake, Rebekah(1), Wiggins, George(1), Morrin, Helen(1), Phillips, Elisabeth(1), Currie, Margaret(1) and Walker, Logan(1)

(1) Mackenzie Cancer Research Group, Department of Pathology, University of Otago Christchurch, New Zealand

Obesity is associated with the high risk of incidence and mortality of postmenopausal breast cancer. Despite this well-established link, the molecular and mechanistic basis of the obesity and breast cancer association still remains unclear. Genetic variation due to copy number differences has become increasingly popular in obesity research. AMY1, the salivary amylase gene, is well-known for its extensive copy number variation (CNV) in the human genome and has previously been correlated with a genetic predisposition toward obesity; however, research surrounding this association is controversial. Despite an established relationship between obesity and breast cancer risk, the recently reported genetic association between AMY1 copy number variation and obesity has not yet been examined in normal and obese breast cancer patients. Furthermore, gene expression changes in breast tumours from obese women remain poorly characterised. We hypothesise that obese breast cancer patients are associated with (1) low AMY1 copy number and (2) differential expression of candidate gene markers.

This study included 55 post-menopausal breast cancer patients from the Cancer Society Tissue Bank, with a BMI (body mass index)>30 (n=28) or BMI<25 (n=27). Quantitative PCR (qPCR) assessment of germline AMY1 copy number status from blood, showed that obese breast cancer patients have a lower average copy number of AMY1 compared to normal weight patients. Examining data from two published studies that compared the breast tumour expression profiles of obese and non-obese patients, identified four candidate genes (GRIA2, DUSP4, NR2F1, and ADH1B) shared between both studies. Analysis of gene expression data from The Cancer Genome Atlas indicated that these four genes are differentially expressed within clinically relevant breast tumour subtypes characterised by oestrogen receptor, progesterone receptor and HER2 status. qPCR analysis of each candidate gene within our study cohort showed that the average expression of GRIA2 and DUSP4 differed between obese and non-obese tumours, however these results were not statistically significant. Our study indicates that BMI may be associated with lower germline copy number of AMY1 in post-menopausal breast cancer patients. Further work with a larger cohort is needed to establish if GRIA2 and DUSP4 are associated
Efficacy of a novel IDO1 inhibitor to suppress kynurenine:tryptophan ratios and tumour growth in combination with immune checkpoint blockades

de Leeuw, Matthew, 1 Henare, Kimiora, 1 Ward, Cameron, 1 Tijono, Sofian 1, Palmer, Brian, 1 Ching, Lai-Ming 1

1 Auckland Cancer Society Research Centre, University of Auckland, Auckland, New Zealand

Tumours adopt a number of mechanisms to evade the patients' endogenous immune system. One mechanism of tumour mediated immune evasion is mediated through increased expression of the enzyme indoleamine 2, 3-dioxygenase 1 (IDO1). This enzyme converts the essential amino acid tryptophan along the kynurenine pathway. Decreased tryptophan or an increase in kynurenine metabolites can lead to increased suppressive T regulatory cell activity and inactivation of immune T effector cells. High IDO1 expression in a broad range of cancers is generally correlated with poor patient survival. IDO1 inhibitors would therefore be expected to be beneficial for restoring tumour immunity in cancer patients. High throughput screening of compound libraries by the ACSRC team has led to the discovery of a number of novel chemical classes of IDO1 inhibitors, from which SN35837 (Inhibitor-3) has been selected for structural optimisation for development into a potential anticancer agent.

Preliminary studies of SN35837 in a preclinical model of glioma have yielded encouraging results. Tryptophan : kynurenine (K:T) ratios is used as an indicator of IDO1 activity, and a sensitive HPLC assay detecting both tryptophan and kynurenine concentrations in tissue samples in the same run was established to measure K:T ratios from tissues collected from mice. K:T ratios in subcutaneous GL261 gliomas increased with size and days after implantation. A single administration of SN35837 at well-tolerated doses was shown to effectively reduce the K:T ratio in plasma and tumour tissue. K:T ratios were significantly decreased 15 min to 6 hours after treatment, but had returned to normal by 24 hours. Daily administration of SN35837 at 75 mg/kg inhibited the growth of both wild-type and GL261 cells engineered to constitutively express human IDO1 (GL261-hIDO1). Pilot studies indicate that SN35837, similar to that demonstrated with other classes of IDO1 inhibitor that are in human clinical trials, that combination therapies with anti-PD-1 or anti-CTLA4 immune checkpoint antibodies provide greater control of GL261 growth than each of the monotherapies alone. Experiments examining the efficacy of these treatments against GL261 tumours implanted orthotopically in the intracranial site are planned.
The role of ascorbate in controlling hypoxia factors in renal cell carcinoma

**Ernst, Christina**¹, Phillips, Elisabeth ¹, Morrin, Helen R ¹,², Vissers, Margreet CM ³, Robinson, Bridget A ¹,⁴, Dachs, Gabi U ¹

1 Mackenzie Cancer Research Group, Department of Pathology, University of Otago, Christchurch, NZ
2 Cancer Society Tissue Bank, Christchurch, NZ
3 Centre for Free Radical Research, Department of Pathology, University of Otago, Christchurch, NZ
4 Oncology Services, CDHB, Christchurch, NZ

Renal cell carcinoma (RCC) is a lethal disease with limited treatment options arising in the epithelium of renal tubules. Clear cell RCC (ccRCC) is the most common and also most aggressive type harbouring alterations in the VHL gene leading to accumulation of pro-survival transcription factors (Hypoxia Inducible Factors, HIF-1 and -2). The second most common form, papillary RCC (pRCC), has a functional pVHL with regulated HIF levels. HIF protein levels and activity are regulated via a family of dioxygenases that require ascorbate (vitamin C) as cofactor. Hydroxylation of two proline residues by proline hydroxylases (PHDs) targets the protein for degradation via the pVHL ubiquitin ligase complex and hydroxylation on an asparagine by factor inhibiting HIF (FIH) leads to inactivation of HIF. Absence of ascorbate impairs activity of the hydroxylases leading to increased HIF activation. Our previous data has shown that higher tumour ascorbate levels are associated with lower HIF levels in endometrial and colon cancer patients.

To investigate the role of ascorbate in controlling HIFs in RCC, we analysed the protein expression levels of HIF-1α and HIF-2α and their selected downstream targets (BNIP3, CAIX, MMP-2, Cyclin D1, CPT1A, PLIN2, GLUT1 and VEGF) with Western Blot or ELISA in banked tumour samples from kidney cancer patients with matched normal renal cortex. Ascorbate concentration in the samples was determined using HPLC-ECD.

Ascorbate levels varied between tumours and normal tissue, between ccRCC and pRCC, and between samples taken from different locations of the same tumour. Protein levels of HIF-1α and 2α and their downstream targets were differentially regulated in ccRCC and pRCC compared to matched normal renal tissue. There was an inverse trend between the tumour ascorbate content and the expression of some of the hypoxia factors, but not all.

This is the first study to analyse ascorbate concentrations in RCC, and to investigate the association between ascorbate and HIF-1/-2 in this disease. The comparison of VHL+ ccRCC and VHL- pRCC provides an excellent opportunity to confirm the mechanism of ascorbate-dependent HIF-regulation in a human VHL gene deficiency setting.

