Cancer Research Institute of Northern Alberta

Inaugural RESEARCH DAY 2014

Saturday, November 15th
University of Alberta
CCIS 1-440
DEAR CRINA RESEARCH DAY ATTENDEE,

Thank you for joining us at the inaugural research day of the Cancer Research Institute of Northern Alberta (CRINA). We are certain that you will enjoy your day of networking with cancer research specialists and their trainees. We hope to foster collaborations among clinicians, cancer investigators and scientists who possess cutting-edge research tools.

CRINA is the newest translational research institute at the University of Alberta. It is a pan-university initiative that will integrate and build upon existing research strengths in cancer research to create a comprehensive cancer program, wherein patient care is intertwined with scientific discovery.

This inaugural meeting will highlight the cancer research capacities at the University of Alberta by featuring oral presentations from a sampling of scientific leaders across campus. Many more trainees and principal investigators will present their work through posters, allowing you an opportunity to further explore what our university has to offer.

Through this networking initiative, we hope that CRINA will enhance the university’s translational science capabilities and competitiveness, leading to new breakthroughs and improved healthcare outcomes for cancer patients.

Sincerely,

Catherine Field, PhD RD
David Eisenstat, MD, MA, FRCPC
Lynne Marie-Postovit, PhD

Interim Co-Directors of CRINA
MESSAGE FROM THE DEAN

Invasive cancers are the leading cause of premature death in Canada and the United States, surpassing death by cardiovascular diseases in the last decade. More than 40 per cent of the North American population develops cancer during their lifetimes. Two Canadians are diagnosed with and one dies from cancer every seven minutes. Canada’s new cancer case rates are projected to increase by more than 50 per cent, to 24,200 per year, by 2030.

Current Alberta cancer occurrences—about 16,200 per year—and death rates—6,100 per year—are daunting. In Alberta, as in the rest of Canada, prostate cancer is the most frequent male cancer, and breast cancer is the most frequent female cancer. Lung cancer is the leading cause of cancer death in both Alberta men (90 per cent fatal) and women (80 per cent fatal). How do we respond to this health crisis?

In April 2014, the University of Alberta established a new Cancer Research Institute of Northern Alberta—CRINA—one of three novel translational science institutes. By 2017, CRINA’s research programs will meet the National Institutes of Health’s National Cancer Institute (NCI) comprehensive designation criteria, setting a new standard for cancer centres across Canada. By leveraging its solid public-private research base towards greater competitiveness for major awards and grants, Alberta will assume its place among the global leaders in breakthrough cancer science by 2020.

In the 15 minutes it will take to read this post, four more Canadians will have developed cancer and two more will have died from it. Cancer is the problem. Research is the cure.

D. Douglas Miller  
Dean, Faculty of Medicine & Dentistry  
University of Alberta
MESSAGE FROM THE VICE PRESIDENT, RESEARCH

An estimated 76,600 Canadians will lose their battles with cancer this year; more than 6,000 of them will be Albertans. To improve outcomes for patients and families with cancer, the University of Alberta has created the Cancer Research Institute of Northern Alberta (CRINA), one of the three multidisciplinary Translational Science Institutes (TSIs) dedicated to fostering collaboration between researchers to move the latest discoveries from the laboratory to the clinic.

This exciting new initiative has tremendous potential—CRINA will redefine the standard of cancer care in Canada by improving our understanding of cancer biology, discovering new therapies and biomarkers to diagnose patients with greater accuracy, and improving relapse rates and prevention.

I congratulate the life sciences faculties on this important interdisciplinary initiative.

Lorne Babiuk
Vice-president, Research
University of Alberta
## Program

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<td>- Liang Li - Development of a Universal Metabolome-Standard Method for Long-term LC-MS Metabolome Profiling and Its Application for Bladder Cancer Urine-Metabolite-Biomarker Discovery</td>
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- Jana Rieger
  *Interdisciplinary translational research in head and neck cancer for improved patient outcomes*

- Shairaz Baksh
  *Pre-clinical Therapeutics for Inflammatory Bowel Disease (IBD) and IBD-related Colorectal Cancer (IBD-CRC)*

- Ing Swie Goping
  *Investigating BAD biology in breast cancer using mouse models*

12:00 PM – 1:00 PM  **LUNCH**  CCIS PCL Lounge

1:05 PM – 1:55 PM  **ORAL PRESENTATIONS**  CCIS 1-440

- Lynne Marie-Postovit
  *From embryonic stem cells to drug screening: Modelling cancer biology with the goal of improving cancer outcomes*

- Russell Greiner
  *Towards Patient-Specific Treatment: Medical Applications of Machine Learning*

- Vera Mazurak
  *Relationship of Fat Metabolism to Muscle Wasting During Cancer*

- Alan Underhill
  *Genetic and epigenetic dysfunction in cancer*

- Atiyah Yahya
  *Measuring Oncologically Relevant Metabolites In Vivo with Magnetic Resonance Spectroscopy*

1:55 PM – 2:05 PM  **SHORT BREAK**  CCIS 1-440

2:05 PM – 2:55 PM  **ORAL PRESENTATIONS**  CCIS 1-440

- Karin Olson
  *A different kind of tired: Fatigue in the context of cancer*
Frank van Landeghem

*Long non-coding RNA (IncRNA) Expression Patterns in Gliomas*

Vickie Baracos

*Body composition as an independent determinant of chemotherapy toxicity and efficacy*

Nicolas Touret

*Plasma Membrane Organization Promotes CD36 signal transduction in Endothelial Cells*

Sarah Hughes

*A genetic model for Neurofibromatosis Type 2*

3:00 PM – 4:00 PM  **POSTER SESSION 2/SNACKS**  CCIS PCL Lounge

Even numbered posters

4:05 PM – 4:45 PM  **ORAL PRESENTATIONS**  CCIS 1-440

Roseline Godbout

*From Model Animals to Modeling Cancer*

Mark Glover

*Structural basis of DNA damage signalling networks*

Mary Hitt

*Preclinical Studies of Vaccinia Virus Mutants for Breast and Bladder Cancer Therapy*

David Brindley

*Targeting autotaxin and lysophosphatidate signalling as a new adjuvant therapy for increasing the efficacy of chemotherapy and radiotherapy*

4:45 PM – 4:55 PM  **AWARDS/CLOSING REMARKS**  CCIS 1-440

5:00 PM – 6:30 PM  **RECEPTION AND CASH BAR**  CCIS PCL Lounge
SPEAKER ABSTRACTS

Pages 8-19

(Poster abstracts are on pages 21-96)
## Oral Presentations

### Alberta Cancer Research Biorepository (ACRB)

**Weinfeld M and Graham K**  
Department of Oncology, Faculty of Medicine and Dentistry, University of Alberta

This presentation will provide an overview of the Alberta Cancer Research Biorepository. The provincial ACRB operates in both Edmonton and Calgary and offers integrated solutions for the collection, processing and storage of cancer related samples. We have frozen tissue and blood fractions from over 40 different cancer types. The largest groups are breast, colorectal, lung, ovarian, thyroid, prostate and kidney cancers. We have blood fractions from 15,000 participants and tissue from 12,000 participants that are currently available for distribution. We can also access the Edmonton clinical paraffin blocks (FFPE) to prepare Tissue MicroArrays. The Open Access samples are available to local, national and international investigators who have ethics-approved research plans. Patients provide consent for their samples to be used in cancer research, other related health studies, and possibly also in pharmaceutical research. The samples are also annotated with relevant clinical data, which increases their utility. Minimal data has been collected on all samples and a comprehensive data set can be collected when needed. The de-identified sample and data are supplied to researchers. Funding for this project is provided by the Alberta Cancer Foundation and their donors.

### The Cross Cancer Institute Cell Imaging Facility

**Hendzel M**  
Department of Oncology, Faculty of Medicine and Dentistry, University of Alberta

The Cross Cancer Institute Cell Imaging Facility was established approximately 15 years ago as a Departmental facility for the Department of Oncology. Since then, the facility has expanded its capacity and capabilities substantially and adopted a policy of preferred user fee rates for anyone at the University of Alberta doing cancer research. There are several rare or unique capabilities that are provided by the CCI facility, particularly instrumentation for quantifying abundance, distribution, and dynamic behaviours. The facility is managed by a dedicated scientist, Dr. Xuejun Sun. He provides experimental advice and direct assistance on the more challenging applications of microscopy. He is assisted by three research technicians to provide support in fluorescence, confocal, deconvolution, and multiphoton microscopies as well as transmission electron microscopy and immunoelectron microscopy. We are also developing techniques to bring “colour” to the transmission electron microscope. In 2014, the CCI Cell Imaging Facility received an operating grant from the Alberta Cancer Foundation. This has further opened our preferred user fee schedule to any cancer researcher in Alberta. I will review the user fee structure and instrument capabilities of the Cross Cancer Institute and illustrate some of these capabilities using data acquired by my laboratory in the CCI facility.
Alberta’s Tomorrow Project: Making the transition from cohort study to health research resource

Robson PJ

Department of Agricultural, Food and Nutritional Science, Faculty of Agricultural, Life and Environmental Sciences, University of Alberta

Alberta’s Tomorrow Project aims to provide a high quality infrastructure platform that will support research in cancer and chronic disease etiology and prevention. Based on a prospective cohort design, the Tomorrow Project is currently enrolling up to 50,000 Albertans aged 35-69y who have not had a diagnosis of cancer. Upon enrollment, participants are asked to complete a health and lifestyle questionnaire and to give broad consent for active and passive follow-up for up to 50 years, including linkage with administrative health data (e.g. Alberta Cancer Registry, Alberta Health etc). They are also invited to attend a study centre to donate samples of blood (50ml, non-fasting) and urine (spot), and have a series of physical measurements taken (standing/sitting height, weight, waist and hip circumferences, grip strength, resting heart rate, blood pressure). Blood samples are processed to multiple aliquots of serum, plasma, buffy coat and red cells, and frozen at -80°C within two hours of venipuncture. In addition to broad consent for data linkage, Tomorrow Project participants have consented to have their data and biological samples used by ‘approved researchers’ who have research questions that will contribute to knowledge in the area of cancer or chronic disease etiology and prevention. Experience to date has demonstrated very clearly that robust procedures and resources must be place to ensure that use of the Tomorrow Project repositories of data and samples can be managed in a way that is transparent, equitable, ethical, streamlined and consistent with the expectations of participants who have donated their data and samples to support research. Currently, Alberta’s Tomorrow Project is developing and refining its systems and procedures, and expects to be open for access in late Spring 2015.

Lowering the Barriers to the Clinical Translation of Cancer Research

Mackey JR

Department of Oncology, Faculty of Medicine and Dentistry, University of Alberta

Background: The path from discovery science to clinical evaluation and utilization in the cancer clinic requires complex choreography among scientific and clinical disciplines, regulatory and ethical oversight bodies, funding agencies, intellectual property experts and commercial partners. This daunting process typically leaves many promising ideas unexplored.

In order to address some of these barriers, the Department of Oncology, Cross Cancer Institute and Alberta Cancer Foundation have put in place programs to help bring potential new cancer therapies into clinical evaluation. These include:

i) The Alberta Cancer Foundation Pre-Phase I Program The mandate of the Pre-Phase I Program (PP1P) is to provide advice, a thorough understanding of the local / provincial capacities and key external outsourcing opportunities, expertise on Health Canada pre-clinical requirements for evaluation of cancer systemic therapies, toxicology and formulation expertise within the steering committee, access to clinical experts to address therapeutic potential and study design, preferential access to the phase I clinical trial stream at the Cross Cancer Institute, and project management expertise. Direct enquires to andrea.rosario@albertahealthservices.ca

ii) The Cross Cancer Institute Investigator Initiated Trial Program In partnership with the Alberta Cancer Foundation, the CCI IIT Program conducts twice annual peer review for approximately ~500K / year funding of intervention clinical trials for cancer patients. In order to streamline the process of conducting, and publishing clinical trials, the IIT program provides study templates with links to local experts for advice, biostatistical support, budget templates and costing, assistance with ethics review and regulatory submission, case report forms, statistical analysis service, and publication coordination. Direct enquiries to casandra.feader@albertahealthservices.ca

iii) The Cross Cancer Institute Clinical Trial Unit The Mission of the Cross Cancer Institute Clinical Trial Unit is “to initiate and conduct clinical trials that improve the lives of current and future cancer patients”. With more than 90 academic principal investigators, 65 expert clinical trial staff and core infrastructure supported by the Alberta Cancer Foundation, the CTU places approximately 600 patients on intervention studies annually. These clinical trials span the spectrum of Phase I first in human testing through registrational phase III studies of novel drug treatments, advanced radiotherapy techniques, physiotherapy, cardioncology, and physical exercise interventions. Our Unit has state-of-the-art cancer imaging capabilities, with clinical laboratory and pharmacy expertise. Direct enquiries to andrea.rosario@albertahealthservices.ca
## Development of a Universal Metabolome-Standard Method for Long-term LC-MS Metabolome Profiling and Its Application for Bladder Cancer Urine-Metabolite-Biomarker Discovery

**Peng J, Chen YT, Chen CL, Li L**  
Department of Chemistry, Faculty of Science, University of Alberta

Large-scale metabolomics study requires a quantitative method to generate metabolome data over an extended period with high technical reproducibility. We report a universal metabolome-standard (UMS) method, in conjunction with chemical isotope labeling LC-MS, to provide long-term analytical reproducibility and facilitate metabolome comparison among different datasets. In this method, UMS of a specific type of sample labeled by an isotope reagent is prepared a priori. The UMS is spiked into any individual samples labeled by another form of the isotope reagent in a metabolomics study. The resultant mixture is analyzed by LC-MS to provide relative quantification of the individual sample metabolome to UMS. UMS is independent of a study undertaking as well as the time of analysis, and useful for profiling the same type of samples in multiple studies. In this work, the UMS method was developed and applied for a urine metabolomics study of bladder cancer. UMS of human urine was prepared by 13C2-dansyl labeling of a pooled sample from 20 healthy individuals. This method was first used to profile the discovery samples to generate a list of putative biomarkers potentially useful for bladder cancer detection, and then used to analyze the verification samples about one year later. Within the discovery sample set, three-month technical reproducibility was examined using a quality control sample and found a mean CV of 13.9% and median CV of 9.4% for all the quantified metabolites. Statistics analysis of the urine metabolome data showed a clear separation between the bladder cancer group and the control group from the discovery samples, which was confirmed by the verification samples. Receiver operating characteristic (ROC) test showed that the area under the curve (AUC) was 0.956 in the discovery dataset and 0.935 in the verification dataset. These results demonstrated the utility of the UMS method for long-term metabolomics and discovering potential metabolite biomarkers for diagnosis of bladder cancer.

## From molecules, mice and man: Radionuclides and molecular imaging for cancer research

**Wuest F**  
Department of Oncology, Faculty of Medicine and Dentistry, University of Alberta

Current biomedical research is revealing the fundamental molecular processes of life and disease. The understanding of how molecular components of living cells are organized, how they interact, how they move and how they are formed and eliminated within the life cycle of an organism represents an integrative approach, which requires the direct observation of biochemical and physiological processes at the molecular and cellular levels in vivo. The molecular processes of life can be studied and visualized at various levels of resolution in humans and other living subjects by means of radionuclides and in vivo molecular imaging techniques. Molecular imaging in living subjects offers distinct advantages when compared with conventional in vitro and cell culture research techniques. Molecular imaging permits measurement of both the temporal and spatial biodistribution of a molecular probe to assess biological processes in a living subject, and to decipher physiological whole-body contributions of proteins and genes. PET, as the most sophisticated and sensitive molecular imaging technology, is based on the application and detection of decaying radioisotopes which are attached to biologically active compounds to form radiolabeled probes or radiotracers capable of imaging and monitoring specific biochemical and physiological events in vivo.

Research activities of the Division of Oncologic Imaging are embedded in the multidisciplinary field of translational cancer research with special focus on the design, synthesis and radiopharmacological characterization of novel radiopharmaceuticals to optimize current diagnosis and treatment of cancer. Research activities are aimed at the evaluation and translation of the diagnostic and therapeutic potential of novel molecular targets and specific biochemical signatures associated with the development and progression of cancer. This especially involves the use of PET radiopharmaceuticals and pre-clinical small animal PET imaging for non-invasive assessment of cancer-related metabolic pathways and biochemical processes at the cellular and molecular level. The presentation will provide an overview on current research activities and infrastructure capacities for the application of radionuclides and molecular imaging in cancer research and cancer patient care.
Interdisciplinary translational research in head and neck cancer for improved patient outcomes

Rieger J
Institute for Reconstructive Sciences in Medicine/Department of Communication Sciences and Disorders, Faculty of Rehabilitation Medicine, University of Alberta

This presentation will provide an overview of the innovative interdisciplinary research that has taken place at the Institute for Reconstructive Sciences in Medicine over the past twenty years to improve outcomes for patients with head and neck cancer. Specific examples of innovations and how they have changed the way patients in Alberta are treated will be presented. Our current research objectives and interests in future collaborations will be highlighted.

Pre-clinical Therapeutics for Inflammatory Bowel Disease (IBD) and IBD-related Colorectal Cancer (IBD-CRC)

Said A1, Fiteih Y2, Salla M1, Gordon M2, Volodko N2, Aldawsari F4, Ortiz RO4, Martinez CV4 and Baksh S1,2,3,5,6

1Department of Biochemistry, 2Pediatrics, 3Oncology, Faculty of Medicine and Dentistry, and 4Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta. 2Member, Alberta IBD Consortium, Alberta, Canada, 3Member, Cancer Research Institute of Northern Alberta, Edmonton, Alberta, Canada

Chronic inflammation has long been associated with a predisposition to the development of cancer with about 30% of all cancer cases being preceded by a state of chronic inflammation. This includes IBD which can predispose individuals to CRC later in life. Current therapeutics reduce or transiently eliminate inflammation but not all IBD patients are responsive to these therapies and side effects are a serious concern.

Currently, it is known that IBD is caused by a combination of genetic factors, environmental influences, intestinal microbial disruptions and immunologic dysfunction. Over 163 susceptibility genes have been identified for IBD including some on chromosome 3p21, the location of the Ras association domain family protein 1A, RASSF1A (or 1A). 1A is a tumor suppressor gene epigenetically silenced in a majority of human cancers (including inflammatory Hodgkin’s lymphoma and colorectal cancer [CRC]) resulting in its functional inactivation. In addition, epigenetic silencing of 1A has also been detected in pre-condition diseases to cancer such as IBD and pancreatitis to suggest regulation of cellular homeostasis beyond a tumor suppressor gene.

Our research group is developing novel therapeutics to inhibit or eliminate acute or chronic inflammation states and interfere with inflammation-induced injury that can lead to malignancy. Therapeutic intervention is being tested in IBD models in both Rassf1a−/− and IL-10−/− mouse knockout models. We are currently exploring inhibition of NFκB activation, inhibition of phosphotyrosine autophagic and metabolic signaling pathways as avenues of treatment for the complex heterogeneous disease that IBD presents as. Our therapeutics include the anti-tumor, anti-NFκB, anti-epigenetic drug resveratrol and its unique derivatives; the established tyrosine kinase inhibitor, imatinib/gleevec; and novel inhibitors to the NOD2 linked protein kinase, RIPK2. RIPK2 inhibitors were designed purely using computational biology and molecular modeling and we are currently synthesizing a RIPK2 inhibitor based on this screen. The RASSF1A molecular pathway may be a key molecular link between chronic inflammation and tumorigenesis. Our expertise in pre-clinical organoid and animal models, inflammation, epigenetic research and rational drug design is what we bring to CRINA and we look forward to new and exciting collaborations with CRINA members.

Funding Sources: AI-IIS, WCHRI and the Stollery Children’s Hospital/Hair Massacre Donation Fund.
**Investigating BAD biology in breast cancer using mouse models**

**Goping, IS**

Department of Biochemistry, Faculty of Medicine and Dentistry, University of Alberta

Breast cancer is the most frequently diagnosed cancer in Canadian women. Taxane chemotherapy is given to thousands of Canadian women every year, although lack of predictive markers prevents personalized treatment. We identified that the Bcl-2 family member Bad is associated with positive outcomes of breast cancer patients after taxane chemotherapy. Thus, in collaboration with basic and clinical scientists, we are pursuing the utility of marker expression as a diagnostic guide to taxane therapy. Importantly, we discovered that Bad has unexpected functions in breast cancer. Using genetic and cell biological approaches, we are investigating the molecular mechanisms of Bad activity.

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**From embryonic stem cells to drug screening: Modelling cancer biology with the goal of improving cancer outcomes**

**Postovit LM**

Department of Oncology, Faculty of Medicine and Dentistry, University of Alberta

Tumours contain populations of cells with stem cell like properties, and it is believed that these phenotypically plastic cells are responsible for cancer progression and metastatic potential. Stem cell-like populations are regulated by dynamic niches, characterized by specific growth factors and extracellular matrices, as well as biophysical features such low oxygen tensions. Moreover, a growing body of evidence suggests that cancer cells co-opt stem cell-associated regulatory networks in order to sustain plasticity. Our lab attempts to define drivers of plasticity so that we can discover biomarkers and targets for therapy. To do this we use an array of model systems. For example, we use developmental models like zebrafish to understand cancer signalling, human pluripotent stem cells to understand epigenetics and dedifferentiation, cancer xenografts to examine tumour growth and metastasis and 3D culture systems with oxygen regulatory systems to better model tumours in vitro. We also use quantitative SILAC-based proteomics together with developmental and cancer model systems to identify potential anti-tumourigenic proteins in stem cell-derived extracellular matrices; and to further understand how cancer cells hijack developmental signalling cascades to facilitate progression. By studying the mechanisms by which cancer cells acquire and sustain phenotypic plasticity, we may uncover novel targets for the prediction and prevention of tumour progression.
Towards Patient-Specific Treatment: Medical Applications of Machine Learning

Greiner R
Department of Computing Science, University of Alberta

Patient-specific treatment requires determining which treatment has best chance of success for an individual patient, based on all available information. As this typically depends on many patient features, finding a single biomarker is not sufficient; instead, we need to find a combination over multiple features, combined using a classifier such as a decision tree. In many situations, these "best treatment" classifiers are not known initially. Fortunately, there is often a corpus of historical data, which includes descriptions of previous patients, as well as the outcomes. The field of Machine Learning provides tools that uses this data to "learn" which treatment is most effective for a given patient, based on his/her specific symptoms. This presentation will use a real example to illustrate the process, then summarize a large number of other successes. It will also describe a novel technology to produce a (meaningful) personalized survival distribution -- like a Kaplan-Meier curve, but specific to an individual patient.

Relationship of Fat Metabolism to Muscle Wasting During Cancer

Mazurak V
Department of Agriculture, Food and Nutritional Science, University of Alberta

My research program is centered around lipid metabolism in disease states with an emphasis on relationships between inflammatory processes and essential fatty acids. My most recent work has focused on defining nutritional requirements for people who have cancer and finding ways to overcome malnutrition during cancer therapies and advanced disease. I use humans, animals and cell culture in a translational research to define nutritional requirements for people who have cancer and determining ways to prevent or overcome malnutrition during chemotherapy and advanced disease. With a focus on essential fatty acids, this research was the first to demonstrate that n-3 fatty acid supplementation improves tumor response to drugs while simultaneously preventing muscle wasting and related pathologies that occur during lung cancer treatment, revealing a new understanding about the role of lipids for muscle health and tumor responses. In people with cancer, muscle loss and fat infiltration of muscle, are each independently predictive of death. Each of these prognostic factors have been modified by providing n-3 fatty acids to humans and in an animal model of cancer.
Oral Presentations

Genetic and epigenetic dysfunction in cancer

Underhill DA, Ziegler KA, MIssiaen K, Shi G

Department of Oncology, Faculty of Medicine and Dentistry, University of Alberta

Our primary focus is deciphering how master regulators of melanocyte development contribute to melanoma pathogenesis using the essential transcription factor PAX3 as a prototype. This factor is required during embryonic development to specify and expand the melanocyte lineage and people with only one functional copy of PAX3 are characterized by pigmentary deficiencies. As a transcription factor, PAX3 regulates the expression of other genes and this is an essential facet of its role in melanoma where it is co-opted to facilitate tumor proliferation and survival. Nevertheless, the identity of these genes is largely unknown. A key goal of our research program is the identification of PAX3 target genes in melanoma, which will provide a molecular picture of a key gene network in disease pathogenesis. In addition, we are examining how histone modifications regulate the balance between cell proliferation and differentiation, and how this safeguard is overridden in breast cancer and melanoma. These modifications have essential roles in the management and functional output of eukaryotic genomes by facilitating gene expression, repression and long-term silencing, replication, recombination, repair of damaged DNA, and mitosis. Within this scheme, our laboratory is examining how the methylation of lysine 20 on histone H4 modulates cell differentiation and how this process becomes aberrant in cancer. We currently make extensive use of biochemical, genetic, molecular, cell imaging, and bioinformatics approaches that incorporate in situ and in vivo models together with analysis of clinical samples. In particular, we have incorporated a next generation sequencing strategy for global analyses of regulatory landscapes and gene expression, and have developed a computational framework to define the architecture of regulatory networks in normal and transformed cells.

