CMRF Currie_PRO2016-001
Does exercise lower systemic inflammation and improve chemotherapy efficacy in cancer patients?
Final Report Feb 2019

Specific objectives for this project:

1. Use immunoassays (ELISAs) to analyse a panel of circulating inflammatory adipokines in stored patient serum samples, and determine associations with clinicopathological variables and disease-free survival.
2. Use in *vitro cell* culture experiments to determine whether inflammatory adipokines (at concentrations detected in obese patients) are capable of altering CYP450 enzyme activity and chemotherapy drug metabolism in HepG2 liver cells.
3. Perform a pilot feasibility study to measure breast cancer patient exercise levels during chemotherapy, as well as circulating inflammatory adipokines and patient metabolism of probe drugs.

Summary of Research:

Our previous research showed that cancer-associated adipocytes (CAA) secrete many inflammatory adipokines that are present in measurable levels in breast cancer patient blood (Dr Elisabeth Phillips). Since it is known that inflammatory factors can inhibit liver CYP450 enzyme drug metabolism, we hypothesized that high circulating levels of CAA-secreted inflammatory adipokines may inhibit the expression of liver CYP450 enzymes, thus decreasing their functional ability to metabolize chemotherapy drugs and produce the toxic metabolites required to effectively treat breast cancer. We further hypothesized that physical activity may decrease systemic inflammation, and thus restore liver CYP450 enzyme function as well as chemotherapy metabolism and efficacy.

We measured circulating inflammatory adipokines in a large cohort (n=120) of breast cancer patients from the Cancer Society Tissue Bank Christchurch, and observed correlations with Body Mass Index (BMI), age and inflammation (Phillips et al. In Prep. Adipocyte 2019). This study aim was extended by a PhD student (Rebekah Crake) who investigated the local effects of these inflammatory adipokines on breast cancer cells. Rebekah performed a global analysis of proteins in breast cancer cells co-cultured with or without CAA. She identified over 1,000 differentially expressed proteins, and pathway analysis showed these proteins are involved in breast cancer cell metabolism, cancer cell invasion and resistance to chemotherapy treatment. This formed an important part of Rebekah's PhD project, and has identified many proteins and signalling pathways for future study by our group (R Crake et al., Submitted Cancer Genomic & Proteomics, 2019).

We aimed to investigate the direct effects of inflammatory adipokines on CYP450 enzymes in liver hepatocytes using cell culture experiments (Aim 2). However, after extensive discussions with our collaborator Assoc. Prof. Nuala Helsby (University of Auckland) and some initial work, we realized this was not technically feasible. Therefore, we decided to concentrate on investigating liver CYP450 function in our patient study (Aim 3). Patient recruitment closed (Dec 2018) with n=12 patients included in the study. A large amount of data has been collected from these patients including clinical data (including co-morbidities/treatments and chemotherapy dosing), anthropomorphic measurements (BMI, Waist:Hip, Body Composition) and measures of physical activity throughout treatment,

blood samples at various timepoints throughout treatment to measure inflammatory adipokines, and blood and urine samples to measure probe drugs and their metabolites (i.e. a surrogate measure of liver CYP450 enzyme function) before and during chemotherapy. We have analysed recruitment capture rates for this study using patient data from Health Connect South (HCS) and clinical oncology letters in MOSAIQ. Although the number of patients recruited to this study was not as high as we had anticipated, our 8% recruitment rate aligns well with overseas recruitment rates for large clinical trials, and we are preparing a short communication to share our New Zealand experience of 'real world' recruitment for an oncology based non-clinical trial (Crake et al., NZMJ 2019). Analysis of data from this study is ongoing - it will be completed by June 30th, with final publications from this study completed and submitted by 30th Sept 2019.

With the agreement of the CMRF, this 18 month project was extended by 6 months (time only) to try to accommodate Dr Elisabeth Phillip's maternity leave. Despite this generous extension of time, we still have approx. $6,520 of Elisabeth's salary and salary related expenses remaining at the close of the account, and we will arrange for this money to be returned to the CMRF with thanks for their generous support for this project.

Outcomes Arising from this CMRF funded Research

The funding and support received from the CMRF for this project has been acknowledged in all outputs.

Manuscripts:

1. *Transwell co-culture with human breast adipocytes alters glycolysis and TCA cycle protein abundance in hormone receptor positive (MCF7) and negative (MDA-MB-231) human breast cancer cells. Crake RLI, Phillips E, Kleffmann T, Currie MJ. Submitted Proteomics 2018; Cancer Genomics and Proteomics 2019.*
2. *Feasibility and patient participation in an oncology-based non-clinical trial to measure liver CTP450 function in breast cancer patients receiving chemotherapy. Crake RLI, Phillips E, Strother RM, Robinson BA, Currie MJ. In preparation, NZMJ 2019.*
3. *Identification of proteins secreted by breast cancer-associated adipocytes, levels in patient serum, and associations with patient outcome. E Phillips, R Crake, RM Strother, BA Robinson, GU Dachs, T Kleffmann, M Currie. In preparation, Adipocyte 2019.*
4. *At least one other manuscript will be published describing the outcomes from the patient study with Rebekah Crake as first author.*

Conference Presentations:

1. *R Crake, E Phillips, T Kleffmann, H Morrin, M Strother, B Robinson, M Currie. Transwell co-culture with human breast adipocytes alters the proteome expression profiles of MCF7 and MDA-MB-231 human breast cancer cells. Poster presentation, European Association for Cancer Research 50th Anniversary Meeting, Amsterdam, 2018. Poster Presentation.*
2. *R Crake, T Kleffmann, H Morrin, M Strother, B Robinson, E Phillips, M Currie. Cancer-associated adipocytes alter protein expression profiles of human breast cancer cells in vitro. Queenstown Molecular Biology Meeting, Cancer Satellite, Queenstown, 2018. Poster Presentation.*

3. *R Crake, M Strother, H Morrin, A Smith, E Phillips, B Robinson, M Currie. An exploratory breast cancer patient study to assess the impact of obesity-related inflammation and physical activity on chemotherapy drug metabolism. Women's Wellbeing Symposium, University of Otago, Dunedin. 2018. Oral presentation.*
4. *R Crake, H Morrin, E Phillips, B Robinson, M Currie, M Strother. An Exploratory Study to Assess the Impact of Inflammatory Markers on Breast Cancer Drug Metabolism in Response to Physical Activity during Chemotherapy. NZ Society for Oncology Meeting, Queenstown, 2018. Poster Presentation.*
5. *RLA Crake, E Phillips, T Kleffmann, MJ Currie. Cancer-associated adipocytes alter proteomic expression of human breast cancer cells. NZ Society for Oncology Meeting, Auckland, 2017. Oral presentation.*

Invited Talks:

Zonta Club Christchurch South	*Oct 2018*
UOC Open Day, Christchurch	*Sept 2018*
Australasian Biospecimens Network Assoc., Melbourne	*Oct 2018*
Maurice Wilkins Centre Meeting, Dunedin	*Dec 2017*
UOC Open Day, Christchurch	*Oct 2017*
Cancer Society & Mackenzie Cancer Research Group Public Event, Christchurch	*July 2017*
3 Minute Thesis Competition	*July 2017*
Mackenzie Cancer Research Group Public Event, Ashburton	*May 2017*
ARA Seminar	*Nov 2017*
UOC Seminar Series	*July 2016*
Department of Surgery, UOC	*May 2016*
Cancer Society NZ, Canterbury Westcoast Division	*May 2016*

Student projects:

Dr Elisabeth Phillips

Elisabeth is an emerging researcher in the Mackenzie Cancer Research Group who has helped to develop the ideas for this project, and performed or supervised the laboratory work involved. She has also supervised (Jess Wise, Devon Bull) and co-supervised (Rebekah, Mohini) many of the student projects that have developed from this CMRF funded research, under the mentorship of Margaret. She has now successfully obtained her own funding from the CMRF for a related project investigating microRNAs in breast cancer cells exposed to cancer-associated adipocytes. Elisabeth is also co-leader of a collaborative project with the Christchurch Regenerative Medicine and Tissue Engineering (CReaTE) Group, developing a novel 3D model of breast cancer that can be used to further study complex interactions between cancer-associated adipocytes and cancer cells, and test drugs that will target the molecules and pathways involved in these interactions.

Rebekah Crake PhD (2016-2019)

Rebekah (Supervisors Margaret Currie, Elisabeth Phillips, Matthew Strother, Bridget Robinson) obtained a University of Otago Postgraduate Scholarship. Importantly, this CMRF grant supported consumable costs for the Breast Cancer Patient Study, which Rebekah has performed as part of her PhD. This feasibility study has provided data on patient body composition, circulating inflammatory cytokines, physical activity and liver CYP450 enzyme

function throughout chemotherapy treatment. It is an invaluable resource and will form the foundation for our future funding applications and ongoing research.

Jess Wise PhD (2017-2020)

Jess (Supervisors Elisabeth Phillips, Khoon Lim, Margaret Currie, Tim Woodfield) obtained a University of Otago Scholarship and is supported by both the Mackenzie Cancer Research Group and the CReaTE Group (UOC). Under the primary supervision of Elisabeth Phillips and Khoon Lim, she is developing a 3D breast cancer cell model using a unique hydrogel system, which we can use for high-through put testing of drugs to target molecules and pathways identified by our CMRF funded research.

Linda Buss PhD (2017-2020)

Linda (Supervisors Gabi Dachs, Margaret Currie, Barry Hock, Bridget Robinson) obtained a University of Otago Postgraduate Scholarship, and is working to develop a mouse model to study the effects of physical activity on immune health. Her project runs in parallel with the patient study in our CMRF funded project, and arose directly from that research.

Mohini Puri MMedSc (2016-2018)

Mohini is a trained anatomical pathologist who joined our group to study cancer-associated adipocytes and breast cancer cells with altered (invasive) phenotype in breast tumour samples (Supervisors Margaret Currie, Elisabeth Phillips). Her Masters of Medical Science ran parallel to this CMRF funded research. Mohini has now returned to her medical training and is currently a House Surgeon at Christchurch Hospital.

Devon Bull Summer Student (2018/2019)

Devon completed a 10-week summer studentship with us (Supervisors Elisabeth Phillips, Rebekah Crake, Margaret Currie). She screened a panel of 10 breast cancer cell lines for PGK1 protein levels, and helped validate this crucial finding from our CMRF funded research. Devon is currently undertaking an BBiomedSc Hons course at University of Otago, Dunedin.