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A synthetic lethal strategy for the chemoprevention of hereditary diffuse gastric cancer

Frick, James¹, Telford, Bryony¹, Chen, Augustine¹, Beetham, Henry¹, Single, Andrew², Brew, Tom¹, Burn, Olivia¹, Armstrong, Kirsty¹, Gastra, Joel¹ and Guilford, Parry¹

¹ Cancer Genetics Lab, Department of Biochemistry, University of Otago, Dunedin, New Zealand

Globally, gastric cancer is the fourth most common form of the disease, affecting almost one million individuals annually. Hereditary diffuse gastric cancer (HDGC) is an autosomal dominant syndrome characterised by predisposition to poorly differentiated adenocarcinoma. Germline mutation of invasion suppressor E-cadherin (CDH1) contributes approximately to one third of HDGC diagnoses. Affected individuals carry an 70% lifetime risk of developing gastric cancer and women an additional 40% risk for lobular breast cancer. Prophylactic gastrectomy is the primary HDGC management strategy due to the unreliability of endoscopic detection.

E-cadherin is a membrane-spanning invasion suppressor protein with key roles in epithelial homeostasis. E-cadherin is involved in intercellular adhesion, cytoskeletal morphology and signalling mechanisms. Abrogation of cell-cell adhesion via biallelic silencing of CDH1 is a common initiating characteristic in a proportion of adenocarcinoma – hallmarked by a diffuse, poorly-differentiated phenotype. CDH1 downregulation is correlated with an upregulation of genetic markers associated with epithelial-mesenchymal transition (EMT).

Targeting of perturbed oncogenes is a relatively straightforward approach to therapy. However, most common tumour mutations result in the loss-of-function of tumour suppressor genes – the absence of which underpins the incompatibility with conventional chemotherapy. One approach that allows the targeting of tumour suppressor genes is synthetic lethal (SL) targeting. A SL interaction exists between a pair of genes where silencing of one is tolerated while silencing of both is lethal.

High-throughput siRNA screening utilising isogenic (CDH1+/+ and CDH1−/−) epithelial cell lines generated by zinc-finger nuclease knockout has elucidated a plethora of SL candidate genes – where candidate gene silencing leads to preferential reduction of CDH1+/+ cell viability. SL candidates are enriched for genes associated with G protein-coupled receptor (GPCR) signalling and cytoskeletal organisation.

In addition to the SL genes, an equivalent number of genes are ‘reverse SL’ (RSL), that is their knockdown leads to greater death in CDH1+/+ cells than CDH1−/− cells. Strikingly, these RSL genes are also strongly enriched for GPCRs. Other highly enriched RSL gene ontology classes include phosphatases, ion channels and translation initiation. The combination of different SL and RSL effects has enabled us to develop a novel model for the mechanism of E-cadherin-associated synthetic lethality. This model, which pivots around central cell survival
pathways, provide a framework for the selection of potential compounds for the chemoprevention of HDGC.
A review of squamous cell vulvar cancers in Waikato Region, New Zealand

Hari Dass, Prashanth¹ and Kuper, Marion¹
¹Medical Oncology Department, Waikato Hospital, Hamilton, New Zealand.

Background
Management guidelines for Squamous cell vulvar cancers are scarce. The objectives of the study were to review the patient characteristics and outcomes of vulvar cancers in the Waikato region.

Methods
Retrospective review of all vulvar cancer cases registered in Waikato Hospital between 1/1/2000 and 31/8/2015. Cases were identified from Waikato Hospital clinical coding department, radiation oncology and medical oncology department database. Patient characteristics, clinical presentation, stage, histology, treatment, treatment related toxicities and outcomes were reviewed. Cases were staged using the TNM and FIGO classifications.

Results
Of the 60 cases, 47 patients with vulvar Squamous cell cancer were reviewed and 13 patients excluded. Of those excluded, 11 patients had other histological diagnosis and 2 patients had insufficient clinical data. Median age was 69 years. 80.5% were of European ethnicity and 19.5% Maori. Vulval pruritis, lump, pain and bleeding were the most common symptoms at presentation. 28% of cases had a background of lichen sclerosus. The most common comorbidities were obesity, hypertension and diabetes. 42% of patients had vulvectomy. Inguinal lymph node dissections were performed in 53% of cases (40% bilateral and 13% unilateral). 3 patients underwent a sentinel node procedure. The most common acute surgical complication was wound cellulitis and dehiscence. The most common late complication was lymphedema. The cases were staged as follows: stage I, fourteen cases; stage II, five cases; stage III, eighteen cases; and stage IV, nine cases. One patient was unstaged. 73% of cases received radiation therapy with curative or radical intent (mean 50Gy) and 13% with palliative intent. 24% of patients were treated with chemotherapy. The combination of 5-Fluororuacil(S-FU) and Mitomycin chemotherapy was the most common regime given concurrently with radiation treatment was seen in seven cases, followed by weekly cisplatin administered to four patients. Grade 3 or higher toxicities were seen in 4 patients receiving S-FU versus none in the cisplatin group. 5 year overall survival was 85.7% (Stage 1), 40% (Stage II), 41% (Stage III) and 14% (Stage IV). Median follow up was 33 months.

Conclusion
Vulvar cancers are rare tumors. Variable treatment approaches are observed from our study. Treatment is associated with high morbidity. This calls for collaboration from treating centres to generate data that will streamline appropriate guidelines to optimise management.
Testicular cancer ethnic disparities in the Waikato region, New Zealand – a thirteen year retrospective review

Hari Dass, Prashanth and Jameson, Michael
1 Medical Oncology Department, Waikato Hospital, Hamilton, New Zealand.

Background
New Zealand Maori have unusually high rates of malignant testicular germ cell tumours compared to individuals of European ancestry, possibly related to higher rates of cryptorchidism in Maori compared to Europeans, suggesting prenatal risk factors. Data from the US also demonstrated ethnic disparities in stage and survival of testicular cancer, for which the reasons are unclear. Within New Zealand, limited data is available with regard to ethnicity differences in stage at diagnosis, treatment and overall survival in patients with testicular cancer.

Methods
Retrospective review of prospectively-collected patient data in the Medical Oncology database at Waikato Hospital, searching for all testicular germ cell tumour patients first seen between 01 January 2001 and 31 December 2013. Patient demographics, disease characteristics, treatment details and outcomes were collected and compared by ethnicity, separately for seminoma and non-seminoma.

Results
A total of 325 patients were seen over the thirteen year period, of whom 95 identified as Maori, 210 as being of European ancestry and 20 of other ethnic origins (including not recorded). 182 were diagnosed with seminoma (147, 24 and 11 with stages 1, 2 and 3 respectively) and 143 with non-seminoma (80, 21 and 42 with stages 1, 2 and 3 respectively). Median age at diagnosis was 39 years (range 18-74) for seminoma and 30 years (range 15-80) for non-seminoma. Maori represented 27.5% of seminoma patients and 31.4% of non-seminoma patients. Median age of presentation with testicular cancer among Maori was 31 years versus 39 years in non-Maori. 3 patients had metachronous testicular cancers and were each counted twice. While stage distribution of seminoma at diagnosis was similar for Maori and non-Maori (chi-square p=0.31), proportionately more Maori had higher-stage non-seminoma than non-Maori (stage 3 in 44% and 22% respectively, chi-square p=0.014). Across all stages overall survival for patient with seminoma did not differ significantly by ethnicity (logrank p=0.18), with 10-year survival being 93.5% in Maori compared to 97.4% in non-Maori. Similarly, overall survival for patients with non-seminoma did not differ significantly by ethnicity (logrank p=0.49), with 10-year survival being 90.1% in Maori compared to 86.5% in non-Maori.

