Rationale for clinical evaluation of tarloxotinib bromide, a hypoxia-activated EGFR/HER2 inhibitor, in NSCLC and squamous cell carcinoma of the head/neck or skin

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Tarloxotinib bromide ("tarloxitinib", TH-4000, previously PR610) is a prodrug that releases a potent irreversible epidermal growth factor receptor (EGFR) and HER2 (ErbB2) inhibitor under hypoxic conditions. Preclinical development at the Auckland Cancer Society Research Centre (ACSRC) led to the Phase 1 evaluation in New Zealand (Auckland City and Waikato Hospitals) and the U.S. establishing a maximum safe dose of 150 mg/m² once weekly IV. Pharmacokinetic equivalent doses were retrotranslated from the human Phase I data into murine (NIH-III nude) tumour xenograft models, demonstrating an improved therapeutic index for tarloxotinib relative to current EGFR inhibitors erlotinib, afatinib and cetuximab. On the basis of these and other data, Threshold Pharmaceuticals, Inc., in collaboration with the Academic Thoracic Oncology Medical Investigators Consortium (ATOMIC), has initiated two Phase 2 clinical trials of tarloxotinib. The first trial is focused on the treatment of patients with mutant EGFR non-small cell lung cancer (NSCLC) who are progressing on treatment with standard EGFR tyrosine kinase inhibitors, but have not acquired the T790M resistance mutation (NCT02454842). Available clinical data indicates dose-intensification is a useful strategy to achieve therapeutic benefit in this context. The second trial is for the treatment of recurrent/metastatic squamous cell carcinoma of the head and neck (R/M-SCCHN) or skin (SCCS), which are considered incurable (NCT02449681). Here, EGFR expression is a poor prognostic indicator that is coordinated with the hypoxic gene signature, particularly in HPV-negative SCCHN. In addition to evaluating a range of target-specific biomarkers, hypoxia status will be measured at baseline in both trials using the PET imaging agent [¹⁸F]-HX4. The studies will be open at 12 sites in the U.S. and Australia.
The discovery of tarloxotinib bromide*: A first-in-class hypoxia-activated tyrosine kinase inhibitor

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We have been interested for many years in imparting increased tumour-selectivity to tyrosine kinase inhibitors (TKI) to improve their therapeutic index. One approach involves exploiting the presence of hypoxia in tumours as a unique physiological target capable of supporting reductive metabolism of hypoxia-activated prodrugs (HAP). We have previously reported the synthesis of SN29966, a prototype HAP of a known irreversible inhibitor of the epidermal growth factor receptor (EGFR). SN29966, bears a 4-nitroimidazole bioreductive “trigger” that fragments following one-electron reduction under hypoxia to release the irreversible TKI (Mol Cancer Ther., 2009; 8(12 Sup), C46; Tetrahedron, 2013, 69, 9130).

During lead optimisation of SN29966 we synthesised structural variations around the 4-nitroimidazole trigger class including electron-donating and electron-withdrawing substituents at the N-1 and C-2 positions of the imidazole ring system. In addition, we investigated heterocyclic trigger variations, such as substituted 2-nitroimidazoles and 2-nitropyrrroles. The HAP analogues synthesised were studied, relative to SN29966, with respect to aqueous solubility and stability. Their one-electron reduction potential, prodrug fragmentation rate and hypoxia-dependent anti-proliferative activity in A431 epidermoid carcinoma, SKOV3 ovarian carcinoma and H1975 non-small cell lung cancer (NSCLC) cells was measured. Preferred derivatives were advanced to plasma pharmacokinetic studies and the biodistribution of each HAP to tumour, liver and skin was assessed along with their efficacy in NIH-III mice bearing subcutaneous H1975 xenografts. These studies identified the 1-methyl-4-nitroimidazole trigger, linked to the TKI through the imidazole 5-position, as possessing optimal properties. This preferred trigger was then conjugated to a series of novel substituted 4-anilinopyrido[3,4-d]pyrimidine irreversible EGFR/HER2 inhibitors, resulting in a pre-lead series of nitromethylaryl quaternary ammonium salt (NMQ) prodrugs that were evaluated in vitro and in vivo. This identified tarloxotinib bromide (TH-4000; “tarloxotinib”) as an optimised hypoxia-activated irreversible EGFR/HER2 inhibitor for clinical evaluation.

A first-in-man Phase 1 dose-escalation trial of tarloxotinib administered as a once weekly one hour intravenous infusion has been completed, demonstrating a maximum tolerated dose of 150 mg/m$^2$/week (NCT01631279). Threshold Pharmaceuticals in collaboration with the Academic Thoracic Oncology Medical Investigators Consortium (ATOMIC) has now initiated Phase 2 clinical evaluation of tarloxotinib for the treatment of patients with mutant EGFR NSCLC who have been previously treated with an EGFR TKI and are progressing on treatment, but have not acquired the T790M resistance mutation (NCT02454842). A second Phase 2 trial of tarloxotinib in patients with advanced squamous cell carcinoma of the head and neck (SCCHN) or skin (SCCS) is also ongoing (NCT02449681).