Measuring Oncologically Relevant Metabolites In Vivo with Magnetic Resonance Spectroscopy

Yahya A

Department of Oncology, Faculty of Medicine and Dentistry, University of Alberta

The purpose of this presentation is to discuss the utility of magnetic resonance spectroscopy (MRS) in the study of cancer and to present some of the challenges encountered in in-vivo MRS. MRS is a non-invasive technique that can serve as a method for measuring biomarkers relevant to the study of cancer. For example, a number of brain metabolites relevant to the study of brain tumours can be quantified with MRS. Some key brain metabolites such as N-acetyl aspartate (NAA), choline (Cho), glutamate (Glu), and myo-inositol (mI) can be measured with standard in-vivo MRS techniques. However, signals from other metabolites, such as glycine (Gly), are obscured by overlapping signal from metabolites present in higher concentrations. Part of my research program is to optimize MRS techniques at 3 T (for human studies) and at 9.4 T (for animal studies) to enable measurement of metabolites that are difficult to detect with standard MRS methods. Standard MRS techniques can be optimized by exploiting differences in scalar coupling interactions (a phenomenon in MRS) of the nuclei of different metabolites. One of the posters being presented describes a technique for resolving the Gly resonance at 9.4 T from the large overlapping signal of mI. Signal from approximately 1 mM glycine was measured and quantified from rat brain in vivo. Lipid quantification is also relevant to the study of cancer. For example, lipid levels have been shown to decrease significantly in spinal bone marrow of patients with leukemia and spinal bone metastases. MRS can enable measurement of lipid signal; however, scalar coupling interactions of lipid nuclei, which are often ignored, can cause errors in quantification. One of the posters being presented investigates of the effect of scalar coupling on lipid quantification with MRS at 3 T.
**Oral Presentations**

### A different kind of tired: Fatigue in the context of cancer

Olson, K  
Faculty of Nursing, University of Alberta

Fatigue is one of the most common and most distressing symptoms experienced by individuals with cancer, but its etiology is not well understood. The central hypothesis of my research program is that fatigue is a behavioural marker for the inability to adapt to biological, psychological, and social stressors associated with cancer and its treatment. I am particularly interested in models that could identify individuals at risk for fatigue, as early intervention could reduce dose delays and dose reductions, and thus improve health outcomes. In this presentation I will outline results of a recent study in which we considered links between obesity, inflammation, and cancer-related fatigue. In a small longitudinal study of 19 individuals, we found that fatigue was correlated with both markers of inflammation and obesity prior to treatment. Findings suggest that patterns of muscle and fat loss may be different for men and women.

### Long non-coding RNA (IncRNA) Expression Patterns in Gliomas

van Landeghem F, Formenti K, Greiner R, Eisenstat D  
Departments of Laboratory Medicine and Pathology, and Pediatrics, Faculty of Medicine and Dentistry, and Department of Computing Science, Faculty of Science, University of Alberta

Gliomas are the most common brain tumors in adults. Only a few prognostic and predictive biomarkers (mutation of IDH-1/-2, methylation of the MGMT promoter, ATRX expression, co-deletion of chr. 1p/19q) are established. Ribonucleic acids (RNA) are implicated in a variety of biological roles in coding, decoding, regulation, and expression of genes. In this pilot study, the following hypotheses will be tested: i) there are differences in patterns of long non-coding RNAs (IncRNAs) between different glioma entities and different WHO malignancy grades of the same glioma entity, ii) specific IncRNA patterns are associated with patterns of known biomarkers, and iii) specific IncRNA patterns can be used as prognostic and predictive biomarkers.

In this preliminary study, 24 gliomas were analyzed for the pattern of 61 IncRNA and transcription variants using total RNA extracts of formaldehyde-fixed, paraffin-embedded (FFPE) tissue using the nCounter platform (NanoString, USA). This platform uses color-coded probe pairs that allow the capture and direct count of individual transcripts (e.g. IncRNA, miRNA or mRNA transcripts) without use of enzymatic reactions (e.g. amplification).

Preliminary results reveal that gliomas with an oligodendroglial component (anaplastic oligoastrocytomas, AOA, and glioblastomas with oligodendroglial component, GBM woc) show an upregulation of ANRIL compared to astrocytomas WHO grade IV (glioblastoma multiforme, GBM). A trend towards an association between the presence of IDH-1 or IDH-2 mutations (R132H and R172K in exon 4, respectively) and an upregulation of ANRIL, C21orf131 (common probe and transcription variant 1), GDNFOS, TUG1-transcription variant 1, and Xist is noted in comparison to gliomas with wild-type IDH-1 and IDH-2. Gliomas with wild-type IDH-1 and IDH-2 show an upregulation of H19 compared to gliomas with IDH mutations. These preliminary results point to potential specific differences of IncRNA patterns in gliomas, dependent on their (i) histopathological diagnosis and (ii) molecular background. Further amendment of this study and validation in another cohort is needed to verify these findings.
Oral Presentations

Body composition as an independent determinant of chemotherapy toxicity and efficacy

Baracos V

Department of Oncology, Faculty of Medicine and Dentistry, University of Alberta

The term sarcopenia denotes depletion of skeletal muscle and the generally accepted definition is an absolute muscle mass >2 standard deviations below that typical of healthy adults. Muscle loss during aging is well known and predicts many poor health outcomes: frailty, falls, fractures, loss of independence, increased length of hospital stay, infectious complications in hospital, and decreased survival. Sarcopenia is not limited to people who appear underweight or thin, and it may be a hidden condition in normal weight, overweight or obese people (i.e. sarcopenic obesity). Our research group has led innovations in the use of computed tomography to study body composition in cancer patients, revealing enormous variation in individual proportions of fat and muscle (Prado et al. Lancet Oncology 2008;9:629-35; Martin et al. J. Clin Oncol. 2013: 31:1539-47). Within this variation, a consistent theme is emerging: that severe skeletal muscle depletion is a novel and powerful predictor of severe toxicity of systemic chemotherapy. Sarcopenia was identified in patients with cancers of the breast, colon, lung, liver, head and neck and kidney and these consistently had worse toxicity when treated with 5-fluorouracil, capecitabine, sorafenib, sunitinib, carboplatin, cisplatin or a regimen (adjuvant FEC, FOLFOX, FOLFIRI). This toxicity resulted in dose reductions and for some patients, definitive termination of treatment.

Plasma Membrane Organization Promotes CD36 signal transduction in Endothelial Cells

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CD36 is a multiligand scavenger receptor that ligates Thrombospondin-1 (TSP-1) at the surface of endothelial cells and induces their apoptosis. Recent evidences have shown that clustering of CD36 is necessary for signal transduction in macrophages and is regulated by the architecture of the cortical actin cytoskeleton apposed to the plasmalemma. Here, we investigated the role of the cortical actin cytoskeleton and plasma membrane nanodomains in the control of CD36 activation in endothelial cells. Stimulation with multivalent ligands (TSP-1 and anti-CD36 IgM) resulted in the downstream phosphorylation of the Src Family kinase, Fyn. Disruption of the actin cytoskeleton or removal of cholesterol blocked this activation. To gain molecular details on the rearrangement of the receptors during TSP-1 binding, we conducted superresolution approaches (based on PhotoActivated Localization Microscopy or PALM) and quantitative spatial distribution analysis. Endothelial cell lines stably expressing CD36-PAmCherry were generated for that purpose. At steady state, CD36 receptors pre-exist in small clusters (average diameter of 100 nm), in which Fyn was also present. Upon TSP-1 binding, CD36 clusters increased in size (average diameter of 140 nm) and also became denser. The average distance between CD36 molecules in these clusters was in the range of 8 nm. F-actin depolymerization or cholesterol depletion reduced the capacity of the ligand to induce formation of larger clusters resulting in a decreased recruitment and activation of Fyn. Our data demonstrate cooperation between cholesterol-dependent domains and the cortical actin cytoskeleton in the organization of CD36 receptors before and during TSP-1 stimulation.
Oral Presentations

A genetic model for Neurofibromatosis Type 2

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Bi-allelic mutations in the Neurofibromatosis Type 2 (NF2) gene encoding the Merlin tumour suppressor lead to NF2, an autosomal dominant disorder that follows a two hit model. NF2 causes crippling cancers of the nervous system, including schwannomas, meningiomas and ependymomas. The accepted treatment is usually repeated surgeries. Bi-allelic NF2 mutations are also linked to most sporadic schwannomas and meningiomas, together constituting 30% of all intracranial tumours in adults. Although the human NF2 gene was first identified in 1993, we do not yet have a mechanistic understanding of how NF2 mutations lead to the formation or progression of NF2 tumours. My research team has shown that Merlin interacts with multiple partner proteins to regulate cell proliferation and cell polarity using Drosophila melanogaster as a genetic model system. This suggests that Merlin functions as part of a larger regulatory complex and that mutation in genes affecting Merlin associated proteins may induce or enhance the progression of NF2 tumours in the central and peripheral nervous systems (CNS and PNS).

We have previously shown that Merlin functions as part of a larger protein complex in different epithelial cells. Protein interactions are mediated via a scaffold protein, Sip1, recruiting a regulatory kinase (Slik) and a phosphatase (Flapwing). Notably, this same regulatory complex co-regulates the Merlin-related protein, Moesin. We are now characterizing the assembly and activity of Merlin and associated proteins within neural cell types, as NF2 is a disease of the nervous system. Use of genetic approaches well established in Drosophila will allow for unique dissection of the molecular mechanisms of Merlin and Merlin associated protein activity within a whole animal.

From Model Animals to Modeling Cancer

Godbout R
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This is an overview of my lab’s basic and translational cancer research activities.

Project 1: Brain fatty acid-binding protein (B-FABP) in malignant glioma. We found B-FABP in a screen designed to identify genes preferentially expressed at early stages of chick retina development. B-FABP was subsequently found: (i) to preferentially bind to long chain polyunsaturated fatty acids such as omega-3 docosahexaenonic acid (DHA), (ii) to be expressed in the migrating radial glial cells of the developing mammalian brain, and (iii) to be highly expressed in human grade IV astrocytoma compared to low grade astrocytomas and normal brain. Work done in my lab demonstrates a role for B-FABP in the migration and infiltration of grade IV astrocytomas. Importantly, the effect of B-FABP on migration can be controlled in vitro by manipulating the ratio of omega-3 to omega-6 polyunsaturated fatty acids in the culture medium. Our next goals are to understand the molecular mechanisms underlying B-FABP-mediated cell migration and infiltration and to test whether in vivo growth of grade IV astrocytomas can be inhibited by altering the fatty acid environment in brain.

Project 2: Lipid binding proteins in breast cancer. We have found a number of associations between FABPs, cellular retinoic acid binding proteins (CRABP) and breast cancer. Of particular interest, our data suggest that the expression of three lipid binding proteins associated with retinoic acid metabolism (FABP5, CRABP1 and CRABP2) may determine response of breast cancer to retinoic acid. Our next goal is to determine whether expression of FABP5, CRABP1 and CRABP2 in breast cancer tissue can indeed predict in vivo response to retinoic acid.

Project 3: Role of DEAD Box 1 in DNA damage response. DEAD Box 1 (DDX1) is a member of the DEAD box family of RNA unwinding proteins implicated in all aspects of RNA metabolism. We have shown that DDX1 can unwind both RNA-RNA and RNA-DNA duplexes in vitro. When cells are irradiated, DDX1 accumulates at sites of DNA double-strand breaks. Our results indicate that DDX1 plays a role in the clearance of RNA at sites of DNA double-strand breaks, thereby facilitating DNA repair by homologous recombination. Our next goal is to determine the mechanism of action of DDX1 at double-strand breaks.

Grant support: Canadian Institutes of Health Research, Alberta Cancer Foundation and Canadian Breast Cancer Foundation.
**Oral Presentations**

### Structural basis of DNA damage signalling networks


Department of Biochemistry, Faculty of Medicine and Dentistry, University of Alberta

Our laboratory probes the structural principles that underlie the protein interactions at damaged chromatin that coordinate DNA repair and regulate diverse cellular responses to DNA damages. I will review three projects in this area:

1. Regulation and targeted inhibition of BRCT-mediated protein signalling in the DNA damage response.
2. Regulation of Lys-63 ubiquitination in DNA damage signalling.
3. Probing the catalytic mechanism of PNKP, a critical DNA repair enzyme.

### Preclinical Studies of Vaccinia Virus Mutants for Breast and Bladder Cancer Therapy

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The Hitt lab is investigating the manipulation of viruses to target and kill cancer cells. One application of viruses in the control of cancer is oncolytic virotherapy, that takes advantage of the virus’ ability to lyse host cells during a productive infection. Normal cells only support virus replication when host defense mechanisms (such as regulation of cell cycling and DNA synthesis) are disabled. Deletions of viral genes that function to block host defenses render the virus inactive in normal cells, but capable of replication in cancer cells that are lacking these host defenses. In collaboration with Dr. David Evans (Medical Microbiology and Immunology) and Dr. Ron Moore (Surgery), we are examining modified vaccinia virus mutants for their activity against breast and bladder cancer in tissue culture and in animal tumor models. Deletion of the gene encoding the viral ribonucleotide reductase small subunit (F4L) is predicted to limit virus replication to rapidly proliferating cells, such as cancer cells, that express the cellular homolog to viral F4L. Here we demonstrate robust delta-F4L virus replication and cancer cell killing in vitro. Promising results of this vaccinia virotherapy in immune competent rodent models of breast and bladder cancer will be presented.
Oral Presentations

Targeting autotaxin and lysophosphatidate signalling as a new adjuvant therapy for increasing the efficacy of chemotherapy and radiotherapy

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Treatment of breast and other cancers by a combination of surgery and chemotherapy is often unsuccessful and patients die. This is explained in several ways. Some cancer cells survive the surgery or they may have already spread to other areas of the body. Secondly, cancer cells gradually develop the ability to avoid being killed by chemotherapy and radiotherapy. Our work concentrates on a growth factor called lysophosphatidate, which signals through at least six G-protein coupled receptors. Lysophosphatidate is produced by a secreted enzyme called autotaxin, which plays an important role in promoting tumor growth, metastasis and the development of resistance to cancer treatments. We show that lysophosphatidate signaling depends on a vicious cycle in which inflammatory cytokines produced by tumors stimulate the production of autotaxin, which then produces lysophosphatidate to stimulate the secretion of more inflammatory cytokines. This inflammatory cycle drives tumor progression, metastasis and causes progressive resistance to chemotherapy and radiotherapy. We now demonstrate that this is because lysophosphatidate increases the expression of the transcription factor, Nrf2, which promotes the expression of anti-oxidant genes and multi-drug resistance transporters. These changes protect cancer cells against oxidative damage caused by chemotherapy and increase the export of chemotherapeutic agents from cancer cells. This knowledge is being used to identify new therapeutic targets that can block these adverse effects of lysophosphatidate. For example, we tested a newly developed compound, ONO-8430506, which produces a prolonged inhibition autotaxin activity and lysophosphatidate formation. This decreases the production of inflammatory cytokines, the growth of breast and thyroid tumors and their metastasis in several mouse models. Combining ONO-8430506 treatment with doxorubicin has a synergistic effect in blocking breast tumor growth and metastasis and in prolonging the efficacy of both treatments. These discoveries demonstrate that targeting the autotaxin-lysophosphatidate axis can be used as adjuvant therapy to increase the effectiveness of chemotherapy, and possibly radiotherapy. We are now negotiating to establish the “first in man trial” of ONO-8430506 in Edmonton. This trial will hopefully lead to the introduction a completely new paradigm for improving the efficacies of cancer treatments and the survival of cancer patients.
POSTER ABSTRACTS

Pages 21-96

(Speaker abstracts are on pages 8-19)
### 1. Dlx transcription factors in the childhood malignant eye tumor retinoblastoma

**Aghazadeh H, Bush J, Eisenstat D**  
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Retinoblastoma is the single most common childhood ocular tumor and is usually diagnosed in children less than five years of age. Interestingly, the oncogenic mutation necessary for the development of retinoblastoma in humans, which can be both hereditary and non-hereditary, is a bi-allelic inactivation of the tumor suppressor gene Rb-1, whereas p107, a related tumor suppressor gene, must also be inactivated for the development of retinoblastoma to occur in mice. Although no cell-of-origin has yet been identified for retinoblastoma in humans, our laboratory has recently discovered that DLX homeobox genes, which code for DLX transcription factors, are expressed in the tumor. The focus of my project has been the expression of these genes in retinoblastoma tumors of humans and mice. We expect to gain new insight into the retinoblastoma cell-of-origin through analysis of DLX co-expression with retinal cell-specific markers. We also expect that DLX2 over-expression will promote differentiation and apoptosis in the Y79 and WERI-RB1 cell lines. Immunohistochemistry methods were used to study DLX2 expression in formalin-fixed paraffin-embedded sections of mouse and human retinoblastoma tumor samples. In addition, DLX2 expression was knocked-down in WERI-1 and Y79 retinoblastoma cell lines using transfection of siRNA. Western blotting will be used to assess transfected cells for levels of DLX2 co-expression with other progenitor and retinal cell-type specific markers. Lastly, flow cytometry will be used to assess markers of proliferation and apoptosis in the transfected cells. As expected, DLX2 is expressed in human retinoblastoma tumors and cell lines. Further analysis of the immunostained patient samples is currently underway that will determine whether DLX is expressed in specific retinal cells that eventually become the tumor. Co-expression of DLX2 with other progenitor and retinal cell-type specific markers will help provide further insight into the identity of the cell-of-origin of retinoblastoma.

### 2. An Evaluation of Potential Novel Biomarkers BNIP3, AIF, DR5, and FABP7 in Glioblastoma Tumors

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Introduction: Glioblastoma (GBM) is the deadliest cancer of the central nervous system. Currently, epigenetic silencing by MGMT promoter methylation is the only confirmed prognostic biomarker for GBM. Previous studies have identified proteins whose level of expression may be associated with GBM patient prognosis or response to treatments, which include surgery, radiation, and chemotherapy. The purpose of this project is to determine if any of these proteins can be used as novel and accurate prognostic biomarkers in GBM patients. Our proteins of interest include BNIP3 (Bcl-2 Nineteen kDa Interacting Factor), AIF (Apoptosis Inducing Factor), DR5 (Death Receptor 5), and FABP7 (Fatty Acid Binding Protein 7). Previous studies from our research group have shown that the mislocalization of cytoplasmic BNIP3 to the nucleus confers resistance to temozolomide, a DNA-alkylating drug, and also represses AIF and DR5 expression, both of which promote apoptosis at high levels. FABP7, one of three fatty acid binding proteins found in mammalian brain, is associated with increasing tumour infiltration and migration. Our hypotheses are that GBM patients whose tumors show decreased FABP7 expression, increased AIF and DR5 expression, and/or cytoplasmic BNIP3 expression rather than nuclear expression will have longer overall survival or better response to treatment.

Methods: Immunohistochemistry was performed on paraffin-embedded slides of GBM tumors from a patient cohort in Manitoba using antibodies against the four proteins. BNIP3 subcellular localization was also determined through dual immunofluorescence using DAPI to mark the nucleus. The tumour samples were scored semi-quantitatively for AIF and DR5 protein expression and BNIP3 localization; immunostaining and grading analysis are still ongoing for FABP7.

Results: There was a non-significant trend of BNIP3 nuclear localization with patient outcomes; other data will be compared to the clinical variables of progression free survival and overall survival to identify if a correlation exists between protein expression and patient outcome.

Conclusions: Depending on the results, one or more of these proteins will be evaluated as a potential biomarker using a different GBM patient cohort to confirm its effectiveness in predicting patient outcomes.
3. Palliative Sedation for Existential Distress? A Survey of Canadian Palliative Care Physicians’ Views

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Aim: Palliative sedation (PS) can be used to treat refractory physical symptoms during end-of-life care. However, use of PS for managing existential distress remains controversial, as it is difficult to determine when the distress is refractory. There are no apparent recent data on the views and practices of Canadian palliative care physicians on the use of PS for existential distress. The aim of this study was to determine the expert opinions and practices of Canadian palliative care physicians regarding PS for the management of existential distress.

Methods: (1) Pilot study: feedback from members of the Division of Palliative Medicine, University of Alberta (n=15); (2) Survey questions (number per section): A. Demographics (n = 9), B. Experience with Palliative Sedation (n = 8), C. Experience with Palliative Sedation and Existential Distress (n = 5), D. Views on Palliative Sedation and Existential Distress (n = 5), E. Additional Comments (n = 3); (3). Online national survey: Available in English and French; Anonymous responses; Target group: E-mails sent to 322 Canadian Society of Palliative Care Physicians (CSPCP) members. Dates: March 24 – April 14, 2014 (allowed two weeks; reminder with additional week).

Results: 81 completed surveys returned, (26% response rate); Average number of years practiced in medicine & palliative care = 22 and 15 (SD 8), respectively; Dyspnea (100%), seizures (95%), & delirium (93%) were the most commonly reported refractory symptoms for which CPS could be indicated; Most (98%) participants believed that CPS is indicated for refractory physical symptoms with coexisting existential distress; 43% believed that it could be indicated for existential distress alone; The majority of respondents reported the use of midazolam (100%), but 22% reported using opioids specifically for CPS; More (71%) respondents were asked to provide, compared to those who actually provided (31%), CPS for existential distress; Loss of dignity (72%) was reported as the main cause of suffering experienced by patients receiving CPS for existential distress; Using a 5-point Likert scale, 40% of respondents either strongly disagreed or disagreed, while 43% either strongly agreed or agreed, with the use of CPS for the management of existential distress when no other refractory physical symptoms are present.

Discussion/Conclusion: A wide variety of responses and opinions appear to exist around palliative sedation for the management of existential distress. Further questions for ongoing consideration include: How is palliative sedation conceptualized in clinical practice compared to the literature? Is there a role for palliative sedation for existential distress alone? To what extent are palliative sedation and euthanasia morally distinct, given variability in practice? Palliative sedation for the management of existential distress continues to be a complex and potentially controversial issue.

4. Growth inhibitory effects of conjugated linolenic and linoleic acid isomers on breast cancer cells in vitro


Department of Agricultural Food and Nutritional Sciences, University of Alberta

Background: Long chain polyunsaturated fatty acids (PUFA) are known to inhibit breast cancer cell growth. The present work focused on determining the effects of three PUFA’s on the viability of breast cancer cells in vitro. Experimental fatty acids included punicic acid (PA), a conjugated linolenic acid naturally found in pomegranate seed oil, and two conjugated linoleic acid (CLA) isomers, t10, c12 and c9, t11, which are found in ruminant derived meat and dairy products.

Methods: Estrogen receptor negative (MDA-MB-231) or positive (MCF-7) cells were allowed to adhere to 24 well flat bottom plates for 48 hours. Cells were then treated for 72 hours with control media (containing 40 μM each oleic and linoleic acid), or control media plus one of the experimental fatty acids. Media was changed every 24 hours. Inhibition of cell growth was evaluated by counting trypan blue excluded cells.

Results: Compared to control media treated cells, PA inhibited growth of MDA-MB-231 cells at concentrations of 15, 20 and 37.5 μM by 87.5%, 89.0%, and 98% respectively (p<0.0009), and MCF-7 cells at concentrations of 16 and 20 μM by 69.5% and 90% respectively (p<0.004). CLA t10, c12 at 250 μM inhibited growth of MCF-7 cells compared to media treated cells by 57% (p<0.018), whereas CLA c9, t11 had no inhibitory effects on this cell line (p<0.4384).