Conclusions
Maori present at an earlier age with disproportionately high rates of testicular germ cell tumours than seen in non-Maori in NZ, particularly with more advanced non-seminomatous tumours. However overall survival appears to be comparable between the ethnic groups for both seminoma & non-seminom.
Neuroendocrine tumours (NET) are complex and variable, making it very difficult for clinicians to determine the best course of treatment. The NETwork project seeks to better understand the epidemiological impact of NETs in New Zealand, and to better characterise the disease to help inform oncologists how to treat it. The estimated incidence rate of patients with NETs in New Zealand is approximately 200 patients per year, however the impact among Māori is not yet known. Māori are disproportionately burdened by cancers of the lungs, stomach, and pancreas, so it is tempting to speculate that NET incidence among Māori could also be high. It is essential that Māori are involved in the study in order to get an accurate indication of the impact of this cancer in New Zealand, what genes are driving the cancers, and how each can be treated.

The multi-faceted NETwork project combines epidemiological analysis and deep genome sequencing of retrospective and prospective NET tissues. Under the guidelines set out in Te Ara Tika, the design of this research project is mainstream, but is likely to involve Māori participants and have direct relevance to Māori. Despite being neither Māori-centred nor Kaupapa Māori in our approach, the NETwork team are dedicated to honouring the Treaty of Waitangi principles of partnership, participation, and protection. Mindful of the past transgressions involving the use of tissues and genetic information obtained from indigenous populations here in New Zealand and overseas, the NETwork group are keen not to repeat these errors themselves, nor facilitate the opportunity for others to do so.

Following ongoing consultation with Te Mata Ira, Maui Hudson, Dr Helen Wihongi, and Associate Professor Papaarangi Reid, we have established a ‘roadmap for safe travel’ to guide all aspects of the multi-faceted project. The framework has three key principles (kawa) underpinning its Governance structure, and three core cultural protocols (tikanga) to be incorporated into the implementation strategies. Adhering to these kawa and tikanga should facilitate the establishment and maintenance of relationships with key stakeholders; a vital aspect to the project.

The roadmap for safe travel is still in its early stages of development, and consultation is ongoing. Nevertheless, the NETwork team have a strong platform from which to further develop their project. Although the presented framework is specific to the NETwork project, it could easily be adjusted and utilised for other clinical and biomedical projects.
An audit of the treatment of metastatic spinal cord compression in Canterbury District Health Board

Jack Hollinghurst¹ and Melissa James²

¹ Radiation Oncology, Canterbury District Health Board
² Radiation Oncology, Canterbury District Health Board

Introduction
Spinal cord compression is a common oncological emergency with significant morbidity and mortality. A recently updated Cochrane review showed that treatment outcomes with single-fraction radiotherapy were equivalent to radiation treatment given in 2 or 8 fractions, for those patients expected to live for less than six months.

Methods
We reviewed the treatment of all patients with MRI-proven spinal cord compression who underwent radiotherapy within Canterbury District Health Board over a 2 year period, from 1st January 2013 to 31st December 2014. We accessed the patients’ online records to assess their: primary diagnosis, radiotherapy dose and fractionation, whether they underwent surgery and the type of surgery, whether they had non-bony metastases and their length of survival. Length of survival was categorised into less than 6 months and more than 6 months.

Results
We identified 44 patients treated with radiation for spinal cord compression in the 2 year period. 1 was excluded as the spinal cord compression was non-malignant. 8 were excluded as they were re-treatments of patients who had already undergone spinal radiotherapy.

Of the 35 which remained the primary cancer type was prostate 37% (n=13), myeloma 14% (n=5), renal 9% (n=3), other 40% (n=14) (melanoma, breast, lung, choleangiocarcinoma, hepatocellular carcinoma, lymphoma, salivary gland, sarcoma and carcinoma of unknown origin). Surgery was performed in 9% (n=3) of cases, with debulking, with or without laminectomy, performed in all cases.

The most common course of radiotherapy performed was 20Gy in 5 fractionations (71% n=25). Other courses prescribed were: 8Gy in 1# - 13% (n=5), 20Gy in 10#s - 3% (n=1) and 30Gy in 10#s – 6% (n=2).

43% (N=15) of patients survived more than 6 months from diagnosis and 57% (n=20) of patients survived less than 6 months. Of the patients surviving less than 6 months only 15% (n=3) received their radiotherapy in a single fraction.

Discussion
Given the recent evidence that the symptoms of spinal cord compression are equally treated with a single fraction of radiation compared to more prolonged fractionation, consideration should be given to increasing the use of the single fraction treatment for oncology patients.
The majority of patients in this series survived for less than 6 months which would suggest the proportion of these patients receiving a single fraction could be increased with possible patient benefits in terms of length of hospital stay and convenience and a small decrease in pressure on radiation treatment resources.
Investigation of bystander effects of hypoxia activated prodrugs using three dimensional cell cultures

Hong, Cho1, Bogle, Gib1, Wilson, Bill1 and Hicks, Kevin1
1University of Auckland, Auckland, New Zealand

Background
Hypoxia is a hallmark of solid tumours and arises as a result of imbalance between the consumption and availability of oxygen. Hypoxia acts as the major barrier to anti-cancer therapies and promotes tumour progression. Hypoxia-activated prodrugs (HAPs) are designed to not only eliminate these resistant cells, but also to exploit hypoxia as a basis of tumour selectivity. A key concept in exploiting hypoxia with HAPs is that active drug metabolite should be able to diffuse from hypoxic zones to maximise effect in surrounding oxic zones. There is some evidence that these ‘bystander effects’ contribute to the antitumour activity of HAPs, but currently there is a lack of robust methods for their detection.

Objectives
To investigate bystander effects from HAPs in a novel spheroid co-culture system.

Methods
A new method for measuring the bystander effect with multicellular spheroids under hypoxic conditions was developed using co-cultures of HCT116 colon cancer cells with high metabolic capacity for HAP metabolism (activators, transfected to overexpress cytochrome P450 reductase [POR]) and cells that cannot activate HAPs efficiently (targets, with POR knocked down by Zn finger nuclease gene targeting) along with antibiotic resistance (puromycin and neomycin) and fluorescent protein markers to enable their quantitation in mixed cultures. Sensitivity of activators and targets to HAPs that are in development (SN30000, PR104A) was determined in these co-culture spheroids by dissociation and clonogenic cell survival assay. Results were also compared to output of a novel agent-based mathematical model of spheroid co-cultures.

Results
Expression of two distinct fluorescent proteins in activator and targets allowed visualisation of their spatial distribution in spheroid co-cultures and confirmation of transfection stability in non-selective medium. In monolayer cultures, activator cells showed markedly higher sensitivity to the HAPs than did targets, as expected. When co-culture spheroids were treated with PR104A, the killing of targets markedly increased with increasing proportion of activators in the spheroids, confirming a bystander effect. However, increased proportions of activators reduced clonogenic cell killing of targets after SN30000 treatment, suggesting that rapid metabolism by POR-overexpressing activators inhibits its penetration. This interpretation was confirmed by the agent based co-culture model using SN30000 diffusion and metabolism parameters from multicellular layer studies.