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Nucleic acid chemical biology and drug design: G4-DNA and Pif1 helicase

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The human genome contains nearly 100 molecular motors that unwind nucleic acids. Only a handful of these helicases are known to preferentially bind and unwind G-quadruplex (G4) DNA, one of the most stable non-canonical DNA secondary structures known. Of these, the SF1B family helicase, Pif1 is the most potent. Recent findings indicate that, unlike normal cells, the demand for cancer cells to proliferate ensures they become dependent on helicases that can unwind (resolve) hard-to-replicate sites, such as G4-DNAs. Knockdown of hPif1 reduces the survival of both p53-deficient and p53-proficient human cells by triggering apoptosis, an effect that is enhanced by co-treatment with replication inhibitors. Moreover, hPif1 supports cell proliferation under oncogenic stress that is crucial during the early stages of tumorigenesis. Interestingly Pif1 knockdown has no effect on non-malignant cells, or, indeed, on mammalian viability. Thus, Pif1 appears to be essential for the proliferation and survival of cancer cells, but not for normal cells.

We seek to confirm the potential of Pif1 as an anti-cancer drug target by i) advancing our knowledge about the interactions of helicases with G4-DNA; ii) analysing the interactions of Pif1 complexed with modified G4-DNA assemblies and iii) utilising this knowledge to synthesise specific G4 mimetics to inhibit Pif1 in cancer cells. Recent results will be presented.


Impact of T cell populations in the tumour microenvironment of people with colorectal cancer

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Colorectal cancer (CRC) is the fourth most common cause of cancer mortality worldwide. Analysis of T cell infiltrate into CRC tumours accurately predicts patient prognosis, but the immune response against CRC is not fully understood. We have analysed the T cell infiltrate in people with CRC by flow cytometry and confocal microscopy. CRC tumours contained distinct T cell populations, which showed suppressed phenotypes compared to bowel tissue from the same patients. T cells isolated from the tumour showed impaired proliferation and had an “exhausted” phenotype. The type of T cell infiltrate was linked to both clinical parameters and disease free survival. The distinct tumour immune microenvironment described in CRC may identify new targets for immunotherapy.
The melanoma microenvironment

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Immune therapy seems set to revolutionise cancer care. The dramatic and durable clinical responses in melanoma patients are beginning to replicate in other indications, and an arsenal of agents targeting the immune system is already under clinical trial. However selection of the best immune therapy for individual cancer patients will require better immunological biomarkers that reflect the status of the interactions between a patient’s tumour cells and the immune system. We have been studying the microenvironment of melanoma cells in metastatic tissue from patients. These studies have highlighted not only the diversity of immune suppressive mechanisms active in different melanoma patients, but also the variety of these mechanisms present within single tumours. This spatial variability is likely to reflect the dynamic battle between tumour cells, stromal cells, and the immune system, and presents challenges in trying to individualise immune therapy based on tissue analysis – especially when limited biopsy material is available.
Can we STING macrophages back into action for the treatment of cancer?

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In cancer, macrophages are often conditioned towards a protumour M2-phenotype, providing immunosuppressive and wound healing functions that facilitate tumour growth, survival, metastasis, and resistance to therapy. Depletion of macrophages can slow tumour growth in mice. Given their common presence within the tumour microenvironment and functional plasticity, tumour-associated macrophages are an attractive target for cancer therapy. Pharmacological “re-education” is an exciting prospect, whereby existing tumour-associated macrophages are repolarised towards a proinflammatory M1-phenotype that can promote antitumour immunity.

Agonists of STING (stimulator of interferon genes) have potential for achieving macrophage repolarisation, based on (i) STING’s role in interferon signalling and antitumour immunity, and (ii) recent discoveries involving mouse STING agonist, 5,6-dimethylxanthenone-4-acetic acid (DMXAA). Firstly, DMXAA alters the cytokine milieu of transplantable murine tumours in a STING-dependent manner. Secondly, DMXAA can repolarise murine macrophages from M2 towards M1, similar to the metazoan STING agonist 2’3’-cyclic guanosine monophosphate-adenosine monophosphate (2’3’-cGAMP). In phase III clinical trials, DMXAA failed to recapitulate the durable antitumour immunity observed in mice. An explanation for this is that DMXAA cannot bind and activate human STING.

We are using human THP-1 macrophages to screen for human-active macrophage polarising agents in vitro. Resting M0, M1-activated (IFN-γ and LPS) and M2-activated (IL-4 + IL-13) THP-1 macrophages are treated with potential STING agonists, and changes in functionality (IP-10 (CXCL10) production) and phenotypes are distinguished based on differential expression of surface markers (CD80, CD86, CCR7, CD163, and CD206) using multi-colour flow cytometry.

M1- and M2-phenotypes can be routinely established among differentiated THP-1 macrophages in vitro. Preliminary results show that 2’3’-cGAMP induces IP-10 production in M0 (75-fold) and M2 (>40-fold) phenotypes, and enhances IP-10 production (6-fold) in M1 macrophages. These findings support the hypothesis that STING is a suitable target for the polarisation of human macrophages, and are consistent with previous observations in mice. Of ten XAA analogues tested thus far, none altered IP-10 production in M0 (<200 pg/mL) or M2 (<150 pg/mL) macrophages, and modest differences were observed among high (>20,000 pg/mL) IP-10 producing M1-macrophages. The XAA analogues tested included 7-MeXAA, 8-MeXAA and 7,8-MeXAA, that we have previously shown to have superior cytokine-inducing activity over DMXAA in human leukocytes. At this stage it seems unlikely that the XAA analogues have potential for polarising human macrophages.
Cancer-associated adipocytes produce inflammatory and matrix remodelling proteins that promote breast cancer cell invasion

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In breast cancer, obesity is linked to invasive tumours that respond poorly to chemotherapy. We have explored relationships between breast cancer cells and adipocytes in the tumour microenvironment using a novel co-culture system. Human breast adipocytes induced chemo-resistance in both oestrogen receptor (ER) positive and ER negative breast cancer cells. In addition, cancer-associated adipocytes induced a partial epithelial-mesenchymal transition, creating a population of breast cancer cells that were highly migratory. In this study, we used discovery mass spectrometry and antibody arrays to analyse the proteins secreted by cancer-associated adipocytes to identify proteins that may contribute to breast cancer metastasis.