Conclusions: PA and CLA t10, c12 appear to have inhibitory effects on breast cancer cell growth in vitro. Further investigation is needed to identify mechanisms responsible for growth inhibition by each of these fatty acids.
5. DDX17, a Sox2 binding partner, regulates Sox2 to sustain tumorigenic and stem-like properties in a phenotypically distinct subset of Breast cancer cells


Departments of Laboratory Medicine and Pathology, Faculty of Medicine and Dentistry, University of Alberta

Background: Sox2, an embryonic stem cell marker, is involved in the pathogenesis of breast cancer (BC). Our lab showed the two different phenotypically distinct cell subsets, based on their differential response to a Sox2 reporter (SRR2). The reporter responsive (RR) cells had more tumrigenic and stem like properties than reporter unresponsive (RU) cells. DDX17 is a transcription co-activator that has been shown to activate many transcriptional factors such as estrogen receptor alpha, P53 and beta-catenin.

Methods: Western blotting, Using liquid chromatography–mass spectrometry and co-immunoprecipitation to identify Sox2 binding partner, and siRNAs were used to query the regulatory relationships between DDX17, Sox2. Mammosphere and colony formation assays were used to assess the phenotypic consequences of DDX17-1 knockdown.

Results: Here, we report that DDX17 regulates Sox2. Using liquid chromatography–mass spectrometry and co-immunoprecipitation we found DDX17 as a Sox2 binding partner in MCF7, ER+ BC cell line. The interaction between DDX17 and Sox2 was significantly higher in RR cell subset than that of RU subset. Moreover, DDX17 was observed to interact equally with beta-catenin in RU and RR cell subsets. Additionally, upon siRNA knockdown of DDX17, protein level of Sox2 was unaffected in RU and RR cell subsets; the activity of Sox2 was significantly decreased in RR cell subsets. However, there were no any significant changes in Sox2 activity in RU cell subsets. Correlating with these findings, siRNA knockdown of DDX17 drastically reduced the tumorigenic and stem-like properties only in MCF7 RR cells as observed by decreased in colony formation and mammosphere formation efficiency.

Conclusions: in a subset of BC cells, namely RR cells, DDX17 regulates Sox2 to coordinately maintain tumorigenic and stem-like properties. The interaction between Sox2 and DDX17 provides a novel mechanism underlying the functional dichotomy of BC cells, which carries potential therapeutic implications.

6. Determining the nuclear localization and targets of nuclear matrix metalloproteinase-2

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Matrix metalloproteinases (MMPs) are zinc-dependent proteases which are known to be involved in extracellular matrix remodeling associated with developmental processes and disease progression. MMP-1,-3,-9,-13,-14, and -26 have all been found in the nucleus. However the function and substrates of nuclear MMPs are mostly unknown. MMP-2 has a C-terminal nuclear localization sequence that is exposed on the protein surface. We hypothesize that MMP-2 is present in the nucleus under physiological conditions but increases during oxidative stress, proteolyzing structural and DNA repair proteins. Lamin A/C, a possible nuclear MMP-2 target, is an intermediate filament protein that provides structural support to the inner part of the nuclear envelope. It has newly identified functions: gene expression, DNA replication, transcription and repair. Cytosolic, membrane and nuclear fractions were extracted from isolated rat hearts that were perfused aerobically. Western blots for lamin A/C (nuclear marker), SERCA2 (membrane marker) and GAPDH (cytosol marker) were used to demonstrate the purity of the nuclear extracts. Gelatin zymography was used to determine MMP-2 activity in fractionated samples. The presence of nuclear MMP-2 was also examined by immuno-fluorescence confocal microscopy in the HT1080 fibrosarcoma cell line. Recombinant lamin A/C was incubated with MMP-2 for 0.5 hr at 37oC. Western blotting showed that the nuclear fraction was free of cytosolic and membrane contamination. Gelatin zymography of the nuclear extracts showed 64kDa cleaved form of MMP-2 in contrast to 72kDa full length MMP-2 in the cytosolic and membrane fractions. Lamin A/C recombinant protein was proteolyzed in vitro by MMP-2 in a concentration dependent manner and this proteolysis was prevented by the addition of an MMP inhibitor o-phenanthroline. MMP-2 is present in the nuclear fraction obtained from aerobic rat heart tissue and in intact nuclei of HT1080 cells. MMP-2 can proteolysel lamin A/C in vitro in a concentration dependent manner. Future experiments will focus on determining MMP-2 colocalization with nuclear bodies, determining nuclear substrates and further discovering the novel function inside the nuclei.
7. Protein and energy intake at recommended levels does not prevent weight loss in head and neck cancer patients

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Background: Patients with cancers of the head and neck (HNC) are at high risk for malnutrition. Information regarding the extent to which these patients meet protein and energy recommendations at various stages of the cancer trajectory is limited.

Objective: To relate energy and protein intakes at diagnosis, during and after radiotherapy treatment (RT) to weight loss in orally-fed HNC patients.

Subjects/Methods: HNC patients (n=38) undergoing RT were prospectively evaluated and completed 3-day food records at diagnosis, after 6 weeks of RT, and at post-RT (follow-up). At each time point, body weight and BMI were recorded; energy and protein intakes were calculated and compared to the ESPEN guidelines of 30-35 kcal/kg/d and 1.2-2 g protein/kg/d.

Results: The majority of patients lost >10% of total body weight (range 0.5-25%) from diagnosis to follow-up. At diagnosis, patients consumed an average of 1.3 g protein/kg/d, declining to 0.9 g protein/kg during treatment and improving at follow-up to 1.5 g protein/kg. Mean energy intakes fell from 30 kcal/kg/d at diagnosis to 23 kcal/kg/d (3.7-84.2 kcal/kg/d) during treatment, and increasing to 30 kcal/kg/d at follow-up. Mean weight loss of patients with protein intakes ≤ 1.2 g protein/kg/d at baseline (45%) through treatment was 11 kg (range 0.6-24.5 kg). Although 32% of patients met or exceeded energy intakes during treatment, 75% of these patients experienced a mean weight loss of 10 kg (0.6-24.5 kg). Ninety percent of patients who met or exceeded the minimum 1.2 g/kg/d recommended protein intake during treatment (32%) lost weight. Despite a restoration of protein and energy intake following treatment, patients continued to lose weight.

Conclusion: Consumption of calories and protein at recommended levels does not prevent weight loss in HNC. Evaluation of guidelines for nutritional support in HNC would be valuable.

8. Investigating J-Coupling Effects on Lipid Methylene Signal in Proton Magnetic Resonance Spectroscopy at 3 T

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Background: Fat to water ratios are a relevant biomarker to the study of cancer. Quantification of fat to water ratios is possible using in-vivo proton magnetic resonance spectroscopy (MRS) but can be complicated by J-coupling interactions of the lipid protons. J-coupling results in signal modulation as a function of echo time (TE), which is a timing parameter of standard in vivo MRS pulse sequences, making accurate quantification of the lipid signal difficult.

Objective: Previously, the effect of J-coupling on quantifying the lipid methyl (CH3) protons has been investigated. The objective of this research project is to modify MRS pulse sequence methodology to reduce J-coupling effects on MRS signal from lipid methylene (CH2) chain protons.

Design and Methods: MRS experiments were conducted on an oleic acid phantom solution using a Philips 3 T MRI scanner using a transmit/receive birdcage head coil. Two PRESS pulse sequences were used, one with standard refocussing pulse bandwidths, ≈550 Hz, and one with narrow bandwidth refocussing pulses, ≈50 Hz. The offset frequency of the pulses was set to that of the methylene protons. Spectra were acquired at a number of echo times and methylene peak areas were measured.

Results: Peak areas from both pulse sequences were plotted as a function of TE and the resulting data was fit to mono-exponentially decaying functions (standard decay in absence of J-coupling). Extrapolated initial magnetization and T2 (transverse) relaxation constants were 23% and 18% higher, respectively, when using the narrow bandwidth PRESS sequence.

Conclusions: We have shown that J-coupling effects are significant when quantifying lipid methylene protons. We will conduct further experiments on other phantom solutions before performing in-vivo scans.

Implications for Research, Practice or Policy: Improving quantification of lipid methylene chain protons will enable more accurate estimates of fat to water ratios in vivo.
9. The oncogenic role of RUNX3 in ovarian granulosa cell tumor (GCT) cells

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Introduction: Granulosa cell tumors of the ovary (GCTs) are the rare form of ovarian cancers which arise from sex cord-stromal cells. GCTs account for approximately 5% of all ovarian malignancies. There are two distinct subtypes of GCTs: the rare juvenile and the more common adult form. Although the prognosis is more favorable compared to the epithelial ovarian cancer, the advanced or recurrent tumors show poor diagnosis. Surgery is the predominant form of treatment. However, the viable treatment options are still limited for patients with advanced and recurrent GCTs. The molecular pathogenesis of GCTs remains poorly understood. Therefore, a better understanding of the molecular pathogenesis of GCTs will help us to develop more effective therapeutic strategies. RUNX3, a member of the RUNX family of transcription factors, regulates gene expression in a tissue-specific manner and plays either a tumor suppressing or an oncogenic role in a cancer-specific manner. Published work has shown that RUNX3 is an oncogene in epithelial ovarian cancer. It has been shown that RUNX3 gene promoter is hypermethylated and the expression of RUNX3 is lost GCT cells. The objective of this project is to investigate whether RUNX3 plays a role in GCTs.

Methods: SVOG (immortalized granulosa cell line), KGN (adult human GCT cell line) and COV434 (juvenile GCT cell line) are used for the study. KGN and COV434 cells retain many characteristics of primary GCT cells. RUNX3 expression in these cell lines was examined by Western blotting. We stably overexpressed RUNX3 in KGN cells which lack expression of the endogenous RUNX3. Because COV434 cells express a high level of RUNX3, we inactivate RUNX3 in COV434 cells by overexpressing a dominant negative form of RUNX3 (dnRUNX3). Overexpression of RUNX3 and dnRUNX3 was confirmed by Western blotting. Cell growth, colony formation, and migration were measured by the neutral red uptake assay, the soft agar assay and the scratch assay, respectively.

Results: Overexpression of RUNX3 significantly increases proliferation, anchorage-independent growth in soft agar and migration of KGN cells. By contrast, inactivation of RUNX3 by overexpression of the dnRUNX3 reduces COV434 cell growth.

Conclusion: Our results suggest an oncogenic role of RUNX3 in GCTs. Ongoing work focuses on the molecular mechanism underlying these effects of RUNX3. In the future, we will determine whether RUNX3 expression affects the tumorigenicity of GCT cells using mouse xenograft models.

10. Epigenetic role of hypoxia and Nodal in dysregulation of cancer stem cells

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Nodal, an embryonic morphogen, and hypoxia are two well-established factors that contribute to pluripotency in Embryonic Stem Cells (ESCs), but are also able to support cancer progression when its signalling pathways are dysregulated. Both of these factors have been shown to alter gene expression via epigenetic changes, and are crucial in the development of Cancer Stem Cells (CSCs), a subpopulation of cancer cells characterized by plasticity and the ability to self-renew. These characteristics afford CSCs with the ability to metastasize and resist therapies, leading to recurrence and reduced survival in patients.

Here we propose to address how the epigenome of CSCs respond to micro-environmental factors such as hypoxia and Nodal by examining the alterations in histone modifications in concert with the resulting transcriptional response. Cell lines that up-regulate stem cell sustaining genes such as Nodal in response to hypoxia will be used, namely H9 hESC and breast cancer cell lines, SUM149 and MCF7. To determine epigenetic changes, Chromatin Immuno-Precipitation (ChIP) with high throughput sequencing (ChIP-seq) will be conducted using antibodies to repressive (H3K27Me3) and active (H3K4Me3) histone marks, both of which were chosen for their association with hypoxia, Nodal, and regulation of the stem cell phenotype. RNA sequencing will be performed to match gene expression changes, after which PCR will be incorporated to validate ChIP-seq and RNA sequencing results.

Elucidating the role of histone modification alterations in the transcriptional response to hypoxia and Nodal, as well as the role of Nodal in hypoxia associated alterations, will better our understanding of how the microenvironment regulates CSCs and ESCs. Highlighting the similarities and differences of this regulation in CSCs and ESCs, and more specifically the dysregulation present in CSCs, may lead to the discovery of potential therapeutic targets.
11. The role of RUNX3 transcription factor and Wnt/β-catenin signaling in carboplatin resistance of epithelial ovarian cancer cells


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Introduction: Ovarian cancer is the leading cause of death due to gynecologic malignancies and the fifth leading cause of cancer-related mortalities in women. Epithelial ovarian cancer (EOC) is the major type of ovarian cancer, constituting approximately 90% of all ovarian malignancies. Despite the initial positive response to the current first-line treatment (platinum-paclitaxel combination), relapse occurs in most EOC patients and the recurrent disease is resistant to current chemotherapy regimens. Identifying the molecular mechanisms underlying chemoresistance will help to develop more effective therapeutic strategies.

Methods: EOC cell line A2780s (cisplatin-sensitive) and its derivative A2780cp (cisplatin-resistant) were studied using DNA microarray, ingenuity pathway analysis (IPA), quantitative real-time polymerase chain reaction (qRT-PCR), Western blotting, neutral red uptake assay, clonogenic assay and LEF/TCF-driven luciferase reporter assay. RUNX3 expression in human primary EOC cells and primary ovarian surface epithelium (OSE) cells was determined by Western blotting.

Results: The gene expression profile analysis showed that RUNX3 expression was elevated in cisplatin-resistant A2780cp cells compared to the cisplatin-sensitive counterpart A2780s cells, which was confirmed by qRT-PCR and Western blotting. Analysis of the microarray data from the Gene Expression Omnibus (GEO) database for RUNX3 and cisplatin resistance showed that RUNX3 expression was significantly higher in EOC tissues from chemoresistant patients compared to EOC tissues from chemosensitive patients. We also confirmed that RUNX3 expression was elevated in human primary EOC cells compared to primary OSE cells. Overexpression of RUNX3 rendered A2780s cells more resistant to carboplatin and overexpression of the dominant-negative RUNX3 (dnRUNX3) resulted in a moderate chemosensitization of A2780cp cells to carboplatin. Mechanistically, dnRUNX3 sensitizes A2780cp cells to carboplatin-induced apoptosis likely by decreasing the expression of the cellular inhibitor of apoptosis 2 (cIAP2). The gene expression profile analysis also suggested that Wnt/β-catenin signaling pathway was more active in A2780cp cells compared to A2780s cells, because the endogenous Wnt inhibitory proteins (DKK1, SFRP1 and FZRB) were down-regulated and Wnt ligands (WNT3, WNT11 and WNT3A) and Wnt target genes (JUN, CCND1, and AXIN2) were up-regulated in A2780cp cells. Using the LEF/TCF-driven luciferase reporter assay, we confirmed that β-catenin transcriptional activity was higher in A2780cp cells than in A2780s cells. Combined treatment of carboplatin and CCT036477 (a β-catenin inhibitor) was more effective in killing A2780cp cells than either agent alone.

Conclusions: Our data demonstrate that RUNX3 and Wnt/β-catenin signaling contribute to carboplatin resistance of A2780cp cells, suggesting that they could be potential therapeutic targets to treat resistant the disease.

12. Enteral nutrition improves micronutrient status but fails to improve protein intakes in patients with cancers of the head and neck

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Background: Patients with cancers of the head and neck (HNC) are at high risk for malnutrition and may be deficient in several nutrients simultaneously.

Objective: To compare intakes of folate, zinc, vitamins D and E, and protein in HNC patients consuming self-selected foods. Intakes at various points in the disease trajectory were compared to ESPEN guidelines (ESPEN-g) for cancer patients.

Subjects/Methods: HNC (n=38) patients undergoing RT had weight, BMI, protein, folate, zinc, vitamins D and E prospectively evaluated at diagnosis, after 6 weeks of RT (treatment), and 2.5 months post-RT (follow-up). Intakes of folate, zinc, vitamins D and E, and protein were compared to ESPEN-g. Nutrient intakes in patients consuming ≥15% daily caloric intakes from enteral nutrition (EN) (n=30) were compared to those not consuming EN (n=8).

Results: At baseline, >80% of all patients consumed below ESPEN-g for folate, and vitamins D and E, 50% of patients were below ESPEN-g for zinc, and 46% of all patients consumed protein below ESPEN-g. Protein intake decreased from a mean of 1.3 g/kg/d at baseline to 0.92 g/kg/d during treatment but was restored to 1.51 g/kg/d post-treatment. Throughout the study, folate and vitamin D intakes were considerably low, with over 60% of all patients well below ESPEN-g. During treatment, patients consuming ≥15% energy from EN increased their intake of folate (4.3%), vitamin D (30.1%), vitamin E (88.4%), biotin (308.5%), and zinc (2.2%) from baseline. Despite improving micronutrient intake during treatment, patients on EN consumed 34% less protein (0.82 g/kg/d) compared to patients not taking EN (1.24 g/kg/d).

Conclusion: Dietary interventions in HNC patients must consider the complete range of deficiencies observed throughout treatment. EN may not be adequate in meeting the nutritional needs of this population, most notably in protein intake.
Background: Pathological accumulation of fat in skeletal muscle (myosteatosis) has been recently identified in cancer patients and independently correlates with survival and treatment outcomes. Our laboratory has previously shown that 1) Fish oil, a concentrated source of EPA and DHA, prevents fat deposition in skeletal muscle during chemotherapy in an animal model for colorectal cancer and 2) Fish oil supplementation in cancer patients undergoing chemotherapy reduces intramuscular adipose tissue content upon treatment completion compared to a control group. The ability for fish oil to reverse fat accumulation once it has occurred has not been investigated.

Objective: To determine if a dietary fish oil intervention may reverse fat infiltration of muscles that occurs in the tumor bearing state and with successive chemotherapy cycles with irinotecan/5-fluouracil.

Methods: Fischer-344 rats bearing the Ward colorectal carcinoma were fed a semi-purified control diet and either received chemotherapy (n=32), or no chemotherapy (n=8). Upon chemotherapy initiation, rats remained on control diet (n=16), or began a fish oil diet (n=16) (2.3% w/w). Diets were isocaloric with equivalent fat content, differing only in fatty acid (FA) composition (fish oil diet contained n-3 FAs, EPA/DHA). Rats were killed before chemotherapy, after 1-cycle, or 2-cycles. Gastrocnemius muscles were isolated. Lipids were extracted, and triglyceride (TG) fractions separated by thin layer chromatography. Fatty acids were identified and quantified with gas liquid chromatography. Gastrocnemius muscles were sectioned with a cryostat, mounted and stained for neutral lipids with oil red O, followed by qualitative analysis.

Results: Compared to healthy rats (847.3 μg/g), tumour-bearing rats exhibited ~3-fold greater concentration of total-TG in muscle (2504.9 μg/g; p=0.001) which was visible in oil red O stained sections. After 1-cycle, muscle TG fatty acids of control-fed animals were 2-fold greater than fish oil-fed animals, and TG content in fish oil-fed animals resembled that of control animals receiving no chemotherapy; this findings was also visible in oil red O stained sections. After 2-cycles, control-fed rats exhibited a greater concentration of total-TG in muscle compared to fish oil-fed rats (p=0.001). Overall, TG content increased with succeeding chemotherapy cycles. Fish oil-fed animals had a greater proportion and concentration of EPA and DHA in TG-FA after both 1- and 2-cycles (p=0.001), which occurred concurrent with reduced TG-FA content and was supported by qualitative histology analysis.

Conclusions: A fish oil intervention during chemotherapy reverses fat accumulation in skeletal muscle of tumour-bearing rats. Fish oil supplementation during chemotherapy may attenuate tumour and chemotherapy-associated myosteatosis.

Therapeutic targeting of the rate-limiting steps in the metastatic cascade may be the key to ending cancer patient death, where it is thought that invasion out of the primary tumour and by metastatic lesions are the bottleneck steps.

To target these steps, we completed the first in vivo whole human genome shRNA screen for direct drivers of metastasis, using a novel intravital imaging platform in avian embryos developed by our group. We identified over 20 genes not previously linked to cancer metastasis, whose overexpression correlates with poor patient outcomes in publicly available datasets. Mechanistically, we found that knockdown of our top 4 genes blocks invasion of primary tumours & metastatic lesions in avian embryos and blocks spontaneous metastasis in mice.

Recently, we developed a Matlab-based software which quantifies cell density within cancer colonies, allowing us to rank our screen hits on their putative effects on metastasis. Through this analysis, we newly identified LAT51 tumour suppressor as a potential driver of metastasis. Using independent shRNAs, LAT51 was knocked down in human bone, breast, head & neck, and prostate cancer cell lines. In vitro proliferation and cell migration were then evaluated in these cell lines after demonstrating knockdown of LAT51 at the protein level using Western blot. Upon intravenous injection of shLAT51-HEP3 cells in shell-less avian embryos, highly compact yet large metastatic lesions formed due to inhibition of tumour cell motility. Further validation of LAT51 may elucidate novel signalling pathways controlling cancer cell migration and lead to development of specific anti-metastatic therapies.
15. Doxorubicin-induced oxidative stress activates intracellular matrix metalloproteinase-2 in human fibrosarcoma cells

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Introduction: Matrix metalloproteinases (MMPs) are a family of extracellular matrix proteases with important roles in both developmental processes and disease progression such as metastasis. MMP-2 can be secreted as a 72 kDa zymogen which is activated upon extracellular release by the proteolytic removal of its autoinhibitory domain to produce a 64 kDa MMP-2. In addition to its important roles in extracellular matrix remodeling, the Schulz lab was the first to show that MMP-2 can proteolyze intracellular targets including sarcomeric proteins. Intracellular MMP-2 can be activated in vitro by peroxynitrite-mediated S-glutathiolation of its Cys102 residue located on the propeptide domain. MMP-2 activity is involved in several heart pathologies resulting from increased oxidative and nitrosative stress including myocardial ischemia-reperfusion injury. Doxorubicin, a potent chemotherapeutic drug used for childhood and women’s cancer, is cardiotoxic in part because it stimulates peroxynitrite biosynthesis. Though effective for the treatment of cancer, cumulative dosing of doxorubicin may cause heart failure in patients undergoing chemotherapy.

Hypothesis: Doxorubicin-induced oxidative stress activates intracellular MMP-2 in both heart and cancer cells.

Methods: To test this, neonatal rat ventricular myocytes (NRVMs) and human fibrosarcoma cells (HT1080 cells) were treated with 0-1 μM doxorubicin for 0.5 and 2 h. MMP-2 activity was measured by gelatin zymography. MMP-2 protein levels were assessed by immunoblotting. Oxidative stress was measured by changes in mitochondrial aconitase activity. Cell death was measured by lactate dehydrogenase release.

Results: In HT1080 cells, 2 h treatment with 0.1-1 μM doxorubicin increased intracellular MMP-2 activity by 60% without any change in MMP-2 protein level. In NRVMs, doxorubicin did not significantly increase intracellular MMP-2 activity. However doxorubicin significantly increased oxidative stress as measured by aconitase activity in a concentration and time dependent manner. At the conditions used, doxorubicin did not induce significant cell death.

Conclusions: Our results support the hypothesis that oxidative stress, stimulated by doxorubicin, may activate intracellular MMP-2 by peroxynitrite-mediated post-translational modification. Doxorubicin-induced intracellular MMP-2 activity can cause intracellular remodeling, which may explain the cases of heart failure in patients undergoing chemotherapy. To visualize changes in intracellular MMP-2 activity in real time, future experiments will use a genetically encoded fluorescence resonance energy transfer (FRET)-based MMP-2 biosensor. This construct contains the MMP-2-selective cleavage site in troponin I genetically fused between two fluorescent proteins. The FRET-based biosensor will allow us to better understand how oxidative stress affects intracellular MMP-2 activity and its specific substrates.