Discussion
Using this novel spheroid co-culture model, a bystander effect of PR104A was demonstrated while SN30000 showed no bystander effect. This is the most direct demonstration of a hypoxic bystander effect for PR104A to date. The model can be used to provide a direct comparison on the magnitude of bystander effects for HAPs in 3D cultures.
Colonoscopy Referral Criteria in Faster Cancer Treatment Pathway patients at Southern District Health Board

Ibrahim, Aisyah 1,3, Jackson, Christopher 1,3, Derrett, Sarah 2, Samaranayaka, Ari 2.

1 Southern Blood and Cancer Service, Southern District Health Board, New Zealand
2 Department of Preventive and Social Medicine, University of Otago, Dunedin, New Zealand
3 Department of Medicine, Dunedin School of Medicine, University of Otago, Dunedin, New Zealand

Introduction
Upon the establishment of the Faster Cancer Treatment (FCT) targets in 2012, patients who were referred as having ‘high suspicion’ of colorectal cancer (CRC) were flagged to monitor compliance with Ministry of Health targets. ‘High-suspicion’ was not explicitly defined at the time – instead the National Direct Access Colonoscopy (NDAC) guidelines were used as a surrogate. We aimed to investigate concordance between ‘high-suspicion’ referrals, NDAC criteria and eventual diagnosis of CRC.

Methodology
Patients referred with high-suspicion of colorectal adenocarcinoma from 1st July 2012 to 30th June 2014 were identified from the Southern District Health Board (SDHB) FCT database. Patients were retrospectively triaged into categories A or B as per NDAC criteria. We then compared them to patients who had confirmed diagnosis of CRC collected from the NZ Cancer Registry to obtain the sensitivity and positive predictive value (PPV) of the high-suspicion flag.

This study has been approved by the University of Otago Ethics Committee (Health) and the Health and Disability Ethics Committee.

Results
494 patients were identified as having CRC from NZ Cancer Registry data. 67 patients were flagged as high-suspicion of CRC. 46 (68.7%) were positive for CRC, while 21 (31.3%) were negative. 22 (32.9%) had Category A criteria, 36 (53.7%) had Category B criteria, and 9 (13.4%) did not fulfil either A or B.

Among those who developed CRC, 13 had Category A criteria, 24 had Category B criteria and 9 had none.

8 (34%) of those with Category A criteria presented with a mass, 2 (8%) with rectal bleeding and iron deficiency anaemia and 12 (58%) with altered bowel habit and rectal bleeding.

With individuals referred as Category B, 13 had altered bowel habit lasting more than 6 weeks and at least 50 years of age (29%), 4 had altered bowel habit, unexplained rectal bleeding and were aged 40-50 years (9%), 7 had unexplained rectal bleeding and were at least 50 years of age (15%) and 21 had iron deficiency anaemia (47%). None were referred with New Zealand Guidelines Group Category 2 or 3 family history, polyps on imaging or inflammatory bowel disease.

There was a significant difference in the proportion of people diagnosed with CRC in those with category A and B criteria versus those who did not fulfil criteria for either category (p=0.03). However, there was no significant difference between those with category A criteria versus those with Category B criteria (p=0.56).
The high-suspicion flag had a sensitivity of 9.3% and positive predictive value of 68.6%.

**Conclusion**
Most patients diagnosed with CRC in Southern District Health Board are referred through routes other than the high-suspicion pathway. The high-suspicion flag has low sensitivity in diagnosing CRC.
A randomized, placebo-controlled, double-blind phase II trial of peri-operative cimetidine in early colorectal cancer

Dr Michael Jameson1, Dr Michael Arendse1, Professor Michael Findlay2, Professor Ian Bissett2, Mr Simone Lolohea1, Ms Judy Warren1, Professor Frank Frizelle3, Mr John Keating4, Dr Gregory Jacobson5, Ms Claudia Romano1, Mr Ralph van Dalen1

1Waikato Hospital, Hamilton, New Zealand, 2University of Auckland, Auckland, NZ, 3University of Otago, Christchurch, NZ, 4Wellington Hospital, Wellington, NZ, 5University of Waikato, Hamilton, NZ

Background
Perioperative CIM reduced mortality (HR 0.53, 95% CI 0.32-0.87) with curative-intent surgery for CRC in a Cochrane meta-analysis of 5 randomised phase 2 trials. The benefit was greatest in patients (pts) whose tumours expressed the sialyl Lewis (sLe) antigens A (CA19-9) or X that bind to endothelial cells via E-selectin, which is induced by inflammatory cytokines and inhibited by CIM. This randomised trial evaluated issues critical to planning a phase 3 trial.

Methods
Patients with stage 1-3 CRC were randomised to take cimetidine 800mg twice daily or placebo orally for 5 weeks, starting 2-7 days before curative-intent surgery. The primary endpoint was 2-year disease-free survival (DFS) in patients with sLe antigen-positive tumours; secondary endpoints included survival, CIM compliance, recruitment, serum inflammatory cytokine profile and tumour expression of sLe antigens by immunohistochemistry.

Results
128 patients (35% female), median 70 (29-86) years, were recruited in 4 centres over 3.5 years and randomised to CIM (n=65) or placebo (n=63); 6 patients took none. 40% of screened CRC patients were eligible of whom 53% were recruited. Of the 45% with a rectal primary, 68% received preoperative chemoradiation (CRT); 15% and 21% of those were downstaged to TNM stage 0 or 1 respectively. Postoperative TNM stage in all patients was 0-1, 2, 3 and 4 in 27%, 38%, 31%, and 2% respectively. 29% of patients had adjuvant chemotherapy. CIM was well-tolerated and feasible to administer orally throughout. At median follow-up of 26 (range 5-56) months, with 18 relapses and 15 deaths, 2-year DFS was 80.3% and 75.8% with CIM and placebo respectively (HR 0.75, 95% CI 0.33–1.69, logrank p=0.54). Serum inflammatory cytokines were elevated up to 2 weeks post-operatively but normalised by 4 weeks. Expression of sLe antigens in >5% of cells was seen in 90% for CA19-9 and 74% for sLe\(^{-}\); all tumours expressed at least one antigen at that level. In 32% of tumours with high expression (>70% of cells) DFS was similar to the whole group. Tumour IHC score for CA19-9 correlated with baseline serum CA19-9 (r=0.30, p=0.014).