Breast adipose tissue samples were collected from patients undergoing therapeutic or prophylactic mastectomy and breast reductions at Christchurch Hospital. Pre-adipocytes were isolated and differentiated into mature adipocytes over two to three weeks, followed by co-culture with human breast cancer cells (ER+ MCF7 and ER- MDA-MB-231) using a transwell co-culture system. Conditioned media was collected from pre-adipocytes, mature differentiated adipocytes and adipocytes co-cultured with either MCF7 or MDA-MB-231 cells. Secreted proteins were analysed using quantitative mass spectrometry (iTraq-MS), antibody arrays (R&D Systems) and ELISAs.

Of the 183 secreted proteins identified using iTraq-MS, 45\% were secreted at higher levels by adipocytes in co-culture with breast cancer cells compared with adipocytes alone, and included proteins associated with inflammation and matrix remodelling. Chitinase 3-like 1 (Chi3L1) levels were increased according to both iTraq-MS and antibody array analysis, and recombinant Chi3L1 treatment (4ng/ml) increased viability, proliferation, migration and resistance to chemotherapy in both breast cancer cell lines (p<0.05). In serum samples from breast cancer patients (n=20), Chi3L1 levels were not associated with body mass index (BMI), but high circulating levels of Chi3L1 were observed in patients with stage III cancers.

Our results support the hypothesis that adipocytes produce a more inflammatory, tumour promoting environment when they are growing in close contact with breast cancer cells. Investigation into the cross-talk between breast cancer cells and cancer-associated adipocytes may aid management of therapy regimens to improve breast cancer patient outcome.
Breast cancer is the most common malignancy in women, accounting for more than 400,000 deaths per year worldwide. Approximately 80% of human breast carcinomas present as oestrogen receptor alpha-positive (ER+ve), which are typically sensitive to hormone deprivation. Oestrogen deprivation, induced by treatment with aromatase inhibitors (AIs) has been identified as the most effective therapy for ER+ve breast cancer in postmenopausal women. Despite this, up to half of patients eventually develop resistance to AIs and understanding of their precise molecular effects and causes of resistance is limited. Using molecular investigations of tumours from post-menopausal breast cancer patients we have identified features of the gene expression in the tumour, stromal microenvironment and hormonal milieu of the tumour that contribute to resistance to treatment.

To better characterise the mechanisms of resistance to oestrogen deprivation, we examined gene expression in 104 patients treated with the AI anastrozole in the neoadjuvant setting. Surprisingly, analyses revealed that pretreatment expression of an inflammatory signature correlated with poor response. Poor response was also associated with higher levels of lymphocytic infiltration in the tumour, suggesting that infiltrating immune cells may play a role in response to aromatase inhibitors. These findings contrast with chemotherapy-treated breast cancers where high levels of infiltration are associated with a good response. Expression of chemokines including CCL5, CXCL16, and CCL22 also increased in response to treatment and this was replicated in vitro in an MCF7 cell model of oestrogen deprivation. In addition, a significant increase in the total number of peripheral blood mononuclear cells migrating to oestrogen deprived cells compared to cells with normal levels of oestradiol (p <0.0001) was observed in a transwell immune cell migration assay. Furthermore, FACS analysis revealed a significant increase in the number of CD4+ cells and a decrease in the number of CD11+ cells migrating to deprived cells (p<0.05). This migration was blocked by addition of a broad specificity chemokine-binding protein and to a lesser degree by a CCL5-specific protein.

These data suggest that oestrogen deprivation-induced chemokine production induces recruitment of immune cells towards ER+ve breast cancer cells and this response may contribute to resistance to anti-oestrogen therapy. Targeting this inflammatory response could be a future direction for therapy.
Contralateral breast cancer (CBC) incidence and outcomes in New Zealand (NZ)

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Background
Patients with unilateral breast cancer (UBC) are at increased risk of developing CBC. Several factors have been identified that influence this risk, however reports are inconsistent and many are now outdated. CBC rates and patient outcome in NZ are unknown.

Methods
NZ cancer registers provided information for patients with UBC diagnosed between June 2000 and December 2012. Patients without metastases who develop a subsequent second primary cancer in the contralateral breast were classed as having CBC. Logistic regression was performed to delineate association between variables and CBC diagnosis. Survival differences were determined by log rank test.

Results
10,374 patients had UBC, median follow-up 4.6 years. 48 women (0.46%) developed CBC; six within a year of UBC diagnosis (12.5% of total CBC), and a further 24 by year 5 (thus 62.5% of CBC are diagnosed within 5 years).