16. CRISPR approach to knockout the DNA repair enzyme PNKP

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CRISPR, a targeted genome-editing tool has been successfully used to knock out genes (CRISPRi), among its various other applications. Using Cas9 and dCas9 (Cas9 with D10A and H840A mutations) and a library of PNKP targeting sgRNAs, we are attempting CRISPRi to knockout PNKP (polynucleotide kinase/phosphatase), a dual functioning DNA repair enzyme critical for maintenance of nuclear as well as mitochondrial DNA integrity. DNA lesions continually arise in both nuclear and mitochondrial DNA (mtDNA) due to various exogenous and endogenous DNA damaging agents, which can inflict a plethora of DNA lesions including DNA strand breaks. These agents often induce breaks with incompatible termini that need to be processed before insertion of the missing nucleotides and strand rejoining. PNKP is a dual functioning protein that contains both a DNA kinase domain to phosphorylate 5'-OH termini and a phosphatase domain to replace 3'-phosphate with 3'-OH termini. PNKP’s role in nuclear repair pathways such as single-strand break repair and non-homologous end joining for double-strand break repair has been well established. We have recently shown that full-length functionally active PNKP localizes in the mitochondria and is important for mtDNA integrity. We have previously shown that downregulation of total cellular PNKP increases spontaneous mutation frequency and sensitivity to radiation and other genotoxic agents. To study the role of mitochondrial PNKP, it will be necessary to achieve greater knockdown of PNKP than can be achieved by shRNA. By developing PNKP-knockout cell lines through CRISPRi, we intend to analyze the effect of genotoxic agents on mtDNA repair and cell survival. We are in the process of developing cell lines with localized expression of PNKP either in the nucleus or mitochondria only. We will examine the relative effects of loss of PNKP from each organelle on DNA repair and cell survival in cells challenged with various DNA damaging and chemotherapeutic agents (eg. IR, hydrogen peroxide, Camptothecin).
17. Regulation of Equilibrative Nucleoside Transporter 1

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Equilibrative nucleoside transporter 1 (ENT1) is a ubiquitously expressed membrane transporter in mammalian cells. Human ENT1 (hENT1) transport activity affects the extent of biological action of its endogenous substrates such as adenosine, as well as chemotherapeutic nucleoside analogues. In fact, hENT1 expression has been studied as a biomarker for individual’s response to pancreatic cancer drug, gemcitabine. Similarly, leukemia pro-drug cytarabine, is taken up into cells by hENT1, where it is phosphorylated into its active form. hENT1 activity is regulated by various endogenous factors. Cells undergoing metabolic stress catabolize ATP to adenosine, which is then transported out of the cell by hENT1 to act on cell surface adenosine receptors and initiate stress-mitigating signalling cascades. Previous studies have implicated protein kinase C (PKC) in the hENT1 regulatory pathway. To determine the role of PKC in the regulation of hENT1 expression and activity, we performed [3H]niribenzylthioinosine (NBMPR; a probe for ENT1) binding and [3H]2-chloroadenosine uptake assays on PK-15 NTD (Nucleoside Transport Deficient) cells, stably expressing wild type hENT1 and S281A-hENT1 (serine 281 has been identified as a canonical PKC phosphorylation site). Our data shows that direct activation of PKC by PMA or indirect activation by the adenosine A1 receptor agonist, 2-chloro-N(6)-cyclopentyladenosine (CCPA), led to a significant increase in the binding of [3H]NBMPR and maximal uptake rate of [3H]-2-chloroadenosine in wild-type hENT1 cells. This effect of PMA on hENT1 activity was not observed when using the S281A-hENT1 cells. To determine whether the increase in hENT1 activity induced by PMA and CCPA was caused by changes in cell surface expression of hENT1, we performed immunocytochemical analyses and cell surface biotinylation experiments. These experiments showed that PKC activation by PMA, but not CCPA, led to a significant increase in the plasma membrane localization of WT-hENT1. However, contrary to what might be expected from the functional activity assays, S281A-hENT1 cells also responded to PMA treatment with an increase in the cell surface expression of the protein as measured by biotinylation ratios. Taken together, our findings suggest that 1) Ser 281, located in the intracellular loop of hENT1 is involved in the regulation of hENT1 activity by PKC; 2) Additional serine/threonines exist in hENT1 as potential PKC targets that contribute to the effect of PMA on hENT1 trafficking to the plasma membrane. 3) Activation of adenosine A1 receptors on the cell surface initiate a regulatory feedback loop that affects hENT1 activity but not hENT1 trafficking. Further investigation regarding hENT1 membrane expression, regulation of trafficking and translocation activity is necessary for optimal use of chemotherapeutic agents that are transported by hENT1.

18. Cancer Cell Inhibition of the Cell-to-Cell Spread of Oncolytic Reovirus

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Reovirus is a dual-capsid, non-enveloped, dsRNA virus that causes pathologically benign infections of the gastrointestinal and respiratory tracts in humans, with a widespread seropositivity of roughly 50-100% by adulthood. Reovirus preferentially infects and replicates in cells with constitutively activated Ras oncogene signalling, which accounts for approximately 80% of all human tumours. As a result of this tropism reovirus is being evaluated as an oncolytic therapy, however, results from clinical trials and current research suggest that Ras-activating mutations do not always confer susceptibility to reoviral infection. Progression of reovirus infection in lung cancer cells (including H1299, A549, and H332) and head and neck squamous cell carcinoma cells (HNSCC; such as SCC9 and A253) was evaluated using a cell-based ELISA assay to quantify reoviral protein levels. Comparisons of infection at 18 versus 48 hours post-infection identified a subset of cancer cell lines permissive to initial infection, yet 8-90 times less permissive to secondary cell-to-cell spread of reovirus relative to the susceptible control, H1299. We hypothesize that some cancer cells resist reovirus dissemination because they produce antiviral factors (e.g., type I interferons) that protect neighboring cells from secondary rounds of infection. A549 lung cancer cells (known to respond to type I interferons) were used to assess the presence of antiviral factors in media of reovirus-infected cells. The media from cell lines less susceptible to reovirus dissemination such as H332, SCC9, and A253 conferred a protective effect upon A549 cells at lower doses of treatment than media from cell lines that are highly susceptible to reoviral spread, suggesting that H332, SCC9, and A253 cells may produce greater amounts of antiviral cytokines. To investigate the identity of the main antiviral molecules produced by resistant cancer cells, antibody neutralization experiments against type I interferons and shRNA knockdowns of the IFN pathway are currently underway. Additionally, comparative titre assays were used to quantify the extent of reovirus release from infected cells, since cell death plays an important role in reoviral spread. In the first 24 hours, H332 and A253 cells released approximately 12 times less virus relative to H522 permissive lung cancer cells, suggesting that limited virus egress may be part of their resistance toolbox. Further experiments are being undertaken to assess the importance of limiting early reovirus release. We propose that limited viral release and the production of antiviral factors likely contribute to resistance to reovirus spread exhibited by some cancer cells.
19. An Optimized Magnetic Resonance Spectroscopy PRESS Sequence for the Detection of Glycine at 9.4 T

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Background: The levels of glycine (Gly), an inhibitory neurotransmitter, are relevant to the study of cancer. Proton magnetic resonance spectroscopy (MRS) can be used to observe Gly levels by quantifying its resonance peak at 3.55 ppm. The point resolved spectroscopy (PRESS) sequence is a commonly employed technique in in-vivo MRS. The PRESS sequence consists of two echo times, termed TE1 and TE2, the sum of which is the total TE. The strong spectral overlap of peaks from myo-inositol (mI) makes the detection and measurement of Gly difficult.

Objective: A long-TE PRESS sequence can be optimized to significantly reduce mI signal in the Gly spectral region by exploiting the J-coupling evolution of mI protons. The objective of this work is to investigate the PRESS {TE1, TE2} signal dependence of mI at 9.4 T to find optimal echo time combinations that sufficiently suppress mI so that Gly levels can be measured more accurately.

Design and Methods: PRESS spectra were acquired from three spherical phantom solutions. Several {TE1, TE2} combinations were used on a 50 mM mI/10 mM Creatine (Cr) phantom. The optimal {TE1, TE2} combination for Gly detection was chosen by examining the 3.52-3.57 ppm mI spectral region and selecting the timing set which yielded a spectrum in which the mI amplitude in the mentioned spectral region was minimal. The optimal timing set was verified on a 10 mM Cr/10 mM Gly/50 mM mI phantom and in vivo on rat brain.

Results: An optimal echo time combination was determined to be {60 ms, 100 ms}. The maximum amplitude and total area of the mI signal in the 3.52-3.57 ppm region is 5.2 % and -6.1 %, respectively, of the corresponding values obtained from the short-TE spectrum. Phantom results showed that the Gly lineshape was relatively unaffected by the presence of mI at the optimal TE; the Gly/Cr area ratio was altered by ≈ 11 % with the introduction of mI when {TE1, TE2} = {60 ms, 100 ms}. The efficacy of the optimized PRESS sequence was verified in vivo on rat brain. The rat brain spectrum showed a Gly peak at 3.55 pm.

Conclusions: We have shown that a PRESS sequence with {TE1, TE2} equal to {60 ms, 100 ms} is suitable for resolving the Gly signal (~3.55 ppm) from overwhelming, overlapping mI signal at 9.4 T.

Implications for Research: The optimization of the PRESS sequence allows for further in-vivo studies of Gly at 9.4 T.

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20. Effect of Human Platelets on Lung Cancer Stem Cell Invasion

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Purpose: According to the cancer stem cell theory of cancer origin, a small population of cancer cells within the tumor bulk has stem cell-like characteristics (CSCs) and is responsible for initiating new tumors following metastasis. Numerous studies have shown that platelets contribute to metastasis, in part by stimulating cancer cell migration via release of chemokines from platelet granules. One of the chemokines released from platelet granules is stromal derived factor-1α (SDF-1α), which is known to mobilize both bone marrow and cancer stem cells via increased matrix metalloproteinase (MMPs) expression. Hence, we hypothesize that activated platelets preferentially induce CSC migration by releasing SDF-1α which then binds to its receptor CXCR4 on CSCs leading to increased MMP production and invasion. Methods: Platelet releasates were collected from collagen-activated human platelets isolated from healthy donors. CSCs were identified within the A549 human lung carcinoma cell line via flow cytometry by staining for Hoechst 33342-negative side population (SP). Total and cancer stem cell MMP-dependent invasion were measured via a modified Boyden Chamber assay in response to platelet releasates. Pharmacological inhibitors AMD3100 and GM6001 were used to investigate the significance of SDF-1α signalling and MMP-dependence during migration. CD133 staining was used to validate CSCs identified by Hoechst negative SP. Results: Platelet releasates preferentially promoted the invasion of A549 CSCs (4.3 ± 0.3% pre-invasion vs. 7.6 ± 0.7% post-invasion SP cells, P < 0.05). Pharmacological inhibition using AMD3100 (10μM) and GM6001 (10μM) demonstrated only AMD3100 (10μM) significantly inhibited total A549 cell invasion (21.38 ± 7.86 x103 cell invaded without vs. 25.15 ± 8.04 x103 with AMD3100, P<0.05). Neither inhibitor significantly decreased SP-identified CSC invasion. Validation of Hoechst SP as CSC marker by CD133 staining showed that although CD133 positive cells are enriched in the SP, only 3.84% Hoechst negative SP cells are also positive for CD133 (1.45 ± 0.59 % in total A549 population vs. 3.84 ± 1.09 % in SP, P<0.05) Conclusions: Activated human platelets preferentially stimulate the invasion of SP-identified cancer stem cells within the A549 human lung carcinoma cell line. Identification of CSC based on both CD133 staining and Hoechst negative SP might be more reliable than using Hoechst SP alone. Further experiments are required to delineate the role of SDF-1α-CXCR4-MMP signalling in platelet-stimulated cancer stem cell invasion.
Introduction: Breast cancer is the most common disease diagnosed amongst Canadian women. Currently, taxane-based chemotherapy is provided for early and metastatic breast cancer patients; however, chemotherapeutic resistance is a major clinical problem with an estimated 50-70% of patients witnessing toxic side effects, yet receiving no benefit from treatment. Identification of biomarkers that predict patient response to taxane therapy would be a major advance in the clinical management of breast cancer.

Our lab examines the pro-apoptotic protein Bcl-2-associated death promoter (Bad), a BH3-only protein of the Bcl-2 family and we have identified Bad as a strong, independent prognostic indicator for disease-free and overall survival of breast cancer patients after taxane chemotherapy. Therefore, it is critical to understand the function of Bad in breast cancer.

Bad is a phospho-protein with conserved serine residues, which have been shown to regulate Bad activity. Its pro-apoptotic activity is apparent when the Ser118 site is dephosphorylated and has the ability to bind and inhibit inhibitors of the mitochondrial apoptotic machinery. However, previous data in our lab suggests the Ser118 site, when phosphorylated, causes Bad to exhibit a proliferative function instead. Understanding the novel proliferative function of Bad in breast cancer is the goal of this project.

Methods: Stable cell lines expressing three Bad mutants (S118A, S118D, and S99A/S118D) in the MDA-MB-231 breast cancer cell background were created to directly test the functional role of phosphorylation at S118. These experiments include cell counts and colony formation assays to examine differences in proliferation, co-immunoprecipitation experiments to examine Bad-binding partners, and western blot analysis of the phosphorylation status of Bad. As well, in vivo tumor growth assays were performed in the subcutaneous flanks and mammary glands of mice. Immunohistochemistry was performed on the tumors to measure proliferative function, regulated cell death, and the vascularization of the tumors.

Results: Our results indicated that phosphorylation of Ser118 on Bad is important for proliferation, as well as downstream phosphorylation of other conserved serine residues, Ser75 and Ser99. Phosphorylation of S99 is necessary for Bad proliferation and binding to 14-3-3 proteins. 14-3-3 proteins sequester Bad into the cytosol and away from the mitochondria. S118D Bad reveals increased proliferation and vascularization, and decreased cell death in vivo.

Conclusion: Understanding the proliferative function of Bad will aid in our understanding of why patients with higher levels of Bad respond better to taxane chemotherapy. Further studies will uncover the molecular mechanism of Bad regulation.

DEAD box 1 (DDX1) is a member of the DEAD box RNA helicase family that function through alteration of the secondary structure of RNA molecules. DDX1 is co-amplified with MYCN and over-expressed in a subset of retinoblastoma and neuroblastoma tumors and cell lines. Recently, over-expression and mislocalization of DDX1 has been shown to be associated with a poor prognosis in breast cancer.

We have found that DDX1 is recruited to a subset of DNA double-strand breaks within minutes of cells being exposed to ionizing radiation. Recruitment of DDX1 is dependent on functional ATM. Treatment of cells with RNase H disassociates DDX1 from DNA double-strand breaks, implying that DDX1 is recruited to sites of DNA damage containing RNA-DNA structures. DDX1 has single-stranded ribonuclease activity as well as ADP-dependent RNA-DNA and RNA-RNA unwinding activities.

To gain further insight into the role of DDX1, we performed co-immunoprecipitation using anti-DDX1 antibody followed by mass spectrometry analysis to identify DDX1-interacting proteins. Rif1 (Rap1 interacting factor 1) was identified as a binding partner of DDX1 and shown to co-localize with DDX1 before and after DNA damage. Using truncated Rif1 proteins, we found that Rif1 interacts with DDX1 through its C-terminal domain. Furthermore, Rif1 is required for DDX1 accumulation at DNA double-strand breaks. Knockdown of DDX1 levels by RNA interference led to impaired cell survival upon exposure to ionizing radiation and defects in homologous recombination repair, a pathway that is required to repair DNA double-strand breaks in an accurate manner. Our results suggest a role for DDX1 in DNA double-strand break repair by promoting homologous recombination.
23. Regulation of the Bmi1 and Ret proto-oncogenes by the DLX2 transcription factor in the developing gastro-intestinal tract

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Introduction: Colorectal cancer is responsible for the second most deaths attributed to cancer. Mutations in the genes responsible for regulating cellular proliferation in the GI tract account for increased susceptibility to this cancer type. Within the intestinal crypts of Lieberkuhn there is a stable, non-dividing stem cell group marked by the oncogene BMI1. The role of these cells is to maintain the epithelial cell lining of the intestine, which undergoes rapid turnover and cannot replace itself mitotically. Unpublished work from the Eisenstat lab demonstrates co-expression of BMI1 and the homeobox transcription factor DLX2 in intestinal crypts. Additional unpublished data support a regulatory role of DLX2 in the expression of the Ret proto-oncogene. The Ret gene is responsible for enteric nervous system (ENS) development. When over-expressed, Ret induces cancers associated with Multiple Endocrine Neoplasia, and Ret loss-of-function leads to Hirschsprung’s Disease characterized by partial or complete loss of intestinal innervation. We investigated the potential for a regulatory effect of DLX2 on both Bmi1 and Ret, with the hypothesis that DLX2 suppresses Bmi1 expression while promoting Ret expression and these interactions are due to direct binding of the targets’ promoters by DLX2 during intestinal and ENS development.

Methods: We investigated interactions between DLX2 and target promoter regions in vivo through Chromatin Immunoprecipitation (ChIP) using our high-affinity DLX2 antibody. Electrophoretic mobility shift assays (EMSA) in combination with Site Directed Mutagenesis of DLX2 binding sites are used to determine the direct binding of the Bmi1 or Ret promoters by recombinant DLX2 in vitro. Ongoing reporter gene assays are being used to determine the effect that DLX2 has on Bmi1 or Ret expression in vitro. Ongoing immunohistochemistry and qRT-PCR assays using Dlx1/Dlx2 double knockout (DKO) mouse-derived tissues are used to determine the role of DLX2 in Ret and Bmi1 expression in vivo.

Results: We demonstrated that DLX2 interacts with both the Bmi1 and Ret promoter in several regions of interest in vivo. EMSA results demonstrate specific binding of DLX2 to the Bmi1 promoter in vitro. Conclusion: ChIP results confirm occupancy of the Bmi1 and Ret promoters by DLX2 while EMSAs demonstrate direct binding of DLX2 to the promoter of Bmi1 in vitro. Future studies, including in vivo gene expression studies comparing wild type expression in the Dlx1/Dlx2 double knockout mouse to the wild type will confirm the biological relevance of the in vitro results.

24. Comparison of Tooth Loss Between Intensity Modulated and Conventional Radiotherapy in Head and Neck Cancer Patients

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Objective: Advanced radiotherapy (RT) such as intensity modulated radiotherapy (IMRT) have become more common in the management of head and neck cancer (HNC). IMRT includes focused target volume coverage while sparing salivary tissues to protect function. However, long-term effects on dentition after IMRT are not well established. This investigation sought to understand dental status by comparing tooth loss after IMRT and conventional RT in HNC patients.

Methods: A retrospective chart review was conducted on individuals who received IMRT or conventional RT (+/− surgery, +/− chemotherapy) for oropharyngeal (OP), oral cavity (O) and nasopharyngeal (NP) cancer between 2000 and 2010 at the Institute for Reconstructive Sciences in Medicine (iRSM). Tooth loss, the primary outcome measure, was assessed using intraoral photographs, radiographs and clinical records. The influence of patient demographics on tooth loss was assessed as well.

Results: Eighty-six patients were eligible for review at baseline: 44 received IMRT and 42 received conventional RT. Twenty-four had data collected up to two years after RT. After adjusting for baseline number of teeth, no significant differences were found between groups two years after RT using RM-ANCOVA (p=0.079.). Site of disease was significantly different between groups.

Conclusion: No statistically significant differences in tooth loss between RT groups were found two years after RT; however, trends in the data suggest that tooth loss increased each year after RT. The early findings need to be viewed with caution, as data beyond three to five years as well as a larger sample size is needed to understand the dental effects after advanced RT.
25. Update on a pilot study of the feasibility and preliminary efficacy of a pre-surgical exercise intervention in rectal cancer patients

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Background: Standard treatment for patients with locally advanced rectal cancer involves 5-6 weeks of neoadjuvant chemoradiotherapy (NACRT) followed by definitive surgery 6-8 weeks later. Unfortunately, NACRT is associated with declines in physical fitness that may impede treatment completion, treatment response, symptom management, and postoperative recovery. To date, no study has examined the feasibility of an aerobic exercise intervention for improving outcomes in rectal cancer patients receiving NACRT.

Objective: The purpose of this pilot study is to assess the safety, feasibility and preliminary efficacy of an aerobic exercise intervention during NACRT and prior to definitive surgery in rectal cancer patients.

Methods: Rectal cancer patients scheduled to receive NACRT are screened for eligibility by radiation oncologists and the study coordinator at the time of their first radiation consultation at the Cross Cancer Institute. All patients are provided with a structured aerobic exercise program from the initiation of their NACRT until their surgery. The exercise training consists of 3 supervised moderate intensity sessions per week during NACRT with the option of continuing with the supervised exercise program or completing an unsupervised exercise program prior to surgery. Patients undergo physical fitness testing and complete a questionnaire prior to starting NACRT, one week after NACRT, and one week prior to their scheduled surgery.

Discussion: Recruitment is now closed for the study but intervention delivery and follow-up continues. Here we report the current flow of participants through the trial and some of the baseline characteristics of the sample. Between April 17 and October 23 2014, 45 patients were assessed for eligibility and 31 (69%) were eligible. Recruitment rate for the trial was 55% (17/31). Reasons for ineligibility include; ischiorectal and perirectal abscesses, bipolar/schizophrenia disorder, the treatment decision being made too late, and patients above the age of 80. Reasons for refusal include; working, fatigue, and being too overwhelmed at time of consult. Of the 13 patients baseline tested to date, 85% have stage III rectal cancer, 92% were/are being treated with oral capecitabine chemotherapy, 69% are males and the mean age is 59. We are collecting valuable information that will aid in the design of future exercise intervention studies for rectal cancer patients.

26. Cyclotron produced 99mTc at the University of Alberta Medical Isotope and Cyclotron Facility (MICF)

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Objectives: With approximately 50 million doses administered per year, the most commonly used medical isotope in clinical decision making is Tc-99m. The current supply chain relies on a handful of ageing nuclear reactor sites across the globe. There have been several major Tc-99m supply interruptions to the healthcare system in recent years, and Canada’s NRU reactor is scheduled to cease medical isotope production in 2016. Therefore, the direct production of Tc-99m using a TR24 cyclotron (Advanced Cyclotron Systems International) is being explored. A cyclotron is a particle accelerator – i.e. a completely different technology for making the same Tc-99m end product. To this end, our objective is to demonstrate and produce a commercially viable supply of this key medical isotope at the University of Alberta.

Methods: The cyclotron production of Tc-99m entails the proton irradiation of a “target” of a stable isotope – i.e. Mo-100. A small fraction of the Mo-100 is transmuted to Tc-99m. Chemical processing is then required to dissolve the Mo-100/Tc-99m product. Design of the target and chemical extraction are both two major challenges that we have overcome. In order to produce the targets to be irradiated, Mo-100 powder is rolled on a mill to produce thin metal foils. The foils are annealed and pressed on aluminum target supports. The targets are transferred to the cyclotron using an automated transfer system, and subjected to irradiation up to 500 µA. The irradiated Tc-99m/Mo-100 target is then dissolved off the target support in hydrochloric acid, followed by ammonium carbonate. The solution of is purified using an automated synthesis unit by solid phase extraction chromatography and subsequent purification using an alumina column.

Results: The Mo-100 targets on the aluminum target support were able to withstand up to 500 µA irradiation currents (n=8). Based on the yields from these experiments we expect to be able to produce 1.5 TBq of Tc-99m from a 6 hour irradiation. Targets can be easily dissolved within 30 minutes, and Tc-99m recovery experiments using reactor Tc-99m gave a purification recovery of >98% (n=3), and passed all applicable QC tests. These results indicate that we can produce large amounts of Tc-99m on a TR24 cyclotron and we have a high yielding separation system which can deliver clinical quality Tc-99m.
**27. Theranostic evaluations of bioreductively-activated prodrugs for the management of hypoxic solid tumors**

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Background: Hypoxic solid tumors are resistant to conventional radio- and chemotherapy (due to impaired drug delivery) interventions. Therefore tumor hypoxia presents significant human health challenges and contributes to poor overall survival of cancer patients. Tirapazamine (TPZ) is a highly potent hypoxia-selective clinical drug that was used in several clinical trials, but was withdrawn from the clinic due to its severe neurotoxic manifestations and poor patient population selection. We intend to pursue the translational explorations of glucose-TPZ-based conjugates to evaluate their potential in future clinical management of solid tumors in cancer diseases.

Overall objective: We are developing TPZ-glucose conjugates and the corresponding radiohalogenated pharmaceuticals to transform them into multimodal theranostic drugs for the management of hypoxic solid tumors. Glucose moiety will facilitate their transport (through upregulated glucose transporters) in hypoxic cancer cells whereas TPZ will bioreductively activate in hypoxic atmosphere selectively and bind to cytoplasmic macromolecules therein to impart four-fold theranostic features. Healthy cells are oxygenated therefore the drug will not be retained therein, and minimal toxicity will be experienced by them. Overall, the goal is to carry out preclinical translational studies to validate the theranostic potential of our conjugates for the management of hypoxic tumors.