Conclusions
Perioperative CIM for 5 weeks is feasible and covers the period of risk of disseminating metastases attributable to inflammatory cytokines. DFS has improved compared to historical trials. The trend to improved DFS with CIM is consistent with meta-analysis data. This trial is not powered to detect DFS differences of this size. A phase 3 trial appears justified.
Activity of aspirin in PIK3CA-mutant colorectal cancer

Kang, Woo-Jin 1, Wrightson, Emma 1, Shepherd, Peter R. 2,3 and Jamieson, Stephen M.F. 1,3
1 Auckland Cancer Society Research Centre, the University of Auckland, New Zealand
2 Department of Molecular Medicine and Pathology, the University of Auckland, New Zealand
3 Maurice Wilkins Centre for Molecular Biodiscovery, the University of Auckland, New Zealand

The cyclooxygenase-1 and -2 inhibitor, aspirin, has many uses as an analgesic, anti-pyretic and anti-inflammatory agent. It is also used long-term in patients at risk of heart disease or stroke as an antiplatelet drug. Long-term regular aspirin use has been associated with reduced risk of developing and dying from colorectal cancer, with recent data suggesting that aspirin is most effective in patients with PIK3CA-mutant tumours. Since mutations in PIK3CA, the gene that encodes the p110α subunit of phosphoinositide 3-kinase (PI3K), can activate PI3K/AKT signalling and promote cell survival and proliferation, we sought to investigate whether aspirin alone or in combination with PI3K inhibitors could prevent PIK3CA-mutant colorectal cancer cell growth. The antiproliferative activity of aspirin as a single agent was determined in 9 cancer cell lines with PIK3CA mutations, PTEN deletions or EGFR amplifications. Next, a panel of 9 PIK3CA mutant and wild-type colorectal cancer cell lines were treated with aspirin in combination with the p110α-selective inhibitor A66 or the pan PI3K/mTOR inhibitor BEZ235 to investigate whether aspirin could interact with PI3K inhibitors to prevent cell proliferation and AKT phosphorylation. The in vivo antitumour activity of aspirin alone and in combination with A66 or BEZ235 was also evaluated in an HCT116 (PIK3CA-mutant colorectal cancer) tumour xenograft model. Aspirin prevented cell proliferation in all cell lines with low mM IC50 values and no difference in activity between PIK3CA-mutant, PTEN-null or EGFR-amplified cells. Combining aspirin with either A66 or BEZ235 had little effect on cell proliferation or phospho-AKT expression compared to single agent activity in either PIK3CA-mutant or wild-type colorectal cancer cell lines. Furthermore, combined treatment of aspirin with A66 or BEZ235 had no effect on HCT116 tumour growth. These results suggest that at very high doses (>10x higher than standard clinical doses) aspirin alone does have antiproliferative activity, but the lack of any effect in combination with A66 or BEZ235 in colorectal cancer cell lines suggest that PIK3CA mutation status is not a useful biomarker for predicting short term high dose use of aspirin in combination with PI3K inhibitors. However, since cyclooxygenase-produced prostaglandin E2 can drive cancer through evasion of T-cell dependent tumour control, aspirin is likely to produce its anticancer activity by acting on the tumour microenvironment rather than on the cancer cells themselves.

2 Zelenay et al., Cell. In press, 2015
Does fat provide energy for breast tumour cell invasion and metastasis?

Jones, M.¹, Phillips, E.¹, O’Connor, K.², Hampton, M.B.², Currie, M.¹

¹ Mackenzie Cancer Research Group, Pathology Department, University of Otago, Christchurch, New Zealand.
² Centre for Free Radical Research, Pathology Department, University of Otago, Christchurch, New Zealand.

Breast cancer is the most commonly diagnosed cancer in New Zealand women. Obese breast cancer patients are more likely to have tumours with advanced clinical stage and high vascular and lymph node involvement. The tumour microenvironment provides vital support for tumours during development and progression of cancer, yet the local effects of stromal adipocytes on breast cancer cells have been largely overlooked. Recent studies have shown that breast cancer cells co-cultured with adipocytes become more resistant to radio- and chemotherapy, and more invasive¹-³. However, little is known about the metabolic changes that occur in breast cancer cells when they are cultured with adipocytes. Nieman et al. (2011) determined that lipids were transferred from omental adipocytes to ovarian cancer cells and were consequently used in β-oxidation⁴. We hypothesise that β-oxidation is increased in breast cancer to exploit the fatty acids released by lipolysis from adipocytes in order to support the migration and invasion of breast cancer cells.

In this study, breast cancer cell lines MCF7 (ER+) and MDA-MB-231 (ER-/PR-/HER2-) were co-cultured with adipocytes isolated from breast adipose tissue. Adipose tissue samples were collected by the Cancer Society Tissue Bank from patients at Christchurch Hospital undergoing surgery for therapeutic mastectomy, prophylactic mastectomy and breast reductions. Western blotting was used to assess differences in the expression of proteins involved in β-oxidation between breast cancer cells grown alone or in co-culture with adipocytes. Carnitine palmitoyltransferase 1 (CPT1A) is a protein involved in translocation of fatty acids into the mitochondrial matrix for β-oxidation. Phosphorylation of the key metabolic enzyme, acetyl-CoA carboxylase (ACC1), relieves inhibition of CPT1A to allow fatty acid translocation. A Seahorse XF24 Analyser was used to measure oxygen consumption and extracellular acidification, as indicators of oxidative phosphorylation and glycolysis respectively, between breast cancer cells grown alone or in co-culture with adipocytes. Preliminary data shows that while both MCF7 and MDA-MB-231 cells have similar global metabolic profiles, they have increased levels of phosphorylated ACC1 after co-culture with adipocytes. This suggests that breast cancer cell metabolism is being altered in the presence of adipocytes to utilise the fatty acids released in lipolysis.


**Differential effects of organic versus inorganic selenium species on BRCA1-mutated and non-mutated breast cancer cell lines**

Annie Ko1, Kirsty Mayall1, Holly Sprosen1, Linda Peters1, Michael Jameson2
1 Laboratory of Molecular Biology, University of Waikato, Hamilton, New Zealand
2 Department of Oncology, Waikato Hospital, Hamilton, New Zealand

**Background**
Selenium (Se) is an essential non-metal trace element with an anticancer chemopreventive effect through protecting healthy cells from oxidative damage. Twenty-five proteins in mammals are found to harbour Se in the form of selenocysteine, some of which are antioxidants that prevent reactive oxidative species from damaging the cell membrane and DNA, and also maintain the redox balance within cells. However, the effect of Se is dependent on the dosage and the chemical form of Se intake. High levels of Se (especially inorganic forms) can result in increased DNA damage, whereas low levels can cause decreased immunity, increased cancer incidence and mortality risk. Previous research showed that selenite supplementation reduced bleomycin-induced DNA damage in individuals carrying a BRCA1 mutation but a trend to increased breast cancer incidence was seen in a randomised trial in this population using supra-nutritional doses of selenite.

**Methods**
The aim of this research is to evaluate the interaction of Se dose and chemical form in BRCA1-mutated and non-mutated breast cancer cells in vitro. In this study we applied Se (as organic methylseleninic acid (MSA) and inorganic selenite) to four commercially-available BRCA1 or non-BRCA1 mutated breast cancer cell lines. Se sensitivity was measured by examining DNA damage and cell viability with the comet assay and the colorimetric MTT assay, respectively.

**Results**
Comparison of the comet assays between two BRCA1-mutated breast cell lines (MDA-MB-231 and Sum149pt) and two non-BRCA1-mutated breast cancer cell lines (MCF-7 and MDA-MB-231) showed significantly (p=0.01) greater DNA damage in the cells with BRCA1 mutation for each Se compound. For cell viability, measured with the MTT assay, the IC50 of both selenite and MSA is lower in BRCA1-mutated cells than the non-BRCA1-mutated cells.