Variables associated with CBC development include being postmenopausal (OR 2.2, p=0.02), pT stage 4 (OR 8.6, p=0.00), and prior treatment with endocrine therapy (OR 2.0, p=0.04). Having a first degree relative with breast or ovarian cancer was associated with CBC (OR 2.1, p=0.02), however diagnosis in any less immediate relative/s was not (OR 1.2, p=0.53). Lobular histology was not associated with CBC development (OR 1.4, p=0.45). Compared to all other ethnic groups, Maori showed a non-significant trend toward CBC development (OR 2.1, p=0.06). Being premenopausal and having fewer than three nodes removed at UBC diagnosis were associated with reduced CBC odds (OR 0.4 and 0.24 respectively, p=<0.05).

BRCA testing was performed infrequently in this population (1.8% of patients), and no patient who developed CBC had a documented BRCA mutation. Also no patient who underwent a contralateral prophylactic mastectomy developed CBC.

There was no significant difference in overall survival (Log Rank HR 1.3, p=0.34), disease specific survival (Log Rank HR 1.5, p=0.26), and distant metastases free survival (Log Rank HR 1.4, p=0.29) between patients who developed CBC and those who did not. Development of metastases was far more common than CBC (ratio 26:1).

Conclusions
The incidence of CBC in the NZ population is much lower than in historical series, and is vastly outnumbered by the incidence of metastatic cancer. Patients have similar survival outcomes independent of whether they subsequently develop CBC. Several factors were identified in our population that were associated with CBC development. The risk of CBC in patients with a positive family history but no BRCA mutation remains undefined.
Evaluating *BRCA1* and *BRCA2* sequence variants that modulate isoform expression

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Routine diagnostic *BRCA1* and *BRCA2* gene screening is typically performed for individuals from high-risk breast-ovarian families. A significant number of *BRCA1/BRA2* sequence variants in or near splice sites and splicing regulatory regions, such as ESEs (exonic splicing enhancers), result in a disruption of the mRNA splicing process. Splicing assays currently undertaken to assess the clinical relevance of rare sequence variants in these genes are often limited in their ability to quantify isoform expression changes that may be associated with cancer risk.

Our study aims to utilize a series of new transcriptomic approaches to generate comprehensive expression profiles of *BRCA1/BRA2* isoforms, and measure allele-specific expression changes of rare and common *BRCA1/BRA2* variants. We have profiled mRNA isoforms in lymphoblastic cell lines (LCLs) carrying common and/or rare variants predicted to alter splicing using targeted RNA-sequencing technology (Illumina), MassARRAY (Sequenom) and RNA *in situ* hybridisation (RNAscope).

Our data show a larger number of mRNA isoforms expressed by *BRCA1* compared to *BRCA2* despite *BRCA1* having smaller number of exons. Quantitative assessment indicates that individual *BRCA1* isoforms are less variable in expression compared to *BRCA2* isoforms suggesting more stringent regulatory control for *BRCA1* splicing. Analysis of rare and common variant carriers also identified the expression of several mRNA isoforms with relative expression levels outside of the expected range observed in controls. We have further demonstrated the variability of *BRCA1* and *BRCA2* isoform expression levels between individual cells using a new *in situ* hybridisation assay, RNAscope.

Although *BRCA1* and *BRCA2* have been well studied over the past two decades, next-generation technologies are generating important new information about these breast cancer susceptibility genes. This new information will establish the ‘normal’ expression variability of each mRNA isoform, enabling aberrant splicing events to be detected in cells of potential mutation carriers. Such guidelines will be critical for the future interpretation of splicing analyses results in a diagnostic setting.
Scoping for RNA biomarkers in colorectal cancer

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Colorectal cancer is the most commonly registered cancer in New Zealand. Significant prognostic variability exists with this disease due to tumour heterogeneity and clinicopathological staging limitations. Molecular techniques offer promise in improving staging and treatment. However, limitations in current technology have seen few molecular biomarkers implemented into clinical practice. RNAScope® (Advanced Cell Diagnostics, Inc., Hayward, CA) is a powerful new in-situ hybridisation technology that measures RNA in histologically preserved cells, whilst overcoming limitations of existing techniques. Our aim is to establish RNAScope® as a valid method to identify RNA biomarkers for improved prediction of colorectal cancer prognosis by assessing the expression patterns of candidate RNA markers in colorectal tumour cells using semi-quantitative and quantitative image analysis methods in relation to established protein markers and clinical outcomes. Cases used in the study were selected across surgical stage and tumour grade from the Cancer Society Tissue Bank. RNA expression levels of three genes (*MLH1, TNFRSF11A* and *GFI1*) associated with colorectal cancer subtype and/or prognosis, were microscopically assessed in 110 histologically preserved colorectal cancer cases using RNAScope®. Semi-quantitative analysis was determined using the manufacturer’s scoring system whilst quantitative analysis was performed using ImageJ and SpotStudio™ image analysis software. RNAScope® was successful in detecting RNA molecules from lowly expressed genes. Expression of all genes in non-tumour cells was found to be higher than tumour cells in a minority of cases. Image analysis provided assistance in automating assessments of tumour heterogeneity. However both analysis software packages failed to discriminate between probe clusters and strong chromatin and nucleolar staining in some cases leading to over-calling and inaccurate counts. We show that RNAScope® is a powerful technology to explore tumour heterogeneity and microenvironment interactions, with potential for colorectal cancer diagnostics.
Screening for vulnerability in older adults with curable head and neck cancer: the Waikato protocol

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Background
Management of cancer is increasingly complex in older adults, with shorter life expectancy, more frailty and typically multiple co-morbidities. Comprehensive geriatric assessment (CGA) has been shown to improve selection of patients who can successfully tolerate aggressive cancer treatment, but has major resource implications, given the ageing population of cancer patients. Rather than referring all elderly patients, tools are available that could identify vulnerable older patients who may benefit from CGA. In this pilot study we assessed the utility of incorporating such a tool in the assessment of older adults for curative-intent treatment for head and neck cancer.