Hypothesis: Our prodrugs, when containing a diagnostic radionuclide (e.g., F-18, I-124), will allow diagnosis of hypoxic tumors by positron emission tomography (PET) and, when labelled with a therapeutic radioiodine (e.g., I-131), will impart hypoxia-targeted in situ molecular radiotherapy (MRT). The drug can also be used as a radiosensitizer and possible chemotherapy agent. The aim is to provide better theranostic options for cancer patients diagnosed with therapy-resistant solid tumors.

Experimental Approach: Initial studies will be carried out on selected cancer cell lines to validate the hypothesis, followed by pre-clinical studies in hypoxic tumor-bearing animal models. After determination of the pharmacodynamics and kinetics of the drug’s expression, cytotoxicity will be checked in-situ and in-vitro. Basically, validation of a series of theranostic studies will determine their multimodal anti-cancer potential and provide a basis for future clinical trials with curative response.

Potential Outcome and Value: Our innovation contributes to the diagnosis and treatment of hypoxic solid tumors leading to improved patient care, reduced morbidity and mortality, substantial health care cost savings, and thereby consequently improving the quality of life of cancer patients.

**28. Docosahexaenoic acid and doxorubicin act synergistically to disrupt cell cycle and increase cell death of MDA-MB-231 breast cancer cells**

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It is well established that long chain n-3 polyunsaturated fatty acids reduce viability of human breast cancer cells, however cellular mechanisms are not clearly understood. We have previously demonstrated that treating human breast cancer cells with the fatty acid docosahexaenoic acid (DHA) prior to treatment with doxorubicin (Dox) significantly increased the action of the drug (as measured by cell death and apoptosis). The objective of the current study was to explore how pre-treating MDA-MB-231 human breast cancer cells with DHA followed by Dox treatment alter gene expression. Cells were treated with DHA (60 µM) in control medium (containing 40 µM oleic acid / 40 µM linoleic acid (OALA)) or control media alone for 48 hours and then treated for 24 hours with Dox (4.1 x10-7 M, a concentration previously found to reduce cell number by 80%). RNA was extracted for microarray analysis (Affymetrix GeneChip Human Gene 2.0). A selection criterion of p≤0.05 and fold change ≥1.5 was set to define up or down regulated genes compared to cells incubated only with the control media (and no dox) (using Ingenuity Pathway Analysis software). Preliminary analysis found that DHA+Dox treated cells had more genes up or down regulated (155 and 184 respectively) than Control+Dox treated cells (131 and 150). Two canonical pathways found to have multiple genes up or down regulated by the combined DHA+Dox were cell cycle and cell death. More specifically key genes involved in cell cycle were down regulated with DHA+Dox treated cells, but not Control+Dox including Cyclin B1 (-2.1), CDC25C (-1.8) and WEE (-1.5). Genes in the apoptosis pathway were also assessed. For DHA+Dox treated cells, CD95 (1.3), TNFSF15 (2.8) and Caspase 10 (1.3) were up regulated and BCL2 (-1.3) down regulated. Changes in gene expression were confirmed by protein quantification using western blot analysis. Our results suggest that treating MDA-MB-231 cells with DHA may be improving the cytotoxic effects of Dox through several cellular pathways (cell cycle regulation and apoptosis).
Objective: Success of an anticancer treatment often depends on its ability to induce apoptosis in tumor cells; therefore, a molecular imaging agent that can detect and image apoptosis non-invasively would provide important information on treatment efficacy. Phosphatidylserine (PS), a membrane phospholipid that is externalized to the outer leaflet of the cell membrane during the early stages of apoptosis, is a particularly promising target for such an agent. The most common PS-binding ligand used to identify apoptotic cells in vitro is the protein annexin V; however, its use as a non-invasive molecular imaging agent is limited by its dependency on extracellular calcium, short blood half-life (less than 7 min), and poor uptake into solid tumours. Here we report the application of a novel radiometric binding assay using copper-64 ([64Cu]-labeled annexin-V as radiotracer in order to investigate various phosphatidylserine (PS)-binding peptides as potential leads for the development of an apoptosis-detecting molecular imaging agent.

Methods: [64Cu]-labeled annexin-V ([64Cu][Cu-NOTA-annexin-V) was used in a radiometric binding assay to screen various PS-binding peptides. PS-binding peptides were analyzed for their inhibitory potency towards PS, with unlabeled annexin-V used as an internal reference. Half maximum inhibition constants (IC50) were then calculated.

Results: As expected, unlabeled annexin-V displayed the highest inhibitory potency to compete with [64Cu][Cu-NOTA-annexin-V binding to immobilized PS (IC50 52 nM). Several hexapeptides exhibited IC50 values in the range of 1-15 mM, whereas 14mer peptides based on PS-binding motif FNFKAKAGKIRG (PSBP-6) displayed more favorable potencies (IC50 9-600 µM). We are now investigating the applicability of PSBP-6 as an in vivo apoptosis-detecting tracer. To develop PSBP-6 as a molecular imaging agent, the peptide is first conjugated with 1,4,7-triazacyclononanetriacetic acid (NOTA), a metal chelating group. NOTA-conjugated PSBP-6 can then trap positron-emitting radioisotope gallium-68 (68Ga3+) to produce [68Ga]NOTA-PSBP-6. We have investigated the in vivo metabolic stability of [68Ga]NOTA-PSBP-6 in mice, and found that 36% of the tracer remains intact after 60 minutes in the blood, determined by HPLC analysis of blood plasma samples. [68Ga]NOTA-PSBP-6 resides primarily in the blood plasma rather than blood cells or proteins, demonstrating a good blood distribution profile.

Conclusion: The novel radiometric binding assay with [64Cu][Cu-NOTA-annexin-V as radiotracer can be used as a versatile tool to screen compounds for their PS-binding potency. Favorable inhibitory potency of PSBP-6 has prompted us to investigate this peptide as a potential lead for the development of a PET radiotracer for Ca2+-independent molecular imaging of PS as a biomarker of early apoptosis.

30. Application of Quantitative Metabolomics in Cancer Biomarker Studies


The Metabolomics Innovation Centre, Department of Computing Science, Faculty of Science, University of Alberta

Increasingly it is being realized that cancer is fundamentally a metabolic disorder (characterized by cellular aerobic glycolysis and glutaminolysis) driven by genetic mutations at key metabolic control points. This makes metabolomics ideally suited for uncovering the metabolic basis of cancer and identifying novel treatments, novel targets or novel diagnostic/predictive biomarkers. The Metabolomics Innovation Centre (TMIC) is Canada’s national platform for metabolomics research and services. TMIC has several mandates including the provision of low-cost metabolomics services to academic and industry researchers, the maintenance of freely available metabolomics databases and web servers (HMDB, DrugBank, T3DB, MetaboAnalyst) and the development of innovative metabolomics technologies for a wide range of applications, including cancer and human health. TMIC specializes in developing quantitative metabolomics assays on human, animal, plant and microbial samples using a wide range of technologies including NMR, GC-MS, GCXGC-TOF, LC-MS/MS, LC-FT/MS, HPLC-UV/FD, ICP-MS and HPLC-ELSD-FAMES-MS. We will describe comprehensive approaches for absolute quantitation and untargeted profiling of tens to hundreds of metabolites in biofluids utilizing in-house developed methods, technologies and bioinformatics tools. We will also describe novel mass spectrometry-based tissue imaging approaches by MALDI-FTMS. This technique can be used to image up to 800 metabolites, including lipids and nucleotides, in tissues. Identification of biomarkers for cancers and human health conditions will be presented. Bioinformatics resources, including the Human Metabolome Database, and Small Molecule Pathway Database will be described as tools to assist in biomarker discovery, validation and translation. Metabolomics holds great promise for improving cancer diagnoses and contributing to an improved understanding of cancer biology, treatment and prevention. TMIC provides analytical services, bioinformatics support and collaborative opportunities for the discovery of novel biomarkers in cancer.
**Poster Presentations**

### 31. Synthesis of Thermosensitive Galactose-Decorated Nanogel for Targeted Drug Delivery of Iodoazomycin Arabinoside (IAZA) to Hypoxic Hepatocellular Carcinoma

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Hepatocellular carcinoma, more commonly known as liver cancer, is currently the leading cause of all cancer deaths worldwide, mostly affecting Southeast Asian and African communities, with more than half a million new patients being diagnosed each year. While earlier stages of the disease can be treated by modern surgical excision, more advanced stage of the disease, such as the development of hypoxic regions, generally leads to poor prognosis and high mortality. Hypoxic regions within tumors present a huge challenge for researchers, as these changes in the microenvironment promote the progression to a more metastatic phenotype and increase the resistance 2-3 fold to the radio/chemotherapeutic treatments. Therefore, a novel molecular imaging and multimodal therapeutic approach to manage these hypoxic tumor cells will turn hypoxia from problem to advantage, which would be of great clinical significance to provide personalized therapy and improve patients survival.

Over the past decade, several hypoxia-specific imaging agents have been developed and tested in preclinical and clinical settings, however nitroimidazole-based bioreductively activated molecules have been extensively studied. They have the ability to bind to subcellular macromolecules within viable hypoxic tissues with a linear relationship to decreasing oxygen concentration to create desired theranostic (therapy-diagnostic) effects. Mechanistically, nitroimidazoles sensitize hypoxic tumors to the killing effects of ionization radiation by mimicking oxygen within the cell, thus generating reactive oxygen species in hypoxic environments. Radioiodinated Iodoazomycin Arabinoside (IAZA), a 2-nitroimidazole-based nucleoside, has been developed in our lab that has demonstrated serious clinical potential in imaging cancer patients diagnosed with solid tumors. IAZA however demonstrates some deiodination under physiological conditionand non-specific interactions with circulating blood proteins that need to be overcome to maximize its theranostic benefits.

Recently, nanogels have demonstrated to be efficient drug-carrying scaffolds due to their highly tunable surface, multivalent ligand display, biocompatibility and targeted payload delivery. Interestingly, much research has shown that hepatocytes display an overexpression of an endocytic cell surface receptor called the asialoglycoprotein receptor (ASGPR). The primary physiological role of ASGPR in hepatocytes is the receptor-mediated binding, clathrin-coated vesicle internalization and trafficking to the lysosome for degradation of asialoglycoproteins and glycoproteins containing terminal galactose and N-acetylglucosamine moieties. By exploiting this physiological characteristic, we have developed a thermosensitive galactose-decorated nanogel for the targeted delivery of IAZA to hypoxic hepatocellular carcinoma, which will maximize its theranostic effects. Related evaluations are being carried out.

### 32. Smoking and risk of colorectal adenoma and hyperplastic polyps in Japanese Brazilians

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Background & Aims: Substantial differences in colorectal cancer (CRC) incidence and mortality between Japanese Brazilians and Japanese living in Japan or Hawaii have been reported over the past decades. Variations in environmental risk factors for CRC are being investigated as a reason for these differences. In a colorectal adenoma study in São Paulo, Brazil, participants with Japanese ancestry were assessed for their smoking history in two general hospitals (Nipo-Brasileiro Hospital and Sociedade Beneficente de Cotia Hospital).

Methods: Based on pathology and colonoscopy reports, participants were classified into four groups: control (normal colon/rectum), CRC/adenoma(s), hyperplastic polyp(s), and other lesions (excluded). Data on smoking (status, duration, dose, age starting and quitting, time since quitting, and passive smoking) were also collected. The associations between each smoking variable and risk of CRC/adenoma and hyperplastic polyp were examined in multinomial logistic regression models adjusted for potential confounders.

Results: Among the 844 participants included in the analysis (62% female), prevalence of former and current smoking was higher among CRC/adenoma than in the control group (29.8% vs. 19.6%, P=0.001); however, smoking status was not significantly associated with CRC/adenoma risk. Current smoking was associated with increased risk of hyperplastic polyps (OR: 2.24, 95%CI: 1.02 – 4.90; P=0.043). A 21% (95%CI: 1.02 – 1.42; P=0.026); increase in hyperplastic polyps risk was observed for every additional 10 yrs of smoking. Every 10 yrs delay in starting smoking was associated with 63% (95%CI: 0.15 – 0.89; P=0.026) reduced risk of hyperplastic polyps. No statistically significant association was found between smoking status and size or location of adenomatous polyps. There was no evidence of any effect modification by lifetime alcohol intake on the association between smoking status and CRC/adenoma or hyperplastic polyps.
Introduction: Cancer metastasis is responsible for 90% of cancer-related deaths. For metastasis to occur cells must shed their epithelial phenotype and acquire a mesenchymal phenotype in order to gain the characteristic motility and invasiveness. These alterations are referred to as “Epithelial-Mesenchymal Transition (EMT)”. The Wnt/β-catenin signaling pathway is central to the progression of many cancers. β-catenin is a dual functional protein that is involved in cell-cell adhesion and in regulating the gene expression of key functional proteins in the cell. The latter function is associated with β-catenin’s role in promoting the genesis and progression of cancer (oncogenic properties). A significant component in the gene regulatory function is the transcriptionally active form of β-catenin called Active Beta-Catenin (ABC). Phosphatidylinositol 3’ Kinase (PI3K) pathway is another pathway commonly deregulated in many cancers. PTEN, a dual function (lipid/protein) phosphatase dephosphorylates lipids phosphorylated by the kinase activity of PI3K and maintains control over the PI3K pathway. Loss of expression or activity of PTEN is seen in many cancers and allows constitutive activation of the PI3K pathway resulting in pro-survival and anti-apoptotic cellular response. We hypothesize that ABC/β-catenin is a significant component in regulation of cancer progression and its cellular levels/activity is jointly regulated by PI3K and Wnt signaling pathways.

Methods: We used a panel of cell lines as model of EMT representative of the various phases of melanoma progression. Using this panel of cell lines we determined cellular expression, sub cellular distribution and activity of β -Catenin (ABC) and and components of the PI3K pathway using Western blotting (WB) and Immunofluorescence (IF) analysis.

Results: We determined that there was a loss in the expression of the tumor suppressor PTEN with melanoma progression. This was correlated with an increase in total cellular levels of ABC. We then carried out transient transfection of the PTEN-null metastatic cell line with a vector containing wild type PTEN tagged with GFP (pEGFP-PTEN). Our results showed a decrease in ABC levels with re-expression of PTEN. There was no change in total cellular β-Catenin levels or in the phosphorylated forms (β-Catenin at ser-33/37 and thr-41 and at ser-45) of β-Catenin.

Conclusions: Our results illustrate the significance of ABC in cancer progression and a regulatory role of PTEN/PI3K pathway upon the levels of ABC.

Significance: Components of the PTEN/PI3-Kinase pathway involved in regulating ABC levels represents potential molecular targets, which could be intercepted to limit progression to metastatic disease.

34. Gamma Delta T Cells and Nodal in the Breast Tumour Microenvironment

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Gamma delta T cells (GDTc) kill transformed cells, and increased circulating GDTc levels correlate with improved outcome in cancer patients. However, GDTc tumor infiltrating lymphocytes (TIL) were recently deemed the most significant independent factor predicting negative clinical outcome in human breast cancer. We hypothesize that GDTc become functionally altered by Nodal, an embryonic morphogen secreted by breast tumour cells in the hypoxic tumour microenvironment and implicated in aggressive disease. We have identified GDTc TIL in serial sections of breast cancer tissue in which Nodal is also expressed. Under hypoxic compared to normoxic conditions, GDTc viability and cell density increase, as does expression of activating receptor CD56, the gamma delta TCR, HLA-I and CD95; conversely, the inhibitory receptor CD94 is down-regulated. Thus GDTc can survive and proliferate in a hypoxic environment, and are armed with increased activating receptors implicated in cytotoxicity. Blood-derived in vitro expanded primary human GDTc kill breast cancer cell lines; however, Nodal-expressing breast cancer cells (231shC) resist GDTc killing compared to those in which Nodal has been silenced (231shN). Thus, Nodal expressed by breast tumour cells appears to suppress GDTc cytotoxicity. Preliminary results from chick chorioallantoic membrane assays suggest greater infiltration of GDTc into 231shN compared to 231shC tumours. We now plan to employ additional cell lines as well as examine GDTc therapy and TIL in xenograft mouse models of breast cancer. Understanding the dynamic interplay between Nodal and GDTc infiltration in breast cancer lesions will be of utmost importance to develop safe and effective GDTc immunotherapies.
DEAD box proteins are RNA helicases that are involved in all aspects of RNA metabolism including RNA processing, translation, decay and splicing. DEAD Box 1 (DDX1) is a RNA unwinding protein that was originally cloned from a subtracted retinoblastoma cDNA library and found to be amplified in a subset of childhood tumours such as neuroblastoma and retinoblastoma. DDX1 has been implicated in the transport of RNA granules in neuronal cells, RNA processing and repair of DNA double strand break. Knock-out of DDX1 in mouse causes embryonic lethality at the two-cell stage suggesting that DDX1 plays a critical role in early mouse embryo development. Although no lethality has been observed in DDX1 knock-out flies, adult flies are smaller than wildtype flies and infertile.

Microarray cDNA analysis was previously carried out in the Godbout lab comparing gene expression in control HeLa cells and siDDX1-transfected HeLa cells. A high percentage, approximately 25%, of the 400 differentially expressed genes was found to involve splice variants. These data led us to hypothesize that DDX1 may be involved in alternative splicing.

In order to test our hypothesis, we have selected the most differentially expressed gene in our microarray experiment: ‘apolipoprotein B mRNA editing enzyme catalytic polypeptide-like 3H’ (APOBEC3H) for further analysis. The APOBEC3H gene has 4 splice variants (variants 1 - 4). First, we transfected HeLa cells with a scrambled siRNA and two different siRNAs targeting DDX1. Using RT-PCR analysis, we observed a reduction in APOBEC3H variant 1 and an increase in APOBEC3H variant 4 upon DDX1 knockdown. Second, we transfected HeLa, neuroblastoma IMR-32, human embryonic kidney HEK293 cell lines with the following expression constructs: Control, No tag DDX1, HA-DDX1, MYC-DDX1. Based on RT-PCR analysis, we found a correlation between DDX1 overexpression, reduced levels of APOBEC3H variant 1 mRNA and increased levels of APOBEC3H variant 4. These data indicate that DDX1 may indeed play a role in alternative splicing.

Out next objective is to investigate the mechanism through which DDX1 regulates alternative splicing. It has been reported that DDX1 interacts with Drosha, a critical player in microRNA biogenesis that also functions in splicing. We will examine whether DDX1 and Drosha regulate splicing in the same pathway. Furthermore, because DDX1 and APOBEC3H are both implicated in cellular responses to stress, we will study the functional relationship between these two proteins under stress conditions. As different variants of certain genes can have completely different functions, understanding of the role DDX1 has in alternative splicing could help us better understand the role of amplified DDX1 in neuroblastoma and retinoblastoma.

**Abstract:** [Purpose] The 20 and 22 carbon n-3 long-chain polyunsaturated fatty acids (LCPUFA) inhibit the growth of tumors in vitro and in animal models, but less is known about the 18 carbon n-3, stearidonic acid (SDA). This study aimed to establish and determine a mechanism for the anti-cancer activity of SDA-enriched oil (SO). [Methods] SO (26% of lipid) was produced by genetically engineering flax and used to treat human tumorigenic (MDA-MB-231, MCF-7) and non-tumorigenic (MCF-12A) breast cells. Nu/nu mice bearing MDA-MB-231 tumor were fed SO (SDA, 4% of fat). Cell/tumor growth, phospholipid (PL) composition, apoptosis, CD95 and pro-apoptotic molecules were determined in SO treated cells/tumors. [Results] Compared to a control lipid mixture (oleic acid, OA and linoleic acid, LA), SO reduced (P<0.05) the number of tumorigenic, but not MCF-12A cells, and resulted in higher concentration of most of the n-3 fatty acids in PL of all cells (P<0.05). However, docosapentaenoic acid (DPA) increased only in tumorigenic cells (P<0.05). SO diet decreased tumor growth and resulted in more n-3 LCPUFA, including DPA, and less arachidonic acid (AA) levels in major tumor PLs (P<0.05). Treatment of MDA-MB-231 cells/ tumors with SO resulted in more apoptotic cells (in tumors) and in vivo and in vitro, more CD95+ positive cells and a higher expression of apoptotic molecules caspase-10, Bad or Bid (P<0.05). [Conclusion] Supplementing SO alter total PL and PL classes by increasing membrane content of n-3 LCPUFA and lowering AA (in vivo), which is associated with increased CD95 mediated apoptosis, thereby suggesting a possible mechanism for reduce tumor survival.
37. Next generation approaches to PAX3 motif and target gene discovery

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The PAX3 transcription factor (TF) contributes to birth defects and cancer. Specifically, loss-of-function mutations in PAX3 cause Waardenburg syndrome types 1 and 3 (WS1 and WS3). These congenital conditions exhibit a range of neurocristopathies and can also be associated with musculoskeletal deformities in WS3. Moreover, PAX3 and the related PAX7 protein undergo chromosomal translocations with the gene encoding the forkhead transcription factor (FKHR) to produce oncogenic fusion proteins (PAX3 or PAX7-FKHR) in ~80% of pediatric alveolar rhabdomyosarcomas (ARMS), and PAX3 is frequently deregulated in melanoma. The normal and pathogenic effects of PAX3 are determined by the target genes it regulates. PAX3 contains two sequence-specific DNA-binding domains, the paired domain and homeodomain, and can interact with a range of target sites through differential use. We have established that a combination of alternative splicing and post-translational modification influences mode of binding, which is further modulated by the ARMS-associated translocation. We therefore hypothesize that PAX3 exists as a population of structural variants with distinct DNA-binding properties and target gene preferences.

DNA-binding profiles for human and mouse PAX3, as well as the oncogenic fusion protein PAX3-FKHR were derived from previously published sequence datasets. Complementary motif discovery (MD) methods, including the Weeder enumerative and exhaustive search algorithm and the Motif Discovery Scan (MDScan) alignment algorithm, were used to identify DNA-binding patterns for each protein. MD was subsequently validated using a discriminative support vector machine, kmer-SVM. Pattern frequencies from each computational experiment were aligned and aggregated into log-odds matrices called position weight matrices (PWM), each representing a single PAX3 probabilistic DNA-binding model. Regularized matrices were used to quantify the enrichment and distribution of PAX3 DNA-binding sites across different tissues and disease states.

The resultant PAX3 DNA-binding libraries represent the first sets of optimal motifs described for full-length PAX3 variants. Furthermore, the results of this computational analysis have elucidated key differences between the binding specificity of normal PAX3 and pathogenic PAX3-FKHR proteins, which were previously undefined. Our ongoing work leverages PAX3 PWMs to query the distribution of target sites across genome-wide regulatory element datasets with the goal of defining specific target gene networks and how they may be altered in WS, ARMS and melanoma. Significantly, differential binding specificity described for PAX3-FKHR yielding an altered target gene network could underlie pathogenesis. Further validation of identified targets will give key insight into how PAX3 governs cell fate decisions and reveal strategies for manipulating PAX3 activity to therapeutic benefit.

38. Single cell omics for breast cancer: heterogeneity and metastasis

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All cancers exhibit intra-tumoral heterogeneity that increases the complexity of basic research, clinical diagnosis and treatment. We are performing genomic and transcriptomic profiles for breast cancer, from different sites of the disease within each patient, initially in small clusters of cells and eventually in single cells. Not only will this define the level of heterogeneity, but also from comparative analyses, we may be able to classify cancer cells into more accurate subtypes, identify metastasis related biomarkers, evaluate the risk of recurrence, and develop effective individualized treatment. For each patient, we are using laser capture microdissection (LCM) to isolate cancer cells from 3 sites: 1) primary tumor, 2) blood vessels around the primary tumor (lymphovascular invasion or LVI), and 3) metastatic sites in lymph node. Due to the resolution of the LCM, we collect 10 to 50 cells instead of single cells, but we do so from multiple locations within each site. We use the MDA protocol for DNA pre-amplification, and a modified SmartSeq protocol for RNA pre-amplification, to generate enough materials for library construction and nextgen sequencing. Preliminary results indicate: 1) high levels of heterogeneity, copy number variations in regions of the chromosomes, over 700 differentially expressed genes and about 300 genes with high numbers of mutations in their protein coding regions. A functional analysis revealed an enrichment for categories like phospho proteins/kinases, cell membrane proteins related to ion channels and cell-cell junctions, etc.
We are also developing a probe capture based cancer gene panel to enrich patient plasma samples for cell-free tumor DNA sequences. This will be used to monitor cancer metastasis and recurrence after surgery and chemo/radiotherapy.
### 39. Fatty Acid Binding Proteins in Retinoblastoma: Implications for Tumour Morphology and Cell of Origin

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Retinoblastoma (RB) is a rapidly-growing pediatric eye tumour most commonly affecting children under the age of 3, with over 97% of cases caused by homozygous loss of the retinoblastoma gene, RB1. The cell type and developmental stage in which retinoblastoma emerges is unknown, though its presentation in infants suggests that the oncogenic insult occurs during fetal development. Despite its characterization as a “genetically simple” cancer, RB tumours have numerous clinical presentations and morphologies, and display a wide spectrum of cellular phenotypes in vitro. Heterogeneity of RB tumours may be a product of the cell type from which they are derived in the developing retina.