**Conclusions**
From this study, the BRCA1-mutated breast cancer cell lines appear to be significantly more sensitive than non-BRCA1-mutated cells towards Se treatment. We will explore whether this differential sensitivity is maintained in conjunction with chemotherapy. If so, this could potentially be exploited in patients with BRCA1-mutated metastatic breast cancer.
Pancreatic enzyme replacement therapy (PERT) for symptomatic management in advanced pancreatic cancer

Landers, Amanda 1,2 and Brown, Helen 2 and Strother, Matthew 1
1 University of Otago, Christchurch, New Zealand
2 Nurse Maude Hospice Palliative Care Service, Christchurch, New Zealand

Background
In pancreatic cancer, late diagnosis, poor surgical outcomes, and limited benefit from chemotherapy lead to a poor prognosis, with only 11% of patients surviving one year past diagnosis. Additionally, due to pancreatic exocrine dysfunction, most pancreatic patients suffer from malabsorption (MA), which may develop without obvious clinical signs. Advanced MA has hallmark symptoms of bloating, flatulence, pain and steatorrhoea. The use of pancreatic enzyme replacement therapy (PERT) in those with advanced malignancy is underutilized in pancreatic cancer, with minimal clinical studies defining appropriate dosing, schedule, or effectiveness in pancreatic cancer. In a recent study one in five patients with metastatic pancreatic cancer was not on PERT in spite of the majority of patients exhibiting symptoms consistent with MA.

Aim
To determine the efficacy of PERT in improving MA symptoms and quality of life (QOL) in metastatic pancreatic cancer patients.

Methods
Patients referred to the Nurse Maude Hospice Palliative Care Service with the diagnosis of advanced pancreatic cancer, and not previously taking PERT, were invited to participate in this study. Creon 50,000 units with meals and 25,000 units with snacks were initiated following assessment by a specialist dietician. Baseline weight, symptom inventory, concurrent medications, Palliative Performance scale, and QOL using the EORTC-QLQ30 and EORTC-PAN26 were determined prior to PERT initiation. The measures were repeated at 1 and 3 weeks following PERT initiation.

Results
Between June 2013 and May 2015, 97 patients were assessed by the dietician; 44 consented to the study; 29 completed all study assessments. The average age was 69.8 years and the majority were female (66%). Two participants had undergone surgery, and 6 had undergone biliary stenting. Preliminary analysis shows that 1 week following PERT initiation 13/29 participants (45%) maintained or increased weight, but this had declined to 12/29 (39%) by week 3. Improvements post –PERT were the hallmark symptoms of MA: reports of bloating changed from 17/29 (60%) to 6/29 (20%); and flatulence changed from 23/29 (79%) to 17/29 (60%) over the duration of study. Similar trends were seen for steatorrhoea and pain. QOL scales also showed marked improvements which were sustained. Adverse effects of the medication were minimal.

Conclusion
Preliminary results suggest PERT improves problematic symptoms potentially related to MA in pancreatic cancer patients. Further analysis revealed improved QOL that was maintained through the study period. Use of PERT appears to be a viable option for symptom management in pancreatic cancer patients.
Cell culture research with implications for clinical practice in breast cancer: Potentiation of growth inhibitory responses of the mTOR inhibitor everolimus by dual mTORC1/2 inhibitors

Leung, Euphemia¹² and Baguley, Bruce¹

¹Auckland Cancer Society Research Centre, and ²Molecular Medicine and Pathology Department, University of Auckland, Auckland, New Zealand

The mammalian target of rapamycin (mTOR), a vital component of signaling pathways involving PI3K/AKT, is an attractive therapeutic target in breast cancer. Everolimus, an allosteric mTOR inhibitor that inhibits the mTOR functional complex mTORC1, is approved for treatment of estrogen receptor positive (ER+) breast cancer. Other mTOR inhibitors show interesting differences in target specificities: BEZ235 and GSK2126458 are ATP competitive mTOR inhibitors targeting both PI3K and mTORC1/2; AZD8055, AZD2014 and KU-0063794 are ATP competitive mTOR inhibitors targeting both mTORC1 and mTORC2; and GDC-0941 is a pan-PI3K inhibitor. We have addressed the question of whether mTOR inhibitors may be more effective in combination than singly in inhibiting the proliferation of breast cancer cells. We selected a panel of 30 human breast cancer cell lines that included ER and PR positive, HER2 over-expressing, and “triple negative” variants, and determined whether signaling pathway utilization was related to drug-induced inhibition of proliferation. A significant correlation (p = 0.005) was found between everolimus IC50 values and p70S6K phosphorylation, but not with AKT or ERK phosphorylation, consistent with the mTOR pathway being a principal target. We then carried out combination studies with four everolimus resistant triple-negative breast cancer cell lines, and found an unexpectedly high degree of synergy between everolimus and the other inhibitors tested. The level of potentiation of everolimus inhibitory activity (measured by IC50 values) was found to be cell line-specific for all the kinase inhibitors tested. The results suggest that judicious combination of mTOR inhibitors with different modes of action could have beneficial effects in the treatment of breast cancer.
Development of a metabolic syndrome mouse model of breast cancer

Mandani, Anishah1, Phillips, Elisabeth1, Scott, Nicola2, Currie, Margaret1 and Dachs, Gabi1

1Mackenzie Cancer Research Group, Department of Pathology, University of Otago Christchurch, New Zealand
2Christchurch Heart Institute, Department of Medicine, University of Otago Christchurch, New Zealand

Metabolic syndrome is a cluster of disorders, including obesity, atherosclerosis, inflammation and insulin resistance. It is associated with increased risk of various types of cancers including breast cancer. Obesity in particular is a risk factor for an aggressive tumour phenotype and reduced survival of patients with breast cancer. To understand the underlying mechanisms we aimed to develop and characterise a metabolic syndrome mouse with an orthotopic model of breast cancer.

Apolipoprotein E (ApoE) is involved in the catabolism of triglycerides and cholesterol, and the ApoE knockout mouse model is prone to obesity and development of atherosclerosis. The double knockout ApoE/ArKO mouse displays all features of metabolic syndrome. At 6 months of age, wild type, ApoE and ApoE/ArKO C57BL/6 mice were inoculated with the murine breast cancer cell line E0771. Growth of tumours in the mammary fat pad and mouse weight were measured until tumours reached ethical endpoint. The hypoxia marker, pimonidazole, was injected 90min prior to euthanasia, and plasma, organs and tumours were harvested and weighed. Half of each tumour was formalin fixed and paraffin embedded for immunohistochemical (IHC) analysis of cancer associated adipocytes (perilipin), proliferation (phosphohistone-H3), oestrogen receptor status (ERα) and hypoxia (pimonidazole adducts). The other half was frozen and processed for tumour lysates, which were used to measure hypoxia inducible factor 1 (HIF-1α) by Western blotting, and adipokines, using an antibody array. Hif-1α levels in EO771 cells were analysed by subjecting the cells to hypoxic conditions.