Methods
The study recruited patients aged 65 years or older with a potentially-curable primary malignancy of the head and neck region who were being presented at the Head and Neck Multidisciplinary meeting (MDM). The G8 questionnaire was administered by a nurse prior to the MDM. Clinicians at the MDM made a treatment recommendation, including referral for CGA if considered advisable, prior to the G8 score being revealed. Patients considered candidates for curative-intent treatment whose G8 score indicated vulnerability (<15) were referred for CGA. Subsequent treatment and outcomes were recorded. Informed consent was gained.

Results
Over 6 months 35 patients were recruited into the study, median age 74 (range 65-93) years, 63% male, 11.4% Maori, 80% with squamous cell carcinoma and 63% with upper aero-digestive tract primary tumours. Seven (20%) patients were considered vulnerable by the MDM and referred for CGA, but 17 (49%) patients were assessed as vulnerable by G8 score, and were generally older than those with higher scores (median age 79 vs. 72 years). CGA was performed in 7 patients, of whom 86% continued to curative-intent treatment. Of 10 patients with G8 scores <15 but no CGA, 60% proceeded to curative-intent treatment. Average length of stay after surgery was 16 vs. 3.6 days in patients with G8 scores < 15 or ≥ 15, respectively (p=0.03), and the completion rate for radical radiotherapy was 71% vs. 100% in each group, respectively.

Conclusions
In this pilot study approximately 50% of patients aged over 65 years were identified as vulnerable by the G8 score (using a threshold of <15), twice the proportion identified by MDM clinicians. However most patients who had a CGA continued to curative-intent treatment. Tolerance of treatment was poorer in those with lower G8 scores. This tool merits assessment in a larger study in this setting.
A prospective audit of venous thromboprophylaxis in hospitalised oncology patients

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Background
Venous thromboembolism (VTE) is a common complication in cancer patients, and has significant implications on morbidity and mortality for those affected. Compared with hospitalised patients who do not have cancer; patients with cancer are at higher risk of VTE development. The benefit of venous thromboprophylaxis for this subset of patients is therefore considered to be greater. Internationally accepted clinical practice guidelines (American College of Chest Physicians [ACCP] Antithrombotic Guidelines 9th Ed) recommend venous thromboprophylaxis for acutely unwell hospitalised oncology patients.

Objective
To provide a systematic prospective audit assessing current compliance with internationally accepted guidelines regarding venous thromboprophylaxis in hospitalised non-surgical oncology patients.

Methods
Three authors (CN, SW, AR) conducted an independent review of clinical records for adult patients (≥18 years) admitted to Christchurch Hospital under the care of the oncology department over a three month time period. Information included: age, date of admission, reason for admission (acutely unwell with complications of cancer or cancer treatments; versus elective admissions for cancer treatment or minor procedures), primary site of malignancy, stage of disease, VTE diagnosis, prescription of venous thromboprophylaxis, and if not prescribed, the presence of contraindications to its use.

Results
Between 1st April and 30th June 2015, there were 265 admissions to the oncology ward at Christchurch Hospital. Average age was 62 years. 48% (N=127) of patients were male; 52% (N=138) were female. 86% (N=228) were acutely unwell on admission, while 14% (N=37) were elective admissions. Of the 265 patients, 73% (N=194) were eligible for thromboprophylaxis according to ACCP guidelines (excluding elective admissions and those anticoagulated prior to admission). Of 194 patients, 9% (N=18) received prophylactic anticoagulation. 11% (N=22) patients had contraindications to the use of prophylactic anticoagulation, and 5% (N=1) were subsequently prescribed mechanical thromboprophylaxis (graduated compression stockings, intermittent pneumatic compression, inferior vena cava filter). No bleeding complications were recorded. VTE was diagnosed in 7.5% (N=20) of all patients during their admission. A further 13% (N=34) were on active treatment for VTE prior to admission.

Conclusion
VTE is a common complication in cancer patients. Internationally accepted thromboprophylaxis guidelines promote its use in acutely unwell hospitalised patients with cancer. Overwhelmingly,
these recommendations are not reflected in current practice within our locality. Potential improvements may be achieved through ongoing education, standardisation of risk assessment and venous thromboprophylaxis prescription, clinical prompts, and closer collaboration with junior medical staff, pharmacy and nursing services. After a twelve month period, we recommend the audit be repeated to ensure sustained improvement.
Colorectal tumours dysregulate gut macrophage conditioning

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A high infiltration of macrophages in colorectal cancer (CRC) has been associated with better prognosis, which is the opposite of what is observed in many other cancers. However, the effect macrophages may have in altering CRC progression is not well understood.