Fatty acid binding proteins (FABPs) are a family of 9 proteins involved in the uptake and transport of hydrophobic ligands. FABPs have tissue and cell-specific patterns of expression and broadly influence cell character by modulating membrane structure, metabolic processes, and the expression of genes involved in proliferation and differentiation. Three FABPs (3, 5, and 7) are expressed in specific cell types and developmental stages in the retina. FABPs may therefore be key markers for identifying the cell of origin in retinoblastoma. Expression of FABPs may also influence key tumourigenic processes such as migration and angiogenesis, which can profoundly impact treatment decisions and clinical outcomes.

The purpose of this study was to determine the expression patterns of FABP3, 5, and 7 in 18 RB cell lines using reverse transcriptase polymerase chain reaction (RT-PCR) assays, and elucidate cell of origin based on the spatio-temporal expression patterns of FABPs during retinal development. Our results indicate that RB tumours show evidence of failed differentiation and are unlikely to originate from glial precursors or FABP7(+) stem cells. Additionally, some RB cell lines express FABP3 and exhibit a cellular phenotype that suggests they are derived from a cell that is committed to a neuronal lineage. Heterogeneity between RBs in their cell morphologies and FABP expression profile indicates that there is likely more than one possible cell of origin in retinoblastoma.

### 40. The potential of small molecule drugs as an effective treatment for a rare ovarian cancer

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Introduction: Granulosa cell tumour (GCT) is an uncommon form of ovarian cancer, constituting ~5% of ovarian neoplasms. While typically diagnosed in early stage, with 5-year survival >90%, it is marked by late recurrence and an 80% mortality rate for women who relapse or have advanced disease. Evasion of apoptosis is one hallmark of cancer. Caspase-3 (CASP3) is at the hub of multiple apoptotic pathways and, when active, contributes to an irreversible cascade of protease activity leading to programmed cell death. X-linked inhibitor of apoptosis (XIAP) facilitates cell survival by inhibiting CASP3 permitting unchecked proliferation of cancer cells. Targeted, small-molecule cancer therapies are drugs designed to interact with the enzymatic activity of proteins affecting tumour growth and progression. Traditional cytotoxic chemotherapies usually kill rapidly dividing cells in the body by interfering with cell division, while targeted therapies fight cancer cells with more precision and potentially fewer side effects. Procaspase activating compound-1 (PAC1) is a small-molecule drug which induces cleavage of Procaspase-3 into active CASP3 by sequestering an inhibitory zinc ion. Embelin is a small, monovalent inhibitor of XIAP. This study reports on preliminary experiments testing our hypothesis that use of complementary, small-molecule drugs affecting the apoptotic pathway will result in increased killing of GCT cells, and may represent a novel therapeutic approach.

Methods: GCT cell line, KGN, was treated in vitro with various concentrations of PAC1, for selected time points, and evaluated for viability using a metabolic assay. High-content screening of similarly-treated cells was used to quantify PAC1 activation of CASP3-mediated apoptosis. KGN cells were then treated in vitro with selected concentrations of embelin to assess the effect of XIAP inhibition, and combined with 10 µM PAC1 to look for additive effect by combining these small-molecule drugs.

Results: Preliminary in vitro tests showed PAC1 induces significant cytotoxicity in KGN in both a dose and time-dependent manner (p<0.05) with an estimated effective concentration (EC50) of ~10 µM. High-content screening confirmed significant reduction in cell number and increased CASP3-mediated apoptosis in both a dose and time-dependent manner (p<0.05). Combination of 10 µM PAC1 with selected embelin concentrations produced significant increase in cytotoxicity in a dose-dependent manner (p<0.05).

Conclusions: These in vitro results support the hypothesis that combining small-molecule drugs to affect CASP3 activation and XIAP inhibition is potentially an effective, novel treatment for GCT that warrants further study.
41. The potential of small molecule drugs as an effective treatment for a rare ovarian cancer


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Purpose: Over the past 15 years, fibular free flap reconstruction (FFFР) involving oral rehabilitation of the jaws has evolved in the planning and reconstructive surgery technique. This shift has reduced the number of surgeries and time to treatment completion. In the advanced technique, a virtual surgical plan is created using a 3D application and Surgical Design and Simulation (SDS) to plan the resection and reconstructive surgery. This strategic pathway uses advanced digital computer programs, planning and rapid prototyping modalities. Both FFFР techniques aim to reconstruct the patient’s defect, restore function and aesthetics with oral rehabilitation. The purpose of this study was to investigate the utilization and loading rate of osseointegrated implants in traditional and advanced FFFР techniques in head and neck cancer (HNC) patients.

Methods & Materials: Retrospective analyses of 19 subjects with HNC who underwent FFFР of the maxilla or mandible were selected. Eight of the subjects were included in the SDS group and 11 were in the non SDS group. In the SDS group, two of the eight subjects had a history of radiation therapy (RT) and six of the 11 subjects in the non SDS underwent RT as part of their HNC treatment. Two examiners evaluated the outcome data using archival records and clinical photographs. The outcome data involved recording the number of implants installed and the utilized in the implant retained prosthesis. Patient demographic data, prosthesis utilization and months since connection were also recorded. Health Research Ethics Board approval was obtained from the University of Alberta.

Results: There was a significant difference between the two groups (t= 2.456, df= 12, p= 0.03, two-tailed) In the SDS group, 97% of the implants were utilized compared to 76% of the implants in the group without SDS. Between the two groups, there was a 21% higher loading rate of the implants utilized in the SDS group. Eight subjects in the SDS group were recorded to have 33 implants pre-planned for installation, 32 were installed and 31 were connected to an implant retained prosthesis. Eleven subjects in the non-SDS group had 36 implants planned to be installed, 49 were installed and 37 were connected to an implant retained prosthesis.

Conclusion: This study showed a higher loading rate and utilization of implants in the SDS group. Longer follow-up data is required to assess implant and prosthesis utilization as the advanced reconstructive technique has been applied in the past few years. Future studies on health economics and health technology assessment for advanced techniques in jaw reconstruction and rehabilitation are required to bring advanced jaw reconstruction techniques to the international HNC reconstruction community. SDS and guided FFFР reconstruction provides important treatment plan and surgery communication within the treatment teams.

42. Intestinal Uptake and Transport of Vitamin B12-loaded Soy Protein Nanoparticles


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Background: Intestinal absorption of vitamin B12 (VB12) is a major challenge in combating pernicious anemia due to intrinsic factor (IF) deficiency. The aim of this study was to explore the feasibility of using soy protein isolates (SPI) nanoparticles to improve the intestinal transport and absorption of VB12.

Methods: Three different sized VB12-loaded SPI nanoparticles were produced by modulating preparation conditions using a cold-gelation method. The intestinal uptake and transport mechanisms of SPI nanoparticles for VB12 delivery were investigated and related to particle size.

Results: SPI nanoparticles were not cytotoxic to Caco-2 cells and were effectively internalized into the cytoplasm via multiple endocytosis pathways including clathrin- and/or caveolae-mediated endocytosis and macropinocytosis. VB12 transport across the Caco-2 cell monolayers was increased to 2-3 times after nanoencapsulation, which was dependent on particle size, in the increasing order of 30 > 100 > 180 nm. Using inhibitor block method, the transport of 30 and 100 nm SPI nanoparticles appeared to be clathrin-mediated transcytosis and macropinocytosis routes. The intestinal transport of VB12, assessed using rodent jejunum in Ussing chambers, was improved up to 4-fold after being encapsulated into 30 nm SPI nanoparticles.

Conclusions: The findings suggest that SPI nanoparticles could be a promising carrier to facilitate the oral delivery of VB12.
Ovarian cancer ranks fifth in cancer deaths among North American women, accounting for more deaths than any other cancer of the female reproductive system. At present there are no ideal biomarkers for early diagnosis, 90% the ovarian cancer could be cured if patients could be diagnosed at an early stage. Clinically, the main biomarker used to detect ovarian cancer is CA125. Approximately 80% of ovarian cancer patients present with high levels of serum CA125, and this marker is elevated upon recurrence. However, it is not specific to ovarian cancer, making healthy background levels difficult to establish. Hence, CA125 cannot accurately predict early disease. Therefore, this study is aiming to discover new biomarkers for ovarian cancer that will enable early detection and diagnosis. We reasoned that an ideal marker would be specific to cancer. Hence, one would expect it to be in low abundance, necessitating methods that can detect low abundance proteins and/or involve a purification or fractionation. We addressed these requirements by focusing on stem cell related proteins that re-emerge in cancer and by focusing on extracellular vesicles (Evs), a fraction that can enrich for cancer-derived proteins. We have studied several biomarkers/potential biomarkers, including CA125, HE4, Nodal, Notch1 and Notch3 in ovarian cell lines, cultured ascites cells and primary ascites cells, and their EVs using Western blotting. We have found that: (1) CA125 is only detected in 1 of 7 ovarian cancer cell lines and 4 of 9 cultured ascites cells from ovarian cancer patients. CA125 protein expression is eventually lost with increasing cell culture passage. Gene expression data from the TCGA support our observations, such that CA125 mRNA is present in primary ovarian cancers, but is generally absent in ovarian cancer cell lines. In addition, there is a small portion of CA125 in ascites EVs derived from patients. (2) HE4 (human epididymis protein 4) is another potential biomarker of ovarian cancer, overexpressed by serous and endometrioid epithelial ovarian carcinomas. Our results show HE4 is expressed in ovarian cancer cell lines, cultured and primary ascites cells, but cannot be detected in ascites EVs. (3) Nodal, an embryonic protein and a potential tumor marker, is expressed in ovarian cancer cell lines, cultured ascites cells and primary ascites cells. While Nodal can be detected in EVs derived from cultured cells, it cannot be detected in fresh ascites derived EVs. (4) Notch1, a member of Notch family, playing a role in developmental processes by controlling cell fate decisions, is expressed in most of cultured ascites cells, but is not detected in primary ascites cells or in EVs. (5) Notch3, is expressed in a few of ovarian cell lines, is lowly expressed in some cultured ascites cells, but is not detectable in primary ascites cells or EVs. Our results reveal that cell culture can dramatically alter the expression of potential biomarkers, causing the loss of some, like CA125, and the acquisition of others like the NOTCH receptors.

XRCC4/DNA Ligase IV induces conformational changes in the polynucleotide kinase/phosphatase active sites and increased kinase catalysis on DNA substrates

If unpaired or misrepaired, DNA double-strand breaks (DSBs) can lead to genomic instability and cell death or neoplastic transformation. The major DSB repair mechanism in higher eukaryotes is non-homologous end-joining (NHEJ). In NHEJ, polynucleotide kinase/phosphatase (PNKP) is the primary enzyme for processing abnormal 5'-hydroxyl and 3’-phosphate ends that preclude the final repair step by XRCC4/DNA Ligase IV (Lig IV). This processing step is thought to be mediated by an interaction between the PNKP-FHA domain and CK2-phosphorylated XRCC4 C-terminal tails. However, our results from protein-protein binding experiments show that tight binding occurs between XRCC4/Lig IV and PNKP both in the presence and absence of phosphorylation of XRCC4. We have also shown through activity assays that these interactions of PNKP with both phosphorylated and non-phosphorylated-XRCC4/Lig IV both contribute to an increase in its kinase activity on double-stranded DNA substrates. We were able to purify phosphorylated-XRCC4/Lig IV/PNKP and study the ternary complex by Small-angle X-ray scattering (SAXS) experiments. The ensemble structures from our SAXS results also suggest a secondary interaction between the PNKP and XRCC4/Lig IV that does not depend on the PNKP-FHA domain and is phosphorylation-independent. To get more detail on the nature of this interaction, we used hydrogen-deuterium exchange experiments to measure changes in deuteration in solvent-exposed regions of PNKP upon binding XRCC4/Lig IV. The HDX results showed decreases in deuteration/increased protection from solvent in non-FHA regions and a simultaneous increase in deuteration/increased accessibility to solvent at the kinase active site. We have shown evidence for a novel phosphorylation-independent interaction between PNKP and XRCC4/Lig IV that contributes to both conformational and functional changes in PNKP. This functionally related secondary interaction seems significant for driving increased DNA double-strand break repair in cells and may prove an interesting target for small-molecule disruption of NHEJ toward radio- and chemo-sensitizing therapies in cancer treatment.
Poster Presentations

45. POLE exonuclease domain mutations in endometrial and ovarian cancer

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Background and Aims: POLE exonuclease domain (EDM) mutations were recently found to occur in a subset of endometrial endometrioid-type carcinomas and result in defective proof-reading function during DNA replication, leading to a very high mutation rate in the tumor genome. The aims of this study are to characterize POLE EDM mutations in endometrial and ovarian cancers, and to screen established endometrial cancer cell lines for POLE EDM mutations.

Methods: We assessed for EDM mutations in POLE by Sanger sequencing in 99 endometrial cancers (53 high-grade endometrioid, 25 serous, 16 clear cell and 5 dedifferentiated-type) and 90 ovarian endometrioid-type cancers (67 low-grade and 23 high-grade). We correlated POLE mutation status with clinicopathologic features and molecular parameters. Univariate and multivariate survival analysis were performed using Kaplan-Meier and cox regression analyses. 12 endometrial cancer cell lines were examined by custom Truseq panel (Illumina) to screen for mutations in the coding regions of POLE and 25 other endometrial cancer genes in addition to microsatellite instability analysis and copy number analysis (Affymetrix SNP 6.0).

Results: POLE EDM mutations were identified in 8 of 53 (15%) high-grade endometrial endometrioid carcinomas and not in any other histotypes of endometrial carcinomas. When analyzed together with published cohort of high-grade endometrioid carcinomas by The Cancer Genome Atlas (TCGA), the presence of POLE EDM mutation is associated with significantly better progression-free survival in univariate analysis (p=0.025) and multivariate analysis (p=0.010), such that none of the patients with POLE mutated tumors experienced disease progression. In contrast, POLE EDM mutations were identified in 4 of 67 (6%) of low-grade but not in any of the high-grade ovarian endometriod-type carcinomas. Our cell line analyses identified 2 POLE EDM mutated (P286R) endometrial cancer cell lines (HEC-251 and HEC-88nu) that were microsatellite stable and both POLE mutated cell lines demonstrated a high number of point mutations in the genes surveyed, thus confirming a hypermutation genotype. Of the remaining 10 endometrial cancer cell lines, 8 exhibited high level of microsatellite instability (MSI-H) and 2 showed a high number of copy number aberrations in combination with TP53 mutations.

Conclusions: POLE EDM mutations occur in a significant subset of high-grade endometrial endometrioid-type carcinomas and are associated with excellent clinical outcome. POLE mutation screen may provide valuable prognostic information in the clinical management of patients with high-grade endometrial endometrioid-type cancer. We also identified experimental cell line models corresponding to the different molecular types of endometrial cancers for further functional characterization. POLE EDM mutations were found only in a minor subset of low-grade ovarian endometrioid-type carcinomas but not in high-grade tumors, and further study is needed to elucidate its clinical significance in the setting of ovarian carcinomas.

46. Reactivity of phenylbutazone towards superoxide dismutase-peroxidase activity: Toxicological implication in HepG2 and HT-29 cancer cells

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Purpose: To investigate if superoxide dismutase (SOD)-peroxidase activity which is known to produce carbonate radicals would cooxidize phenylbutazone and lead to cytotoxicity in two cancer cell lines. Methods: In an enzymatic system, we utilized UV-Vis spectrophotometry to determine if SOD-peroxidase activity would enhance the oxidation of phenylbutazone. Also, we used electron paramagnetic resonance (EPR) spectroscopy spin trapping using 5,5-dimethylpyrroline-1-oxide (DMPO) to detect the effects of phenylbutazone on the spectrum of carbonate radical- spin adduct that was generated by SOD-peroxidase activity. In a cellular system, we used EPR to detect phenylbutazone radical- spin adduct (PHZ/DMPO) in both human colorectal cancer cells (HT-29) and human hepatocellular carcinoma cells (HepG2). Also, we utilized HT-29 and HepG2 to evaluate the cytotoxicity of phenylbutazone using the alamarBlue assay. Results: The oxidation of phenylbutazone was enhanced by the peroxidase activity of SOD. UV-Vis measurement showed that λmax for phenylbutazone (λ=260 nm) showed a decline in intensity. SOD-omitted reactions produced no oxidation. The spectrum of spin adduct of carbonate radical was attenuated with an increase the concentration of phenylbutazone. The resulting phenylbutazone radical was also trapped (PHZ/DMPO) in both cancer cells, HepG2 and HT-29, however, this spectrum was attenuated with the presence of 4-aminobenzoic hydrazide (peroxidase inhibitor) and diethylidithiocarbamate (SOD inhibitor). Lastly, cytotoxicity of cells treated with a combination of a hydrogen peroxide generating system and phenylbutazone showed synergistic cytotoxicity when compared to cells treated with either reagent alone. Conclusion: SOD appears to play a role in phenylbutazone cytotoxicity through a cooxidation reaction that involves the carbonate radical. These findings may represent a novel mechanism of phenylbutazone-induced toxicity in both HepG2 and HT-29 cancer cells.
47. Optimizing nanodroplet formulation and fabrication for enhanced biodistribution and cell uptake

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Sheeran et al (Langmuir 2011) previously described the creation of nanodroplets which are nanosized perfluorobutane liquid droplets stabilized by a phospholipid shell. Nanodroplets exposed to high mechanical index ultrasound can phase-change into microbubbles which can provide ultrasound imaging contrast and cavitation nuclei for enhancing sonoporation of cells.

Our group investigated the in vivo kinetics and biodistribution of Sheeran's nanodroplets and realized that these nanodroplets (containing positively charged 1,2-dipalmitoyl-3-trimethylammonium-propane) have significant non-specific cell binding properties which greatly reduces their kinetics and biodistribution. Our goal was to create new formulations of nanodroplets optimized for enhanced 1) in vivo biodistribution, and 2) receptor specific uptake.

Methods: Cationic and anionic (based on Definity® microbubbles) nanodroplets were created with lipids containing Rhodamine B and injected in the chorioallantoic membranes (CAM) of embryonic chicks followed by microscopy imaging of the CAM immediately after and 24 hours after nanodroplet injection. Chicks were sacrificed 24 hours after nanodroplet injection and tissues were dissected and imaged with microscopy.

A second anionic nanodroplet formulation was created containing lipids conjugated with folate. Unconjugated and folate conjugated anionic nanodroplets (also containing lipids with Rhodamine B) were created and some nanodroplets were forced through a 200 nm filter using a mini-extruder and sized using a Malvern Zeta sizer. Nanodroplets were incubated for 2 hours with folate receptor negative and positive ZR-75-1 and HeLa cells, respectively, and cells were washed and imaged with confocal microscopy.

Results: Cationic nanodroplets displayed very little flow when injected in the CAM membranes of chicks. Much of the cationic nanodroplets remained near the injection site as large clumps. In contrast, the anionic nanodroplets displayed rapid flow and widespread distribution through all of the CAM vasculature. Anionic nanodroplets also displayed significantly greater accumulation in the brain, heart, and especially lungs of the chick.

ZR-75-1 and HeLa cells displayed no uptake of either the folate-conjugated or unconjugated nanodroplets which were ~530 nm in diameter. Extruded nanodroplets were ~370 nm in diameter and only the folate-conjugated extruded nanodroplets were taken up by HeLa cells. Extruded nanodroplets also had ~half the polydispersity index, suggesting greater size uniformity.

Conclusion: Based on our results, cationic nanodroplets have high non-specific cell binding properties and incorporation of anionic lipids in nanodroplets is necessary for proper kinetics and biodistribution. Nanodroplets can be sized with extrusion and must be < 500 nm for cell uptake. Ongoing experiments involving identification of nanodroplet size requirements for accumulation in tumors will also be discussed.

48. Microcantilever Sensor Arrays for Real-time Detection of Breast Cancer Cells

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In the early stages of breast cancer, circulating tumor cells (CTCs) are very rare and present in the blood stream at very low concentrations. Their presence carries valuable information about primary tumors and serves as potential biomarker for disease diagnosis and progression. Analysis and early detection of CTCs is a promising field for identifying the disease, estimating the metastatic regressions and monitoring risk of progressions in cancer patients. In this study, we report a nanomechanical assay based on peptide functionalized microcantilever arrays for quick detection of breast cancer cells in blood without enrichments or complicated preprocessing. A sensing cantilever, functionalized with cancer targeting peptide (WxEAAYQrFL) was able to distinguish cancer cells (MCF7 and MDA-MB-231) from noncancerous cells (MCF10A and HUVEC), and detect breast tumor cells (MCF7) at very low concentrations – down to 25±5 and 50 ±10 cells/mL in buffer and blood, respectively. The assay was further confirmed by surface plasmon resonance through monitoring the amplified refractive index changes associated with target cell capture by the peptide. The peptide-based microcantilever approach offers a great potential in tracking of tumors and monitoring efficiency of treatments.
49. Handling of liquid biopsy samples can dramatically change the numbers of PSMA+ microparticles

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The use of PSA as the gold standard for diagnosing prostate cancer (PCa) has been challenged recently and the search for new PCa biomarkers has increased. Although PSA testing has acceptable sensitivity, it lacks the necessary specificity to discriminate benign prostatic diseases, resulting in overdiagnosis and overtreatment. New biomarkers for PCa are needed to prevent unnecessary surgical biopsies. Analysis of extracellular microvesicles in biofluids is an exciting new area of investigation. We define microvesicles as submicron vesicles shed from the plasma membrane which can contain mRNA, microRNA, or membrane proteins. Any diagnostic test must differentiate microvesicles originating from PCa cells versus non-cancerous origins, and also define a biomarker enriched in this prostate cancer microvesicle (PCMV) population. In our assay, the prostate specific membrane antigen (PSMA) which is highly specific for PCa cells, is used. The microvesicle field is new, so no standard methods for specimen isolation, handling or even proper controls have yet been accepted. Initial studies examined plasma preparation and storage as a source of variation. Blood was collected from five prostate cancer patients and plasma prepared under different handling conditions. Microparticles were assessed directly in plasma using the Apogee A50 micro-flow cytometer and the Nanosight LM10, and ranged in size from ~80-200nm. Matched isotype controls were used for gating purposes. Fresh, never frozen plasma had dramatically more PSMA positive microparticles than any other treatment group. However, assaying fresh patient plasma is not practical; development of any biomarker assay will utilize retrospective samples, so frozen samples resemble true sample availability. Plasma prepared within 2hrs of collection, aliquoted and frozen at -80C was considered as “control”. Plasma or whole blood stored at RT overnight significantly increased PCMV (ie PSMA+) counts (2-4 fold respectively) compared to control. Whole blood that was stored at 4C for 30 min or overnight had significantly more PCMVs. Plasma stored at RT (6hrs) or at -20C O/N and then frozen at -80C had similar PCMPs compared to control. In agreement with other published data, isotype controls yielded significant numbers of “positive” microvesicles. The size of vesicles consistently ranged from 80-200nm with a sharp drop off at both ends and a significant peak at ~90nm. After freezing, microvesicle numbers <80nm increased but the sharp cutoff at 200nm remained. In conclusion, storage and handling of patient plasma can significantly affect microvesicle numbers which stain positive for PSMA and hence be classified as prostate cancer microvesicles. Standardization of processing and handling of patient samples is necessary to effectively analyze any potential biomarker for diagnostic value.