ApoE mice weighed more than wild type and ApoE/ArKO mice, and showed increased tumour growth rates. ApoE/ArKO mice had the least omental fat and the smallest tumours. IHC analysis showed that EO771 tumours in ApoE mice had the highest number of intratumoral, perilipin positive adipocytes (p<0.01), and tumours from ApoE/ArKO had the highest percentage of phosphohistone-H3 positive cells (p<0.05). All tumours stained negative for ER-α. Pimonidazole staining was prominent in all tumours, with secondary tumours staining strongly. From Western blot analysis, tumours grown in ApoE/ArKO mice had the highest level of HIF-1α, and HIF-1α levels were higher in secondary tumours compared to primary tumours (p<0.05). A range of adipokines was differentially expressed in tumours grown in the three different genetic backgrounds.

Our findings show that breast tumours grown in either ApoE or ApoE/ArKO mice have an aggressive tumour phenotype, with increased proliferation, tumour hypoxia and intratumoral adipocytes. These models represent valuable tools for research that will bridge the gap between cell culture models and breast cancer patients.
Investigation of oxygen and nutrient dependence in a growth model of multicellular spheroids

Mao, Xinjian\(^1\), Hicks, Kevin\(^1\), Wilson, Bill\(^1\) and Bogle, Gib\(^2\)

\(^1\)Auckland Cancer Society Research Centre, University of Auckland, Auckland, New Zealand
\(^2\)Auckland Bioengineering Institute, University of Auckland, Auckland, New Zealand

Background
Hypoxia activated prodrugs (HAPs) are a promising strategy to target hypoxia in tumours, which is associated with poor prognosis. We have used studies in three-dimensional cultures combined with pharmacokinetic/pharmacodynamic modelling to select SN30000, an analogue of tirapazamine with improved transport and complementarity with radiation. However our current models have important limitations, such the inability to simulate time-dependent drug effects, tumour regrowth and response of cellular subpopulations. To overcome these limitations we have developed an agent-based model (ABM) of multicellular spheroid growth where the fate of each cell can be followed as it reacts to its microenvironment, including concentrations of oxygen, glucose and drugs.

Objectives
To investigate the oxygen and glucose dependence of HCT116 multicellular spheroid growth and compare to ABM predictions.

Methods
Spheroid growth is simulated in the ABM as a function of oxygen, glucose and drug concentrations determined by solving diffusion equations using oxygen consumption measured in HCT116 monolayers and glucose and SN30000 diffusion and metabolism measured in HCT116 multicellular layers, linked with intracellular reaction equations. Cell fate (growth rate, division, death) is determined by concentrations of oxygen, glucose and drug within the spheroid. Cell counts, diameter, volume and cell survival of experimentally grown HCT116 spheroids at a range of oxygen and initial glucose concentrations were compared to model predictions, as was glucose depletion from the culture medium. Finally, the ability of the model to simulate cell killing and growth inhibition induced by SN30000 was investigated.

Results
The model reproduced known features of spheroids including oxygen and glucose gradients, rapid cell proliferation at the periphery and central hypoxia and necrosis. Growth curves of HCT116 spheroids, quantified by spheroid diameter, were linear as a function of time. Spheroid growth was slower when cultured in 5% and 1% O\(_2\), than in 20% O\(_2\). Glucose was rapidly consumed from the culture medium and spheroids stopped growing when glucose was less than 0.5 µM. The growth of spheroids and depletion of glucose from the culture medium was well-fitted by the ABM. The ABM simulated spheroid regrowth after the exposure to SN30000 under 20% O\(_2\).
Discussion: An ABM depending on oxygen and glucose has been developed and calibrated using *in vitro* metabolism and diffusion parameters and has been shown able to predict spheroid growth under different nutrient and O\(_2\) conditions. This model will be further developed and used for optimising treatment by SN30000 combined with radiation or other anti-cancer drugs.
The effect of vitamin D on gene expression in colorectal tumours and normal colon

Munro, Fran¹, McCall, John¹, Black, Michael¹
¹University of Otago, Dunedin, New Zealand

Background
Colorectal cancer (CRC) remains the second leading cause of cancer death in New Zealand. Epidemiological studies have reported an inverse association between vitamin D status and incidence of CRC and higher vitamin D levels at the time of diagnosis or post-surgery have been associated with improved long-term outcome in CRC. Vitamin D deficiency is common in New Zealand, particularly at lower latitudes and during the winter months. The primary aim of this study was to examine whether pre-operative vitamin D supplementation has a measurable effect on vitamin D responsive genes in the tumour and normal colon.

Methods
The study was a randomised, double-blind, placebo-controlled trial of 200,000 IU of vitamin D administered orally 7-21 days prior to surgery for CRC at Dunedin Hospital. RNA was isolated from fresh resected normal and tumour specimens and profiled on microarray gene-chips. Array gene expression data were analysed to identify differentially expressed individual genes and to identify differences in pathway expression.

Results
Pre-incision vitamin D concentrations were higher in the treatment than in the placebo group (mean 87 +/- 22 vs 49 +/- 19 nmol/L; p<0.001). There were no significant differences in single gene expression between the treatment and control groups. In the normal tissue patients randomised to receive vitamin D had down-regulation of a number of pathways compared to the placebo group including fatty acid and cytochrome P450 metabolism, vitamin processing and regulation of growth pathways. In the tumour tissue the treated group also had down-regulation of several pathways compared to the placebo group including the Fatty Acid Metabolism and Fatty Acid beta-Oxidation. In the paired analysis the expression of the Ribosome and Translational Termination pathways were enhanced by vitamin D in the tumour tissue of some patients and reduced in others, compared to the normal tissue.

Conclusion
In this randomised clinical trial potentially significant biological differences between the vitamin D and placebo groups were identified, in particular the down-regulation of Fatty Acid Metabolism and Fatty Acid beta-Oxidation pathways in the tumour tissue. The down-regulation of these pathways may lead to reduced proliferation and increased apoptosis via butyrate induced HDAC inhibition. These findings indicate that some biological effects of vitamin D supplementation are detectable in tumour and normal tissue, even after short term administration.
3D breast cancer models: in vitro investigation of adipocyte/tumour interaction

Phillips, Elisabeth¹, Lim, Khoon², Woodfield, Tim², and Currie, Margaret¹.

¹Mackenzie Cancer Research Group (MCRG), University of Otago Christchurch, Christchurch 8011, New Zealand.
²Christchurch Regenerative Medicine and Tissue Engineering (CReaTE) Group, University of Otago Christchurch, Christchurch 8011, New Zealand.

Recent evidence shows that obesity is a negative prognostic factor for all breast cancers, independent of tumour hormonal receptor status, tumour stage and patient menopausal status. It is also reported that excess adiposity may favour tumour invasiveness and poor patient outcome. Using a two-dimensional (2D) transwell cell co-culture method, we have shown that adipocytes can interact with breast cancer cells in vitro, and that this interaction enables breast cancer cells to survive chemotherapy doses that would otherwise be lethal.

The aim of this study was to develop novel, three-dimensional (3D) co-culture systems by growing human breast adipocytes and breast cancer cells together. Breast adipose tissue samples were collected from patients undergoing therapeutic or prophylactic mastectomy, or breast reduction, at Christchurch Hospital. Mature adipocytes were isolated, washed, and used immediately in experiments. The adipocytes were either encapsulated in 5% gelatin hydrogel alone, or in a mixed co-culture with breast cancer cell lines (ER+, PR+, HER-; MCF7) (ER-, HER-, PR-; MDA-MB-231) and (ER-, PR-, HER+; HCC1954). Breast cell lines were also grown alone in 5% gelatin hydrogel. Cell viability and metabolic activity of the cells and the co-cultures was assessed using Alamar Blue assay. Immunofluorescence was used to detect markers of cancer progression and proliferation; including Ki67 and proteins related to epithelial to mesenchymal transition.