Myeloid populations were assessed by flow cytometry in both tumour and non-transformed bowel (NTB) tissue collected fresh from patients undergoing surgery for CRC (n=11). Cells were assessed for expression of the macrophage marker combination CD45, CD64 and CD11b and then analysed for expression of CD33, CD14, CD206 and CD163 to determine phenotype. This revealed a heterogeneous macrophage population ranging from gut conditioned to a more conventional monocyte-derived macrophage phenotype. The frequency of gut conditioned macrophage subsets was lower in tumour compared to matched NTB (7.47% and 3.87% in NTB vs 2.17% and 0.690% in tumour, median of gut resident subset 1 and 2 respectively, n = 11), whereas the more conventional phenotypes were higher in tumour than NTB (19.3% and 1.55% in NTB vs 26.8% and 5.54% in tumour, median of gut resident subset 3 and 4 respectively, n = 11). Based on analogous in vitro data, the two conventional macrophage populations represent pro-inflammatory and anti-inflammatory phenotypes respectively.

Microenvironment strongly affects macrophage phenotype. Patient PBMCs were also cultured in both tumour and NTB conditioned media, which were assessed for cytokine content using a multiplex assay. Conditioned medias that contained high levels of IL-6 and/or TNF-a better supported an anti-inflammatory macrophage phenotype. Furthermore, conditioned medias only affected monocyte to macrophage maturation, not mature macrophage populations. This study provides new insight into macrophage function in CRC and demonstrates useful methods to examine them ex vivo in human tissue.
Does a variant of the tumour suppressor p53 play a role in tumour metastasis?

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p53 is universally acknowledged as the canonical tumour suppressor gene, crucial for preventing cancer development at the earliest stages. Mutations of p53 are commonly found in many human tumours, and result in a ‘gain-of-function’ phenotype of aggressive disease. A number of naturally occurring isoforms of p53 have been discovered which appear to have differing roles in tumour prevention or promotion.

Rather than preventing tumour development, the p53 isoform Δ133p53 has been implicated in tumour progression, and is elevated in a number of cancer types, as well as recently being shown to promote tumour growth and angiogenesis. A mouse model of the isoform, the Δ122p53 mouse, has a pro-inflammatory profile and a spectrum of aggressive tumours, resulting in early death. We are interested in determining whether Δ122p53 promotes invasion and metastasis, and by extension make inferences about the actions of the Δ133p53 isoform in human cancers.

Using wound closure assays we found that the addition of conditioned media from p53Δ122p53/+ MEFs to p53+/+ MEFs significantly increased migration compared to conditioned media from p53−/− MEFs, with a time to close of 28 hours compared to 37 hours. Using multiplex assays, we identified candidate factors for the promotion of the increased migration, including the pro-inflammatory cytokine IL-6. To test this, we employed MEFs from Δ122p53 mice crossed onto an IL-6−/− background. In both wound closure and Transwell assays we found that p53Δ122p53/+ IL-6−/− MEFs had a reduction in cell migration compared to p53Δ122p53/+ IL-6+/+ MEFs. These results confirm the requirement of IL-6 for the pro-migratory phenotype of Δ122p53. In addition, p53Δ122p53/+ IL-6−/+ mice had tumours which metastasised to other organs more frequently than tumours from p53Δ122p53/+ IL-6−/+ mice.

Using organotypic assays we found that Δ122p53 cells were more invasive in a collagen-fibroblast matrix than control cells, with an invasive index of ~50% compared to ~25% for controls. In vivo metastasis assays using B16 melanoma cell lines showed that B16/Δ122p53 administration resulted in a greater number of metastatic colonies both on and within the lungs earlier than controls. In addition, B16/Δ122p53 cells had significant invasion into adjacent adipose tissue, something not seen in B16/vector cells.

Taken together, these results suggest that Δ122p53, and by extension Δ133p53, promotes a pro-migratory and pro-tumorigenic phenotype dependent on a panel of signalling factors, and suggests a mechanism for the poor survival of cancer patients with Δ133p53 expressing tumours.
Inhibitors to the immune-suppressive enzyme indoleamine 2,3-dioxygenase 1 (IDO1) for cancer therapy: a drug discovery journey

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IDO1 is an immune-suppressive enzyme adopted by cancers to evade the patient’s anti-tumour immunity. High IDO1 expression correlates with poorer patient outcome. Development of IDO1 inhibitors is currently an active area of research as an approach to restoring tumour immunity.