50. The Effect of Nodal on Human Fibroblast


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Background: Metastasis is the main cause of cancer death for breast cancer patients. However, the causative factors and the mechanisms by which cancer cells spread and metastasize remain unknown (unclear). Some studies have showed that activation of cancer/tumour associated fibroblasts (CAFs/TAFs) may be pivotal in providing the appropriate microenvironment to support tumour progression and metastasis. Studies from our research team and others have demonstrated the promoting effects of Nodal, another critical component of tumour microenvironment, and an embryonic morphogen, in breast cancer tumorigenesis and metastasis.

Objective: Therefore, the current study using human foreskin fibroblast (HFF) as cell model, investigates the potential involvement of Nodal in fibroblast activation and cancer metastatic promotion.

Results: Our preliminary data reveals that Nodal induces HFF activation; and HFF responds to Nodal in a dose dependent manner which is indicated by several parameters:

1. Western blot demonstrated that Phospho-ERK1/2 is induced in response to increasing concentrations of Nodal;
2. RT-PCR showed the gene expression elevations of main fibroblast activation markers, FAP, CTGF, alpha-SMA, Vimentin and Desmin, with treatments of increasing Nodal concentrations;
3. Functional assessments also indicate that Nodal promotes HFF cell migration and invasion dose-dependently;
4. Nodal also accelerates HFF cell proliferation even though dose-dependent effect is not as prominent as other functional aspects;

Conclusions and Significance: Our data demonstrate that Nodal can signal to fibroblasts, activating mitogenic ERK signalling. Nodal can also lead to an activated phenotype, associated with altered gene expression, increased invasion and more proliferation. Future studies will determine the effects of Nodal-induced CAF phenotypes on breast cancer growth and metastasis.
51. Polymeric Nano-Micelles for Delivery of STAT3 Inhibitors to Multiple Myeloma

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Purpose: Signal transducer and activator of transcription 3 (STAT3) is a transcription factor that is constitutively activated in many types of human cancer including multiple myeloma (MM), for which there is currently no cure. Despite the universal acceptance of STAT3 inhibition as a promising strategy in cancer treatment, STAT3 inhibition has yet to be successfully translated to clinical settings. This is mostly due to toxicity and inefficient delivery of STAT3 inhibitors to tumor cells. S3I-201 and its derivative S3I-1757 are effective inhibitors of STAT3 dimerization that have shown activity against MM, but their further development to drug candidates has been hampered due to their low water solubility and poor tumor selectivity. The aim of this research was to design and develop polymeric micellar nano-formulations for delivery of these STAT3 inhibitors to MM tumors. Polymeric micelles have shown promise in solubilisation and tumor targeted delivery of poorly water soluble drugs.

Methods: Diblock copolymers of poly(ethylene oxide)-block-poly(ε-caprolactone) (PEO-b-PCLx) or PEO-b-poly(α-benzyl carboxylate-ε-caprolactone) (PEO-b-PBCLy) having similar degree of polymerization in PEO segment (114 ethylene oxide units) and different degrees of polymerization in the PCL (x = 22, 44, 66) and PBCL (y = 15, 20, 30) were synthesized by ring-opening polymerization. S3I-201 and its derivative, i.e., S3I-1757 were encapsulated via co-solvent evaporation in the PEO-b-PCL or PEO-b-PBCL block copolymer micelles. Drug-loaded micelles were characterized for their size, encapsulation efficiency, drug release profile, and cytotoxicity against human U266 MM cell line.

Results: PEO-b-PCL and PEO-b-PBCL block copolymers were successfully synthesized as evidenced by 1H NMR. Block copolymers self-associated to form micelles in aqueous solution. Successful encapsulation of S3I-201 and particularly S3I-1757 in all micellar structures under study was evidenced by HPLC (~25 and 75% encapsulation efficiency, respectively). Dynamic light scattering revealed micelle diameters of 30-70 nm. The release of S3I-1757 from polymeric micelles was slower than that of S3I-201 (~35% in 24 h versus ~100% in ≤ 8 hours, respectively) irrespective of polymer structure. Interestingly, despite slow drug release, significantly better cytotoxicity (lower IC50) for S3I-1757-loaded in PEO114-b-PBCL15 (10.2 ± 0.7 mM) and PEO114-b-PBCL20 (11.0 ± 1.3 mM) than free S3I-1757 (13.1 ± 0.9 mM) was observed against human U266 cells, in vitro.

Conclusion: PEO-b-PCL and PEO-b-PBCL micelles are promising nano-delivery systems for STAT3 inhibitors S3I-201 and S3I-1757 delivery against MM.

52. Calcineurin Regulates Nuclear Factor I in Malignant Glioma

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Malignant gliomas, comprising grades III and IV astrocytomas, are the most common adult brain tumours. These tumours are highly aggressive with a median survival of less than two years. Nuclear Factor I (NFI) is a transcription factor family (NFIA, NFIB, NFIC, and NFIX) that regulates expression of glial genes in both the developing brain and in malignant glioma. In malignant glioma cell lines, NFI regulates expression of two important developmentally-regulated glial genes, brain fatty acid binding-protein (B-FABP) and glial fibrillary acidic protein (GFAP). B-FABP correlates with increased migration in malignant glioma cell lines and is associated with poor survival in grade IV astrocytomas. In malignant glioma cell lines, expression of B-FABP and GFAP correlates with hypophosphorylation of NFI, suggesting that NFI transcriptional activity is regulated by dephosphorylation. Using chromatin immunoprecipitation we have shown that NFI occupies the GFAP and B-FABP promoters in malignant glioma cells with hypophosphorylated NFI. However, upon inhibition of the phosphatase calcineurin with the specific inhibitor cyclosporin A, occupancy of the promoters by NFI is decreased. Treatment of malignant glioma cells with cyclosporin A also results in a decrease in NFI dependent promoter activity. Conversely, when a constitutively active form of calcineurin is expressed, NFI dependent promoter activity increases. Using co-immunoprecipitation, we have found that calcineurin interacts with NFI. Furthermore, western blot analysis of malignant glioma cell lines reveals the presence of a cleaved form of calcineurin that correlates with hypophosphorylation of NFI. This cleaved form has previously been shown to have increased phosphatase activity. Furthermore, nuclear localization of calcineurin correlates with hypophosphorylated NFI in malignant glioma. Our data indicate that phosphorylation of NFI regulates its transcriptional activity, and this phosphorylation may be regulated by an activated form of calcineurin in malignant glioma. This work will help identify upstream regulators of key genes involved in malignant glioma growth properties, thus providing new potential targets for the treatment of malignant glioma.
53. A Randomized Controlled Trial of High-intensity Aerobic Interval Training in Testicular Cancer Survivors (HITTS Trial)

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Background: Testicular cancer survivors (TCS) have a good prognosis but are at high-risk of developing late-onset/persistent cardiovascular (CV) disease within the first two decades post treatment (Abouassaly, 2011). Aerobic exercise interventions have documented benefits in improving multiple CV health endpoints in cancer survivors (Jones, 2011). Regular aerobic exercise has been shown to prevent and reverse metabolic and atherosclerosis-related disease progression in a number of CV disease populations (Currie, 2013; Madden, 2009). Compared to other forms of aerobic exercise, high-intensity interval training (HIT) is associated with greater mortality risk-reductions (Schnohr, 2012) and greater improvements in exercise capacity, cardiac/vascular health and metabolic/lipid-profile changes (Currie, 2012; Tjonna, 2008). To our knowledge, there are no published aerobic exercise interventions targeting TCS. Furthermore, given the specific nature of the disease and treatment-related CV injury, it is unknown whether HIT will have similar benefits for TCS.

Research Question and Hypothesis: Our primary research question is: Does 12 weeks of HIT positively influence the CV health of TCS? We hypothesize that 12 weeks of HIT will improve multiple CV endpoints in TCS compared to usual care.

Methods: Recruited via the Alberta Cancer Registry, 70 of 543 TCS will be randomly allocated to either HIT or usual care (asked to maintain current physical activity levels). Subjects in the HIT group will attend thrice-weekly supervised exercise sessions for 12 weeks. The HIT intervention consists of uphill walking/jogging on a treadmill between 65% and 95% of peak heart rate for 35 minutes (including a 5-minute warm-up and cool-down). Assessments will be made at baseline, two weeks, immediately post-intervention and at three months post-intervention. Participants’ general and CV health will be assessed (in order) using non-invasive measures of autonomic nervous system function, blood vessel structure and function, self-report questionnaires and a maximal exercise treadmill test. Following the blood vessel measures, blood samples will be collected and used to provide additional indices of participants’ CV health. ANCOVAs will be performed to identify a group (HIT vs. usual care) effect for all primary and secondary outcomes.

Significance: The demonstration of HIT’s ability to significantly improve aerobic capacity, reverse blood vessel dysfunction and disease and control CV disease risks may i) directly inform the clinical care and long-term follow-up of TCS at high risk of CV disease development, ii) help substantiate the development of practical and cost-effective community- and clinic-based interventions, and iii) influence provincial and federal cancer care policy by helping confirm a physiologic basis for the therapeutic role of exercise within the cancer survivorship continuum.

54. Designing a mobile device for swallowing therapy: a systematic and collaborative approach

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Introduction: Over 100,000 mobile health applications exist today, yet their use and patient benefit depends largely on the application’s design. Despite this, little documentation exists regarding optimal methodologies.

Objective: This study documents the design process, to date, for a patient-centric mobile health device.

Methods: Detailed documentation of design decisions and reasoning was carried out. Selected methods in the Research and Ideate stages of this formative research involved the core team members (engineer, software developer, researchers, designer, team leads) and primary stakeholders (patient, clinician).

Results and Discussion: Of 67 initial approaches considered, 5 Research design methods helped guide the first Ideation meeting. The collaborative approach ensured (1) informed ideation thorough research and (2) equal consideration of ideas, before resource investment at the Prototype and Validation stages.

Conclusion: The successful design of mobile health applications goes beyond obtaining feedback on a prototype. Inviting as many perspectives early on can expose important creative and technical considerations.
Poster Presentations


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Myosteatosis (triglyceride accumulation in muscle) is associated with wasting syndrome in cancer. We have previously shown in an animal model that myosteatosis develops during Irinotecan (CPT-11) plus 5-fluorouracil (5-FU) treatment but is prevented by a diet containing fish oil (FO) [eicosapentaenoic (EPA) and docosahexaenoic (DHA)]. Mechanisms underlying these observations are not known. This study aimed to measure expression of adipogenesis transcriptional factors within gastrocnemius muscle of Ward colorectal tumor bearing rats receiving two cycles of CPT-11/5-FU that received isonitrogenous, isocaloric, semi-purified diets with or without FO (2.3 g FO/100 g containing 0.64% EPA and 0.16% DHA). CPT-11 (50 mg/kg/day) was initiated on Day 0, and 5-FU (50 mg/kg/day) on Day 1. Rats were euthanized on Day 7 (one cycle). A second group received another cycle of chemotherapy starting on Day 7 and rats were euthanized on Day 14 (two cycles). Tumor bearing rats received control diet served as a reference group. Diets containing FO started either prior to tumor implantation (long-term) or on the same day of the first dose of CPT-11 (short-term). Muscles were isolated prior to starting chemotherapy and after each cycle of CPT-11/5-FU. qRT-PCR used to measure expression of mRNA for CCAAT/enhancer binding proteins (C/EBPs), peroxisome proliferator-activated receptor (PPARs), and sterol regulatory element binding protein 1c isoform (SREBP-1c). CPT-11/5-FU treatment significantly increased PPAR-γ (2.5-fold) after 2 cycles compared to the reference group. No genes were significantly altered between rats fed a short-term FO and rats fed control diet following either one or two cycles of CPT-11/5-FU. Rats fed a long-term FO diet exhibited the lowest expression of PPAR-γ (0.4-fold), C/EBP-β (0.3-fold), C/EBP-σ (0.8-fold), and SREBP-1c (0.7-fold) compared to the other groups (P<0.05) following one cycle of CPT-11/5-FU; mRNA of SREBP-1c (0.04-fold) and C/EBP-β (0.2-fold) were significantly reduced (P<0.05) in rats fed long-term FO diet compared to rats fed a control diet or short-term FO diet following two cycles of CPT-11/5-FU. Only long-term feeding of EPA and DHA reduced the expression of adipogenic transcriptional factors in this animal model of colorectal cancer providing one mechanism to explain reduced fat content of muscles in rats provided FO during CPT-11/5-FU treatment.

56. Differential oncogenic role of anaplastic lymphoma kinase (ALK) in ALK-positive neuroblastoma cells

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Purpose: Anaplastic lymphoma kinase (ALK) is a receptor tyrosine kinase that initially discovered in anaplastic large cell lymphoma (ALCL). Recent researches identified ALK mutation and amplification as an additional form of ALK dysregulation in some types of cancers such as neuroblastoma and glioblastoma. Neuroblastoma is the most common childhood malignancy and most patients have aggressive metastatic disease. In neuroblastoma, ALK was identified as a critical oncogene in which ALK amplification accounts for 3.5% of neuroblastoma patients while activating mutations account for 8.4%. Cell lines with ALK amplification shown to be very sensitive to the ALK inhibitor Crizotinib while cell lines with wild-type ALK or mutated ALK were resistant to the inhibitor. Our focus is to understand why these cell lines differentially respond to the ALK inhibitor.

Methods: Neuroblastoma cell lines NB-1 (harbor amplified ALK), SKNSH (harbor mutated ALK) and IMR32 (harbor wild-type ALK) were used for this study. The ALK inhibitor Crizotinib used to evaluate the effect of ALK inhibition on ALK downstream effector proteins.

Results: Her we report that the Akt was highly phosphorylated at serine 473 in the crizotinib sensitive cells while minimally phosphorylated in the cells that are resistant to crizotinib. Upon siRNA knockdown to ALK or using the ALK inhibitor, the the Akt phosphorylation at serine 473 was abrogated in the sensitive cell line while unaffected in the resistant cell lines. Concurrently, the transcript level of Akt downstream target proteins such as PAK1 (p21 protein (Cdc42/Rac)-activated kinase 1) were substantially downregulated in the sensitive cell line upon ALK knockdown. Additionally, there was a dramatic increase in the transcript of some tumor suppressor genes such as Caspase 9 and CDKN1B (Cyclin-dependent kinase inhibitor 1B).

Conclusion: ALK is a critical oncogene in the pathogenesis of neuroblastoma and plays different roles depending on the form of ALK variant. ALK mediates its oncogenic effect through Akt signaling pathway in ALK-positive neuroblastoma cells that harbor ALK amplification genotype.
57. Autotaxin inhibition suppresses inflammatory cytokine production in mammary adipose tissue and delays breast tumor growth and metastasis and in mice

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Introduction: Breast cancer is the most common malignancy among women and one-third of patients die from metastases once their cancers become resistant to therapy. We provide evidence that the lipid growth factor, lysophosphatidate (LPA), contributes to this treatment failure. Extracellular LPA is produced by the secreted enzyme autotaxin (ATX). ATX is important in tissue remodeling and wound repair, but ATX is overproduced in many inflammatory diseases including cancers. LPA promotes cancer progression, metastasis and resistance to chemotherapy and radiotherapy. No treatment currently targets LPA signaling and this provides an opportunity for introducing novel cancer therapies.

Methods: Female BALB/c mice were injected in breast adipose tissue with 20,000 4T1 BALB/c breast cancer cells. Tumor growth was monitored with caliper measurements and by weighing once excised. ATX mRNA was quantified by RT-PCR and activity was measured. We evaluated the efficacy of ONO-8430506, a novel oral ATX inhibitor (Ono Pharmaceuticals, Japan) by daily gavage. LPA species in plasma and tumors were measured by mass spectrometry. Cytokines in plasma, tumors and mammary adipose tissue were measured by enzyme-linked immunofluorescence assay (ELISA).

Results: 1) Breast adipose tissue expresses >10,000-fold more ATX mRNA than 4T1 breast cancer cells. Adipose tissue ATX activity was induced 3-fold more by the adjacent tumor compared to the contralateral unaffected breast. 2) ONO-8430506 decreased plasma ATX activity by >90%, and almost completely decreased unsaturated LPA concentrations in plasma and tumors. 3) Initial tumor growth and subsequent lung metastasis was decreased by about 60% by ONO-8430506. 4) ATX inhibition decreased tumor and systemic pro-inflammatory cytokine levels such as TNF-α. 5) TNF-α potently increases ATX secretion from breast fibroblasts, which produce the majority of ATX in the breast tumors.

Conclusions: This study demonstrates for the first time that inhibiting ATX activity decreases breast cancer progression. Breast cancer cells produce negligible ATX relative to mammary adipose tissue. Instead, our work presents a novel paradigm where breast cancer progression depends on inflammation-induced ATX production from fibroblasts in the tumor stroma and the surrounding fat pad rather than the cancer cells themselves. ATX inhibition provides a completely new therapeutic strategy for breaking this vicious cycle of inflammatory cytokine and LPA production, which drives breast tumor growth, treatment resistance and metastasis. We now hope to translate this work in a first in man trial for an ATX inhibitor.

58. Prevalence and Interest in Extreme/Adventure Activities Among Gynecologic Cancer Survivors: Associations with Posttraumatic Growth

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Objective: Exercise is associated with posttraumatic growth in gynecologic cancer survivors (GCS) but the role of extreme/adventure activities has not been investigated. The primary objective of this study was to examine the association between extreme/adventure activities and posttraumatic growth in GCS.

Methods: A Canadian provincial registry generated a random sample of 2,064 GCS stratified by cancer type (i.e., cervical, endometrial, and ovarian) who were mailed a self-report survey that assessed demographic and medical variables, posttraumatic growth using several scales, participation and interest in extreme/adventure activities, and exercise growth (i.e., the extent to which the cancer diagnosis itself prompted participation in more challenging, novel, and/or high risk activities).

Results: Of 621 GCS, only 12.1% reported participating in extreme/adventure activities in the past year. Of 309 GCS interested in a future exercise study, 41.1% were interested in trying extreme/adventure activities. After adjustment for key covariates, neither participation nor interest in extreme/adventure activities were associated with posttraumatic growth. All exercise growth items, however, were significantly associated with all posttraumatic growth scales (all p’s<0.05). In multivariate regression analyses, exercise growth items explained 37.2 % of the variance in the posttraumatic growth inventory, 7.2% of the variance in the negative impact of cancer scale, 19.9% of the variance in the positive impact of cancer scale, and 23% of the variance in the benefit finding scale (all p’s<0.001).

Conclusion: Participation and interest in extreme/adventure activities were not associated with posttraumatic growth, however, exercise growth was strongly associated with posttraumatic growth in GCS.
### 59. Syntheses and Evaluation of Carbon-11- and Fluorine-18-Radiolabeled pan-Tropomyosin Receptor Kinases (Trk) Inhibitors: Exploration of the 4-aza-2-Oxindole Scaffold as Trk PET Probes for Cancer Imaging

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Tropomyosin receptor kinases (TrkA/B/C) are critically involved in the development of the nervous system, in neurological disorders as well as in multiple neoplasms of both neural and non-neural origins. In particular, overexpression of TrkA/B/C tyrosine kinases is implicated in the growth and survival of various human tumors such as neuroblastoma as well as breast and pancreatic cancers. In many Instances, expression of specific Trk isoforms is also a major predictor of poor prognosis and aggressiveness of the disease. Trk receptors therefor represent an actively investigated therapeutic target in oncology. In this context, the development of radiopharmaceuticals specifically targeting Trk would represent a powerful tool to investigate in vivo and non-invasively the specific roles of Trk receptors under pathological conditions. To that end, we first developed [11C]GW441756, a high affinity photoisomerizable pan-Trk inhibitor as a lead radiotracer for our PET program. Efficient carbon-11 radiolabeling afforded [11C]GW441756 in high radiochemical yields (isolated RCY, 25.9 ± 5.7 %). In vitro autoradiographic studies in rat brain and TrkB-expressing human neuroblastoma cryosections confirmed that [11C]GW441756 specifically binds to Trk receptors in vitro. MicroPET studies revealed that binding of [11C]GW441756 in the rodent brain is mostly nonspecific despite initial high brain uptake (SUVMAX = 2.0) and provided detailed biodistribution informations. Modeling studies of the 4-aza-2-oxindole scaffold led to the successful identification of a small series of high affinity fluorinated and methoxy derivatized pan-Trk inhibitors based on our lead compound. Out of this series, a fluorinated compound was selected for initial evaluation and radiolabeled with fluorine-18 (isolated RCY, 2.5 ± 0.6 %). This compound demonstrated excellent Trk selectivity in a panel of cancer relevant kinase targets and a promising in vitro profile in tumors and brain sections but high oxidative metabolic susceptibility leading to nonspecific brain distribution in vivo. The information gained in this study will guide further exploration of the 4-aza-2-oxindole scaffold as a lead for Trk PET ligands development.

### 60. Oncolytic vaccinia virus for breast cancer therapy

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Introduction: Breast cancer (BrCa) continues to be one of the leading causes of deaths in women worldwide. Oncolytic viruses designed to selectively kill cancer cells, may emerge as an alternative treatment for BrCa. Vaccinia virus (VV), a large DNA virus, has a proven safety record in human from its use as a vaccine against smallpox. VV encodes more than 200 genes, some of which are involved in deoxyribonucleoside triphosphate (dNTP) synthesis. These genes are critical for replication of the virus in non-cycling normal cells which typically display low levels of dNTP synthesis. Deletion of these viral genes should impair virus replication in normal cells but not in cancer cells since cancer cells have high levels of dNTP synthesis. F4L, homologous to cellular ribonucleotide reductase (R2), is an important viral gene involved in dNTPs synthesis that could be deleted to restrict virus replication to cancer cells. We hypothesize that F4L-mutant VV will be able to replicate in and kill cancer cells with high levels of R2 such as BrCa and it will be harmless to normal cells with low levels of R2.

Methods: Mutant VVs were generated by homologous recombination method. Plaque assay and alamar blue assay were used to determine viral titer and resulting cytotoxicity, respectively. In vitro specificity of the mutant viruses were studied in 2-dimensional and 3-dimensional (spheroid) cell culture using confocal microscopy. siRNA against R2 was used to show the dependence of F4L-mutant on cellular R2 levels. Syngeneic and xenograft model of BrCa in mice were used to test the safety and anti-tumor efficacy of the mutant viruses in vivo.

Results: Replication of F4L-mutant VV depends on the level of cellular R2 which is elevated in BrCa cells. In vitro F4L-mutant VV replicates to high levels and kill BrCa cells while being relatively harmless to non-cancerous cells. In mice, the virus titer was found to be very high in tumor but was undetectable in normal organs (liver, lungs and spleen). Moreover, the virus was able to significantly delay the tumor progression and increase the overall survival of mice both in case of xenograft and syngeneic mice models of BrCa.

Conclusions: Results from our in vitro and in vivo experiments support the hypothesis that F4L-mutant VV specifically replicates in and kill BrCa cells. F4L-mutant VV, therefore, holds a promise to be tested in human for the treatment of BrCa.

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Introduction: Constitutively activated signal transducer and activator of transcription 3 (STAT3) in tumor and dendritic cells (DCs) plays an important role in tumor progression and tumor-induced immunosuppression. JSI-124 has been identified as a JAK/STAT3 inhibitor with potent antitumor activity. The objective of this research was to develop a nanocarrier for the delivery of JSI-124 to cancer cells and immunosuppressed DCs in the tumor microenvironment.

Methods: JSI-124 was conjugated to the core of PEO-b-poly(ε-carboxyl-ε-caprolactone) (PEO-b-P(CL-JSI-124) to form PEO-b-P(CL-JSI-124). The PEO chains were 12 kDa, and the P(CL-JSI-124) chains were 1.5 kDa. Micelles were characterized for their critical micelle concentration (CMC), self-assembled to micelles in aqueous solution. Micelles were characterized for their size, critical micelle concentration (CMC), and release of JSI-124. The cytotoxicity of PEO-b-P(CL-JSI-124) was investigated against murine B16-F10 melanoma cells and bone marrow-derived DCs (BMDCs) from mice using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. The activity of PEO-b-P(CL-JSI-124) micelles in suppressing phosphorylated-STAT3 (p-STAT3) in B16-F10 cells was investigated by FACS. Untreated BMDCs and those treated with B16 supernatant were incubated with polymeric micellar or free JSI-124 and investigated for DC maturation markers (CD40, CD86) and pSTAT3 expression using FACS. Release of cytokines from BMDCs (IL-12, IL-6, TGF-β1) or following co-incubation of DCs with T cells (IL-2, IFN-γ) was examined using ELISA.