The 3D cultured cancer cells, and 3D co-cultured adipocytes and cancer cells remained functionally viable after 7 days of encapsulation in 5% gelatin hydrogels. After 7 days, the breast cancer cells co-cultured with adipocytes appear more metabolically active than the same cells grown alone. Adipocytes appeared to de-differentiate, becoming less lipid rich in the co-cultured hydrogels; compared with adipocytes that were grown alone. This is comparable to our findings using the 2D transwell co-culture, where the adipocytes lose lipid content and take on a more fibroblast morphology.

Development of more physiological 3D models in which to test drug effects and breast cancer-stromal cell interactions will improve our understanding of the mechanisms and pathology involved in breast cancer.
Overall survival in stage four colorectal cancer patients receiving chemotherapy. A comparison of Tauranga Hospital outcomes with international data.

Sinhalage, V.¹  Harris, J.²  Jones, J.³  Head, M.³  North, R.³
¹ 5th Year Medical Student, University of Auckland
² PGY2 House Officer, Tauranga Hospital
³ Medical Oncologist Tauranga Hospital

**Aim**
To compare the survival rates and patient demographics of those undergoing chemotherapy for stage IV colorectal cancer at Tauranga Hospital with existing international data.

**Methods**
Data was collected from patients with stage IV colorectal cancer who were treated with chemotherapy at Tauranga Hospital between Jan 2008 – Jan 2015. The primary outcome was median overall survival (time from first specialist appointment to death) in all patients and stratified by the number of lines of chemotherapy given. This was then compared to statistics from a randomized GERCOR study from 2004 which used similar lines of chemotherapy to what we have available in NZ (FOLFIRI and FOLFOX). Secondary analysis compared patient demographics and characteristics between study populations. We have subsequently compared both data sets to more contemporary data for stage IV colorectal cancer patients.

**Results**
The median survival for patients at Tauranga Hospital undergoing chemotherapy for stage IV colorectal cancer was 17 months (CI 12.8 – 21.2). This improved to 22 months (CI 17.6 – 26.4) and higher with two or more lines of chemotherapy. The patients from the GERCOR study had a median survival of 21.5 months (CI 16.9 – 25.2) in study arm A and 20.6 months (CI 17.1 – 24.6) in study arm B. These results are comparable with Tauranga Hospital data however recent international data shows median overall survivals of 29+ months among similar patient groups with newer chemotherapy regimens.

**Conclusions**
Similar results are seen in comparable patient groups from the 2004 study using FOLFOX & FOLFIRI and Tauranga patients treated from 2008-2015. This suggests that administration of standard chemotherapy regimens at Tauranga hospital is done in a successful fashion. However both of these results are significantly inferior to contemporary data and emphasise the benefit of additional drugs available around the world, but not yet pharmac approved, in maximising survival for this group of patients.
Tumour intracellular ascorbate availability and effects on the regulation of the HIF hydroxylases

Kuiper, Caroline$^2$, Dachs, Gabi$^3$, Hicks, Kevin$^3$, and Vissers, Margreet$^1$.

$^1$Department of Pathology, University of Otago, Christchurch, New Zealand; $^2$Centre for Cellular and Molecular Physiology, Nuffield Department of Medicine, University of Oxford, Oxford, UK; $^3$Auckland Cancer Society Research Centre, University of Auckland, New Zealand.

The hydroxylases that regulate the stability and activity of hypoxia-inducible factor (HIF)-1 depend on oxygen, iron and 2-oxoglutarate availability. These enzymes also require ascorbate as a co-factor and, if this is limiting, their activity is compromised. Under in vitro metabolic stress conditions we found that low intracellular ascorbate levels exacerbated HIF-1 alpha protein accumulation and the transcriptional activity of HIF-1. In addition, our retrospective observational analyses of tumour tissue from patients with colorectal or endometrial cancer indicated that HIF-1 activity is inversely related to tumour ascorbate content. These results suggest that sub-optimal intracellular ascorbate up-regulates HIF-1 activity by modulating the HIF hydroxylases and thereby provides cancer cells with a metabolic and survival advantage.

Ascorbate is delivered to cells via the vasculature, but its ability to penetrate into tissues remote from blood vessels is unknown. Solid tumours often contain regions with dysfunctional vasculature, resulting in impaired oxygen and nutrient delivery. Using a 3-dimensional pharmacokinetic model, we have measured ascorbate diffusion and transport parameters through dense tumour cell layers in vitro. The data demonstrated heterogeneous distribution of ascorbate in tumour tissue at physiological blood levels and provide insight into the range of plasma ascorbate concentrations and exposure times needed to saturate all regions of a tumour. The predictions suggest that only supra-physiological plasma ascorbate concentrations (>100 µM) will result in effective delivery of ascorbate to poorly vascularised tumour tissue.

Taken together, our data is consistent with the hypothesis that tumour ascorbate content influences the activity of HIF-1 and that low ascorbate availability contributes to tumour growth conditions supported by increased tumour hypoxia and metabolic disturbance. Human clinical intervention studies are needed to further investigate this link and to determine whether it is possible to manipulate tumour ascorbate content.
Barriers to specialist palliative care access for cancer patients in Otago

Watt, Lucy¹. Middlemas, Heidi². Tosh, Grahame³. Jackson, Christopher³.
¹ University of Otago School of Medicine, Christchurch, New Zealand
² Otago Community Hospice, Otago, New Zealand
³ Department of Medicine, University of Otago, Dunedin, New Zealand

Background
Specialist Palliative Care has been shown to be beneficial to patients at the end of their life, with the benefits dependant in part on the duration of contact with these services prior to death. Timely access to Specialist Palliative Care services is therefore in the best interest of dying patients.

Objective
To identify potential barriers to Specialist Palliative Care access for dying patients with a cancer diagnosis in Otago.

Methods
A retrospective case review of cancer patients who died within 30 days of registration with the Otago Community Hospice between 1/1/14 and 31/7/14. Data was collected on patient factors, disease factors, treatment factors and referral processing delays. Descriptive analyses of collected data was carried out, calculating central tendency and spread. Where possible this data was compared to data regarding all cancer patients referred to Hospice in the same time frame sourced from the 2014 Otago Community Hospice Ministry of Health Report. Structured stakeholder interviews were also conducted to inform the descriptive analyses.

Results
We identified 54 patients who died within 30 days of being on the Hospice programme, with Maori patients more likely to be registered on the Hospice programme less than 30 days prior to their death, odds ratio 15.0 (95% CI 1.71-131.54, p value 0.0145). Upper GI tumours were also found to be associated with death within 30 days of registration, the difference in proportions being 0.16 (95% CI 0.05-0.29). The median time from referral sending to receipt at Hospice was 0 days (range 0-22), and the median time from receiving a referral to the patient being entered on the Hospice system was 8 days (range 0-43).

Conclusions
Upper GI tumours and Māori ethnicity as well as referral processing delays may be acting as barriers to accessing Specialist Palliative Care services for Otago patients.