As part of the ACSRC’s IDO1 inhibitor drug development program, a highly sensitive fluorescence assay (limit of detection 153 nM N-formylkynurenine) was initially established. The chemical reaction forming the novel fluorophore utilised in the assay was subsequently characterised and the structure of the fluorophore was identified to be a tetrahydroquinolone adduct formed in the previously unreported reaction between N-formylkynurenine and cyclic amines. This assay was automated for use in the JANUS robotic workstation and applied to screen chemical libraries including the National Cancer Institute Diversity Set III library (1,597 compounds) for inhibitors against wild-type IDO1 as well as alanine-replacement mutants of serine-167 and cysteine-129 in the IDO1 active site. The screening of the NCI library provided 35 compounds that inhibited wild-type IDO1 activity > 50% at 20 μM and indicated that serine-167 but not cysteine-129 is important for the interaction with a broad range of IDO1 inhibitors. Screening of 40,000 molecules from a commercial library also provided another thirty hits that were all resynthesized and re-tested and validated using the in-house assay. Hits were then triaged using computational structural filters and experimental assays that detect nonspecific inhibitors to eliminate the compounds unsuitable for drug development. Our three top-ranked, novel IDO1 inhibitors all have excellent cell permeability, good potency (IC₅₀ 0.066 - 8 μM) in cell-based assays and negligible cellular cytotoxicity. Inhibitor-1 blocked IDO1 activity reversibly, in a similar manner to 4-phenyl-1H-imidazole, a well-studied IDO1 inhibitor in the literature. In contrast, Inhibitor-2 and Inhibitor-3 elicited essentially irreversible IDO1 inactivation, by a mechanism not previously documented for IDO1 inhibitors. LC-MS studies suggested that Inhibitor-3 inactivates IDO1 by destruction of the haem co-factor inside the IDO1 active site. Inhibitor-3 was selected as the most suitable candidate for drug development. This hit failed only one out of the four structural filters and shows excellent cell permeability (27-fold higher inhibitory activity in cell-based assay (IC₅₀ = 66 nM) compared to isolated enzyme assay). Inhibitor-3 also exhibits the irreversible mechanism of IDO1 inactivation which would be one of the more favoured mode of enzyme inhibition for a pharmaceutical agent. Inhibitor-3 is currently being optimised by rational medicinal chemistry and development into a potential anti-cancer agent.
The Benefit and Tolerability of Adjuvant Chemotherapy in the Elderly Stage III Colon Cancer Patients: a 3 Year Retrospective Audit

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**Background**  
Colon cancer is prevalent and predominately a disease of older adults. The role of adjuvant chemotherapy in the “very old” is not clearly defined due to competing comorbidities and frailty with advancing age and the greater risk of significant toxicity from chemotherapy. Furthermore, adding oxaliplatin to fluorouracil based regime has been shown to increase survival benefit in the young, however its benefit in the elderly is controversial. This retrospective audit investigated the usage, benefit and tolerability of adjuvant chemotherapy in patients above 60 with stage III colon cancer.

**Method**  
The Medical Oncology database at the Regional Cancer Centre, Waikato Hospital, was searched for patients with stage III colon cancer aged 60 years and older who were referred for consideration of adjuvant chemotherapy between over 3 years from 1 January 2010. Additional data was sourced from electronic patient records. Data on demographics, whether adjuvant chemotherapy was received, its completion rates as well as toxicities and relapse and survival was collected.

**Results**  
109 patients were identified, median age 71 years (range 60-81, 54% >70 years), 63% male, 89% NZ European and 6% Maori. Of these, 9 patients were excluded (most due to metastases being found on staging investigations or due to moving to another DHB before treatment). Adjuvant chemotherapy was administered to 91% of those aged 60-70 years and 75% of those aged >70 years. Reasons for not giving chemotherapy included patient refusal (1 vs. 5 patients) comorbidities (1 vs. 3) and post-operative complications (2 vs. 3) for those aged 60-70 and >70 years, respectively. Of those who received chemotherapy the proportion receiving oxaliplatin-based chemotherapy was 51% vs. 17 % of those aged 60-70 and >70 years, respectively. A higher proportion of those > 70 years completed planned chemotherapy (61%) compared to the younger cohort (45%), mainly due to oxaliplatin-related toxicities leading to early termination in the latter group. Relapse was seen in 29% and 33% of the younger and older cohorts, respectively. More deaths occurred in the older cohort (51% vs. 28%), similarly the proportion with cancer-related deaths (64% vs. 46%).

**Conclusion**  
Adjuvant chemotherapy was commonly offered to older adults with stage 3 colon cancer, though oxaliplatin was largely restricted to those under 70 years. Oxaliplatin-related toxicities accounted for most treatment cessation.
Analysis of specific T cell infiltrate may predict patient prognosis in a NZ cohort of colorectal cancer patients

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Colorectal cancer (CRC) staging in patients is currently based on tumour morphology and does not take into account the complexity of the anti-tumour immune response. Stage I and II CRC patients are treated with surgery alone whereas stage III patients (lymph node positive) are offered adjuvant therapy to reduce recurrence risk. However current staging does not accurately predict prognosis - some stage II patients relapse after surgery alone whereas some stage III patients do not. The Immunoscore has been proposed as a way to incorporate the T cell infiltrate in the tumour with the clinico-pathological stage to improve estimates of disease-free survival. The Immunoscore is currently being validated worldwide. We performed a pilot study to test the Immunoscore in a cohort of stage II patients who underwent surgery at Dunedin Hospital and were followed up long term. We also extended the immunoscore to include effector regulatory T cells (eTregs).

Immunofluorescence was used to analyse immune cell infiltrates in two patient cohorts. First, we compared early stage (II) colorectal cancer patients with (n=5) and without (n=10) recurrence; and second, we compared the immune infiltrate in colorectal cancer patients (n=120) receiving cimetidine as adjuvant therapy, a drug known to influence local immune responses. Analysis of the first cohort revealed that patients with a high Immunoscore (high T cell infiltrate) had increased disease-free survival compared to patients with a low Immunoscore (low T cell infiltrate; Log-rank p<0.0085). The ability to predict patient outcome was improved further by measuring the infiltrate of CD4+FoxP3+Blimp-1+ cells (eTregs; Log-rank p<0.0021). Patients with a low Immunoscore but high infiltrate of eTregs at the centre of the tumour had better disease-free survival than those with a low Immunoscore and a low infiltrate of CD4+FoxP3+Blimp-1+ cells.

These pilot results indicate that the immunoscore was able to predict recurrence in this cohort of stage II patients, and was further augmented by including eTregs. We now aim to validate these findings to a larger cohort of stage II and stage III CRC patients.
Omega-3 fatty acid analogues as potential agents against brain cancers

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The health benefits of fish oils have stimulated scientific interest for many decades and include diseases such as cancer. Fatty acids from fish oils are taken up by the brain and are important for normal brain development and function. In contrast, the majority of systemically administered chemotherapeutics inefficiently penetrate into the brain, which can lead to sub-therapeutic concentrations. This is considered a significant challenge in developing effective brain cancers therapies. We hypothesise that synthetic omega-3 fatty acid analogues can penetrate into the brain and have activity against brain cancer. Synthetic epoxy omega-3 fatty acid analogues, C29 and C15, were tested for anti-tumour activity against the mouse glioma cell line, GL261. C29 decreased cell viability and inhibited cell cycling in vitro. In vivo anti-tumour activity was determined using an ectopic subcutaneous mouse model in C57Bl/6 mice. Treatment with C29 (50 mg/kg, qd, i.p) demonstrated a significant decrease in tumour growth over time (two-way ANOVA, p value < 0.0001), which led to a significant survival benefit compared to controls (log-rank test, p value = 0.05). To associate this response to compound concentrations, plasma and tumour concentration-time profiles were determined in mice using mass spectrometry. Area under the concentration curve from zero to 24h (AUC) calculated in plasma and tumour tissue were 11180.9 ng.h/ml and 1469.3 ng.h/g, respectively. The activity seen in the subcutaneous tumour model was further investigated in the orthotopic intracranial tumour mouse model where anti-tumour activity was measured using bioluminescence from luciferase transfected GL261 tumours and animal survival. These results were comparatively less effective than those observed in the subcutaneous model. Brain AUC (576.8 ng.g/ml) were substantially lower than those measured in plasma. Activity of C15 was also examined, and was not effective against the ectopic GL261 tumours with the dosing regimen 50 mg/kg, q3d, i.p. The AUC for C15 in plasma and tumour tissue were 53.6 ng.h/ml and 579.5 ng.h/g, respectively. In contrast to C29, AUC for C15 calculated in brain tissue (1186.2 ng.h/ml) was higher than those calculated in plasma and tumour tissue. However, in the intracranial model, no significant survival benefit was obtained after treatment with C15 compared to vehicle-treated controls. In summary, the C29 epoxy omega-3 fatty acid analogue has demonstrated promising activity against the GL261 glioma in vitro and in the subcutaneous tumour model. Further understanding of the enhanced brain penetration by C15 may aid the development of analogues with improved activity against intracranial tumours.
Variant classification in “high-risk” breast cancer predisposition genes – the role of the ENIGMA international multidisciplinary consortium

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Identification of a disease-causing mutation in a cancer syndrome gene directs risk management of carrier individuals, and their relatives - including genetic counselling, pre-symptomatic screening, risk-reducing medication and surgery, and possibly choice of cancer therapy. Many sequence changes identified by clinical genetic testing are not obviously disease-causing e.g. missense changes, small in-frame insertions/deletions, potential splice site alterations. It is challenging for individual testing laboratories to collect enough information for robust classification of such variants. Moreover, the problem is escalating in scale as clinical testing moves rapidly from syndrome gene testing in highly selected familial cancer patients to high-throughput panel testing of multiple cancer genes in population-based cases.

Large-scale studies evaluating cancer gene variants in the BRCA1/2 and now other known or suspected breast cancer predisposition genes are being undertaken by the international consortium ENIGMA. Data curation has identified considerable inconsistency in variant nomenclature and classification across clinical testing and research laboratories. Collation and generation of clinical and laboratory information has standardized and promoted variant classification, and led to the documentation of standardised classification criteria for BRCA1/2 gene variants. Results demonstrate the value of international collaborative studies to facilitate evidence-based classification of cancer gene variants to improve clinical genetic counselling, and patient and family management.
Colorectal Cancer in New Zealand: Preliminary Outcomes of the PIPER Project


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Background
Colorectal cancer has the highest incidence of any solid tumour in NZ and is the second leading cause of cancer related death. The PIPER project was a retrospective national cohort study including all patients newly diagnosed with colorectal adenocarcinoma in calendar years 2007 and 2008 and an expanded time cohort to enrich Maori and Pacific peoples. These were linked to national morbidity and mortality databases using a unique patient identifier (the National Health Index, NHI).

Results
5667 patients were identified. Location of primary tumor and stage were consistent with an unscreened population. 31% of patients presented as an emergency, and 19% of all patients presented with bowel obstruction. For those presenting with obstruction, the 5-year overall survival was 35.6% (95% CI 32.6-38.6%) and without obstruction it was 50.4% (95% CI 48.9-52.0%), p = < 0.0005. Differences remained highly significant after adjusting for age, sex, stage and comorbidity. 59% of patients with stage 3 colon cancer received adjuvant chemotherapy. Less than half of patients completed 24 weeks of therapy. 49% of patients with stage 4 colorectal cancer received chemotherapy and 51% of these patients underwent surgical resection of the primary tumour. Surgical mortality at 30 and 60 days for those with any-stage colorectal cancer undergoing primary resection was comparable to internationally reported figures.

Conclusions
This study underlines the importance of detection of colorectal cancer prior to emergency or obstructed presentation, and describes the impact of systemic therapies on survival in a national study.