Results: The conjugated JSI-124 content in the polymeric micelles was 8.8-8.9% w/w polymer as confirmed with HPLC and 1H NMR. The average diameter and polydispersity of the micelles as determined by dynamic light scattering (DLS) was 41.42 nm and 0.357, respectively. The CMC in aqueous media was 7.97 µM. The release of JSI-124 from PEO-b-P(CL-JSI-124) was very slow. By day 14, only 5.5% of JSI-124 was released from micelles, whereas, free JSI-124 showed 100% release within 8 h under experimental condition. Treatment of B16-F10 cells and BMDCs with free or conjugated JSI-124 resulted in significant loss of cell viability and suppression of p-STAT3 levels in a dose-dependent manner. In B16 cells, polymeric micellar JSI-124 was 4.5 times less potent than free drug. In BMDCs, polymeric micellar JSI-124 was ~70 times less cytotoxic than free drug, but >20 times more potent than free JSI-124 in suppressing p-STAT3 levels. Both PEO-b-P(CL-JSI-124) and free JSI-124 showed upregulation of CD40 and CD86 in BMDCs. However, PEO-b-P(CL-JSI-124) significantly increased secretion of IL-12 and decreased secretion of IL-6 and TGF-β1 compared to free JSI-124. PEO-b-P(CL-JSI-124) also significantly increased secretion of IL-2 and IFN-γ compared to free JSI-124 by T-cells.

Conclusion: These findings show the advantage of nano-particulate JSI-124 over free drug in reversing the immunosuppressive effect of STAT3+ tumors on DCs leading to Th1 immune response.

62. The anti-apoptotic form of tyrosine kinase Lyn that is generated by proteolysis is degraded by the N-end rule pathway

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The activation of apoptotic pathways results in the caspase cleavage of the Lyn tyrosine kinase to generate the N-terminal truncated LynΔN. This LynΔN fragment has been demonstrated to exert negative feedback on imatinib induced apoptosis in chronic myelogenous leukemia (CML) K562 cells. Our investigations focus on LynΔN stability and how reduced stability reduces imatinib resistance. As the proteolytical generated LynΔN has a leucine as an N-terminal amino acid, we hypothesized that LynΔN would be degraded by the N-end rule pathway. We demonstrated that LynΔN is unstable and that its stability is dependent on the identity of its N-terminus. Additionally we established that LynΔN degradation could be inhibited by inhibiting either the proteasome or knocking down the UBR1 and UBR2 ubiquitin E3 ligases. Importantly, we also demonstrate that LynΔN degradation by the N-end rule counters the imatinib resistance of K562 cells provided by LynΔN expression. Together our data suggest a possible mechanism for the N-end rule pathway having a link to imatinib resistance in CML. With LynΔN being an N-end rule substrate, it provides the first example that this pathway can also provide a pro-apoptotic function as previous reports have currently only demonstrated anti-apoptotic roles for the N-end rule pathway.
Pattern of change in visceral and subcutaneous adipose tissue mass in advanced cancer patients

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The majority of advanced cancer patients experience adipose tissue loss during cancer progression. Differences between visceral and subcutaneous patterns of change have not been consistently demonstrated. The objective of this study was to assess the intensity and time course of changes in visceral and subcutaneous adipose tissue of advanced cancer patients in the year preceding death. Longitudinal quantitative analysis of computed tomography (CT) images for loss or gain of adipose tissue depots were conducted in advanced colorectal and cholangiocarcinoma cancer patients (n=57) at intervals spanning 9, 6, 3 and 1 month before death. Changes in visceral (VAT), subcutaneous (SAT) and total adipose tissue (TAT) were calculated as the absolute loss or gain of the tissue (change in cross sectional area) and as the rate (change/100d) for each time interval. On average, fat loss is occurring at all time intervals but the intensity of loss increases as patients approach death. Stratification of patients into fat stable, losing and gaining groups showed that fat gain or stability of tissue occurs in some patients mainly at 9 and 6 months prior to death. Nine months from death 42% of patients were losing fat (TAT mean rate of loss= -6.5±8.4 cm2/100d) whereas within one month of death, fat wasting was observed in 78% of patients (-58.6±9.5 cm2/100d). To further elucidate the change occurring in each depot, patients were also classified into VAT and SAT losing, gaining and stable groups. Interestingly, 9 months before death, VAT loss was accompanied by SAT gain whereas, within one month of death loss of both VAT and SAT were predominant. In conclusion, further away from death, VAT and SAT behave differently whereas close to death, the largest and the most accelerated loss occurs for both depots. Identifying the time course of changes and the intensity of VAT and SAT change over the disease trajectory may help to define the onset of wasting and to design interventions to prevent weight loss.

A comparison of physical activity preferences among breast, prostate, and colorectal cancer survivors in Nova Scotia, Canada

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Purpose: Physical activity (PA) preferences may vary by cancer survivor group but few studies have made direct comparisons. The purpose of this study was to identify and compare the PA preferences of breast, prostate and colorectal cancer survivors in Nova Scotia, Canada.

Methods: A stratified sample of 2063 breast, prostate and colorectal cancer survivors diagnosed between 2003-2011 were identified by the Nova Scotia Cancer Registry (NSCR) and mailed a questionnaire assessing PA preferences and standard demographic and medical variables.

Results: A total of 741 completed surveys were received. Overall, Nova Scotian cancer survivors preferred to receive PA information from a fitness expert associated with a cancer centre (51%); via print materials (61%); start a PA program 3-6 months after treatment (34%); engage in PA with friends (53%) or a spouse (50%); at home (55%) or outside around the neighbourhood (67%); in the morning (55%); and at moderate intensity (65%). Chi-square analysis revealed some significant differences in preferences based on cancer site with breast and colorectal cancer survivors more likely to prefer engaging in PA with their friends than prostate cancer survivors (p<.001) and breast cancer survivors more likely to prefer supervised and group PA than prostate and colorectal cancer survivors (p<.001). Differences were also found within each survivor group based on demographic and medical variables including PA behavior, age, and perceived general health.

Conclusions: Breast, prostate, and colorectal cancer survivors have some differences in PA preferences that may influence targeted PA program interventions.
65. Endothelial cell mTOR complex-2 regulates sprouting angiogenesis in vitro

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Vascular endothelial growth factor/-receptor (VEGF/VEGF-R) signaling mediates vascular development and re-vascularization of ischemic tissue and is a key target in cancer therapy. In the endothelium, VEGF/VEGF-R couples to phosphatidylinositol 3-kinase to signal to Akt1 and mammalian target of rapamycin complex-1 (mTORC1). The function(s) of endothelial mTORC2 positioned upstream of Akt and mTORC1 is poorly defined. We sought to investigate the role of mTORC2 in sprouting angiogenesis. We used pharmacological inhibitors and RNA interference to isolate function of mTORC2, Akt1, and mTORC1. Angiogenesis was evaluated in vitro. To elucidate the mechanism of mTORC2-dependent events in angiogenesis, we studied migration, cytoskeleton reorganization, and focal adhesion formation in mTORC2-inactivated ECs. We correlated these with candidate regulatory signaling events. In primary human ECs, sustained inactivation of mTORC1 activity paradoxically up-regulated Akt1 activation that was dependent on mTORC2 activity. mTORC1/2 dual inhibition or selective mTORC2 inactivation in human ECs in vitro inhibited angiogenesis more effectively than mTORC1 inhibition alone. EC migration was impacted greater by mTORC2-inhibition than that observed in Akt1- or mTORC1-deficient ECs. mTORC2 inactivation robustly suppressed VEGF-stimulated cytoskeleton reorganization of adherent EC. Further, mTORC2 inactivation perturbed EC focal adhesion formation, and activation of focal adhesion kinase, independent of Akt1. In conclusion, endothelial mTORC2 regulates angiogenesis by regulation of EC focal adhesion kinase activity, matrix adhesion, and cytoskeletal remodeling, independent of Akt/mTORC1.

66. DEAD box 1: The deep(-seq) secrets of Drosophila shed light on the black box of cancer

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DEAD box 1 (DDX1) is a member of the DEAD box family of RNA helicases. DDX1 is co-amplified with MYCN and over-expressed in a subset of retinoblastoma and neuroblastoma tumors and cell lines. Recently, over-expression and mis-localization of DDX1 has been shown to be associated with a poor prognosis in breast cancer.

Using mouse models, we have shown that Ddx1 knock-outs are early embryonic lethal. In contrast to mouse, knock-out of Ddx1 in Drosophila produces viable progeny which are smaller in size than their wild-type counterparts. Gametogenesis is affected in knock-out flies, with females producing no eggs and males displaying aberrant sperm development. We have also observed disrupted TOR signaling in the absence of Ddx1. By utilizing next generation RNA sequencing of our knock-out and control Drosophila lines, we have identified those transcripts which have modified levels. Notably, we have identified many genes involved in metabolism and growth. In addition to this, we observed a widespread alteration in splicing patterns. Among genes with modified splice patterns, we identified Sirup, which has previously been associated with starvation phenotypes. These results suggest a role for Ddx1 in the regulation of gene splicing, with the ultimate phenotypes we observed due to the aberrant regulation of Ddx1 target genes. Of particular interest is the prospect that Ddx1 may act as a positive regulator of metabolic signalling factors. This possibility may be mechanism underlying the negative prognostic outcome of cancer patients with higher than normal DDX1 levels. Further work into the function of Ddx1 throughout normal Drosophila development will continue to increase our understanding of the role of DDX1 in cancer development and progression.
67. Alternative splicing of human NODAL
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NODAL is a TGF-beta superfamily member important in embryonic development, and aberrantly expressed in numerous cancers, including breast cancer, where it contributes to tumour progression.

There is currently only one annotated human NODAL isoform. However, using qRT-PCR analysis of human pluripotent stem cell lines, and a minigene splicing reporter plasmid, we demonstrate the alternative splicing of a novel transcript for human NODAL. Furthermore, this alternative splicing is regulated by a single nucleotide polymorphism (SNP).

Interestingly, the allele responsible for splicing the novel NODAL isoform is under-represented in male human embryonic stem (hES) cell lines and is positively correlated with XIST expression in female hES cell lines. This suggests that alternative splicing of NODAL may be contributing to dysregulation of X chromosome inactivation—a process that has been tied to cancer susceptibility and pathology.

Ongoing work is also investigating endogenous expression of this novel NODAL isoform in breast cancer cell lines, its role in NODAL-induced tumour progression, and its correlation with clinical outcomes in breast cancer patients.

This work is an example of how genetic polymorphisms can contribute to the heterogeneity and complexity of tumour biology.

68. Regulation of Transcription Factor AP2 in Malignant Glioma
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Malignant gliomas (MG) are highly invasive brain tumours with a dismal prognosis in spite of aggressive treatment involving surgical resection followed by radiotherapy and chemotherapy. Activator protein 2 (AP2) is a family of five transcription factors (AP2α, β, γ, δ and ε) that regulate the expression of genes important for early development, cellular growth, differentiation and apoptosis. AP2 genes have been shown to be retinoic acid and cAMP responsive. cAMP levels in gliomas are lower than normal brain, which may lead to reduced AP2 activity. Phosphodiesterase inhibitors such as Rolipram (increases intracellular levels of cAMP) have been shown to inhibit MG cell growth and invasion. AP2 is primarily localized in the nucleus of normal cells. Intriguingly, loss of nuclear AP2α expression and increased localization to the cytoplasm has been reported in high grade astrocytomas. My recent immunofluorescence and nuclear-cytoplasmic fractionation analyses have revealed that, like A2α, AP2β and AP2γ are also located in the cytoplasm of MG cells. In preliminary experiments, I have found that Rolipram increases the nuclear localization of AP2γ, suggesting a direct link between increased cAMP levels and the subcellular localization of AP2’s. Thus, the previously reported effects of Rolipram on tumour growth inhibition may be mediated through AP2 subcellular localization.

Based on recent data, AP2β and AP2γ may also be sumoylated in MG cells. Furthermore, the sumoylated form of AP2 may preferentially localize to the nucleus of MG cells. Hypothesis: Alterations in the subcellular location of AP2s combined with sumoylation of AP2 proteins in MG inhibits the transcription of AP2 target genes involved in migration and invasion.

My first objective is to further investigate AP2 subcellular localization and sumoylation in MG cells and to determine their effect on MG gene regulation and growth properties. AP2 overexpression and knockdown experiments in the presence or absence of Rolipram and/or SUMO inhibitors will be carried to determine their effects on: (i) the expression of AP2 target genes, and (ii) MG cell growth properties such as proliferation, migration, invasion and apoptosis. My second objective is to study the combined effect of Rolipram and SUMO inhibitors on tumour growth and survival in MG mouse models.

Treatment with Rolipram in combination with radiation therapy and temozolomide has been shown to enhance the survival of mice bearing intracranial xenografts. Combining Rolipram treatment with sumoylation inhibitors, along with traditional radiotherapy and chemotherapy, may improve clinical outcome.
**Poster Presentations**

### 69. The energetic cost of cancer as a result of increased fermentation: implications for cachexia

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Cachexia affects most patients with late stage cancer, is associated with reduced survival, and is a leading attributed cause of death of cancer patients. While much research has investigated the causes of cancer cachexia, the precise mechanisms causing cachexia are still poorly understood. Concurrently, it is increasingly documented that tumors function with elevated fermentation. To derive an estimate of the specific energetic cost of cancer per unit of mass, we model the energetic cost of cancer as a function of the percentage of energy it produces anaerobically. Based on quantitative data from a range of cancer patients, if 25% of energy is generated by the tumor anaerobically, the energetic cost on the body from the tumor is estimated at 394 kcal/day per kg of tumor. Substrate use by the tumor predicts the tumor’s high level of glucose and glutamine consumption causes muscle breakdown to fuel the tumor, especially in the fasting state. Our model suggests the energetic drain caused by the tumor is substantial when anaerobic energy metabolism is taken into account, and that elevated fermentation in cancer cells may be a key contributor to cancer cachexia. Monitoring glucose and glutamine consumption of tumors, along with patient resting energy expenditure and Cori cycling, would potentially predict the onset of cachexia.

### 70. Polymeric Micelles for Tumor Targeted Delivery of siRNA

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Introduction: The objective of this research was to develop a polymeric nanocarrier for the targeted delivery of siRNA to breast tumors following systemic administration.

Methods: Block copolymers of poly(ethylene oxide)-b-poly(ε-caprolactone) with grafted spermine (PEO-b-P(CL-g-SP)) were synthesized. The pendent spermine group was modified with cholesteryl substitution (PEO-b-P(CL-g-SP-Chol)). Block copolymers self-assembled to polymeric micelles with and without complexation with siRNA. The effect of cholesterol substitution on the micellar size, stability, siRNA binding, release, cellular uptake and cytotoxicity was investigated. MCL-1 siRNA was complexed in PEO-b-P(CL-g-SP) (MCL-SP) and PEO-b-P(CL-g-SP-Chol) (MCL-SP-Chol) micelles. The prepared siRNA formulations were assessed for their cytotoxicity and MCL-1 mRNA down-regulation in human MDA-MB-435 cells using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay and qPCR, respectively. Female nude mice bearing MDA-MB-435 xenografts were treated with intratumoral injections of MCL-SP and MCL-SP-Chol and their counterparts containing scrambled siRNA. Tumor growth was followed and MCL-1 mRNA expression in tumor was measured 24h after the last injection. Tumor bearing mice were also treated intravenously with MCL-SP and MCL-SP-Chol with or without RGD4C peptide modification and assessed for tumor growth, tumor MCL-1 mRNA expression, and major organ pathology.

Results: Attachment of cholesterol to PEO-b-P(CL-g-SP) enhanced the stability, siRNA uptake and viability of treated MDA-MB-435 cells. Cholesterol modification also enhanced the transfection efficiency of polymeric micellar complexes of MCL1 siRNA leading to improved silencing of MCL-1 and 2.5 fold reduction of cell viability after MCL-1 siRNA treatment. In mice, following intra-tumoral administration, both MCL-1 siRNA treated groups (MCL-SP and MCL-SP-Chol) showed delayed tumor growth compared to mice that received the vehicle or scrambled siRNA formulations. On day 10, MCL-SP and MCL-SP-Chol treated mice showed 3.5- and 5.4-fold decrease in tumor volume compared to control animals receiving vehicle. MCL-SP and MCL-SP-Chol treated animals showed 34% and 39% down-regulation in relative MCL1 mRNA expression in tumor compared to scrambled siRNA (no down-regulation). Following intravenous administration, MCL-SP and MCL-SP-Chol with or without RGD4C modification on surface showed a similar inhibition of tumor growth. On day 13, MCL-SP and MCL-SP-Chol with RGD4C modification showed a 1.8- and 1.7-fold decrease in tumor volume as compared to 1.7- and 1.5-fold decrease, for groups without RGD4C modification, respectively. MCL-SP and MCL-SP-Chol with RGD4C on their surface showed 30% and 38% down-regulation in relative mRNA expression as compared to 15% and 4% mRNA down-regulation for micelles without RGD4C, respectively. Histology studies showed no signs of toxicity in liver, spleen, and kidney for all treatments.

Conclusion: These findings show the potential of developed nano-carriers for efficient delivery of siRNA to MDA-MB-435 tumors following intravenous administration.
71. Using Extracted Ion Chromatograms from LC-MS/MS Spectra to Semi-quantitatively Characterize in vivo Breast Tumour Proteome Changes in Response to the Chemotherapeutic Docetaxel

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In recent years, there has been a rising trend in profiling gene expression through the use of microarrays, qPCR, and deep sequencing technologies. These techniques have been applied to measure both in vitro and in vivo gene expression in various disease states, and how these levels change in response to specific stimuli/stresses. One caveat however, is that the measured mRNA abundance of expressed genes poorly correlates with the observed cellular protein concentrations. This lack of correlation exists for several reasons, notably post-transcriptional regulation of mRNA/translation and significant variation in proteins’ cellular half-lives. While most diseases ultimately boil down to their genetic components, the phenotypes, symptoms, and lethality often observed in diseases such as cancer are often due to the cellular protein machinery.

Currently, the literature contains a vast number of studies – largely genetic – that have been performed on tissue samples, looking at how individual genes and signaling pathways change in response to specific stimuli such as chemotherapy. To date, the literature is thin regarding investigations into how the proteome of a tissue changes in response to cytotoxic stress. Previously, this has been for good reason; one of the few truly robust methods of protein identification, mass spectrometric peptide sequencing, has been limited in quantitative approaches due to the requirement of chemical labelling techniques such as iTRAQ and ICAT. These additional steps in sample handling greatly reduce the amount of protein recovery when performing whole-proteome analysis. However, recent advances in mass spectrometry technology have led to the development of label-free techniques for protein quantitation. Here we describe a label-free, semi-quantitative, comparative approach to determine relative changes in tissue protein abundance in response to cytotoxic stress using the extracted ion chromatograms (XICs) of proteins observed by liquid chromatography with tandem mass spectrometry (LC-MS/MS) analysis. To illustrate our technique, we have utilized a xenograft mouse model system, in which tumours comprised of MDA-MB-231 cells were grown in vivo and treated with either the microtubule interfering agent docetaxel, or a vehicle (13% ethanol). Our preliminary data identifies upwards of 1500 proteins per sample, and their relative changes in abundance when exposed to cytotoxic stress.

72. Transport of Arsenic Species by Single Nucleotide Polymorphic Variants of the Human Multidrug Resistance Protein 2 (MRP2/ABCC2)

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Arsenic and selenium are toxic compounds, however in vivo exposures to arsenite and selenite result in mutual detoxification. The molecular basis of this can be explained by the formation and subsequent biliary excretion of the seleno-bis(S-glutathionyl) arsonium ion [(GS)2AsSe]- by the ATP-binding cassette (ABC) transporter, multidrug resistance protein 2 (MRP2/ABCC2). The ABCC2 gene is highly variable; >50 single nucleotide polymorphisms (SNPs) have been identified. Many of these nonsynonymous SNPs, including those that result in the amino acid changes; Arg353His, Ser789Phe, Arg1150His, Arg1181Leu, Asp1244Lys, Pro1291Leu, Ala1450Thr and Thr1477Met have been shown to alter the toxicokinetics of clinically important therapeutic agents. This has led us to the hypothesis that these SNPs will display lower ability for [(GS)2AsSe]- transport in comparison to the wild-type MRP2. These variants were generated using site directed mutagenesis and expressed in HEK293T cells. Plasma membrane enriched vesicles were isolated and protein levels were determined by western blotting. All mutants were expressed with the exception of Ser789Phe, Pro1291Leu, Ala1450Thr and Thr1477Met. Protein levels for the remaining mutants were comparable to wild-type MRP2. Transport assays with well characterized substrates of MRP2; estradiol-17β-glucuronide (E217βG) and arsenic triglutathione (As(GS)3) were performed to confirm functionality of vesicles. Future directions of this study are to confirm cellular localization of mutants with variable expression and to characterize [(GS)2AsSe]- transport activity.
Poster Presentations

73. Cachexia evolution in renal cell carcinoma patients and its relation with cardiac ejection fraction evaluated by MUGA scan

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Introduction: Cancer cachexia is a continuum, progressing from absent, to early stage (pre-cachexia) to cachexia, which can then go on to be moderate, severe or refractory. Cachexia is characterized by skeletal muscle loss, and it has recently been suggested that cardiac muscle also atrophies and shows functional loss such as reduced left ventricular ejection fraction (LVEF) in cachexia.

Material and Methods: In this retrospective chart review of renal cell carcinoma (RCC) patients, lumbar L3 CT scan images were used to evaluate skeletal muscle (SM) and adipose tissue (AT) loss over time. Multi gated acquisition (MUGA) scan-defined LVEF were abstracted from medical charts.

Results: Representing the early disease trajectory, 13 patients (55.9±9.6 y, 8 males) from a randomized phase III trial of adjuvant therapy (sunitinib vs sorafenib vs placebo) in resected RCC were evaluated. During 11.0±2.2 months on treatment only 2/13 patients (15%) showed L3 muscle loss (>6 cm2 (~1 kg total muscle)) on CT and also only 2 out of 13 patients showed over all tissue loss (SM+ total AT). All MUGA scans (n=4/patient) were within a normal range during the same time.

Representing more advanced disease, 11 patients (61.8±8.9 y; 9 males) with metastatic disease treated with sunitinib on different clinical trials were evaluated. During 10.4±2.5 months on treatment, 6/11 patients (54%) developed muscle loss > 6 cm2 (17.2±9.3 cm2) and 4/11 had an abnormal MUGA (EF <50% or fell by >10%). Muscle loss and abnormal MUGA findings were overlapping in 3 patients.

Conclusion: RCC patients eligible for clinical trial participation in an adjuvant setting had a low likelihood of cachexia, muscle loss (15%) or altered cardiac EF (0%). In a metastatic setting, muscle loss (54%) and LVEF impairment (36%) were more prevalent and were overlapping phenomena. Further studies are needed to verify these findings and to probe the relationship between muscle loss and LVEF impairment.

74. Glutathione S-transferases P1 (GSTP1) is post-translational modified and regulated by palmitate

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Glutathione S-transferase P1 (GSTP1) is a phase II enzyme that protects cells from carcinogens by catalyzing their conjugation with the tripeptide glutathione (gamma-Glu-Cys-Gly). In addition, GSTP1 is involved in cell signalling and is important for regulating cell proliferation and apoptosis. Overexpression of GSTP1 in tumours is associated with drug resistance and poor prognosis. In contrast, inactivation of GSTP1 due to epigenetic promoter silencing or reduced activity due to single nucleotide polymorphic variants is associated with increased susceptibility to certain cancer types. GSTP1 is classically described as a cytosolic enzyme; however, we have reported that GSTP1 is strongly associated with the plasma membrane and the strength is comparable with an integral membrane protein. We hypothesize that the addition of a hydrophobic component is required to allow its strong interaction with membranes. Palmitoylation is the reversible post-translational addition of a 16-C saturated fatty acid to proteins, most commonly on Cys residues through a thioester bond. This addition can be catalyzed by palmitoyltransferases (PAT) and allows the protein to anchor to cellular membranes. In the current study, we found that GSTP1 is modified by palmitate. GSTP1 has four Cys residues (Cys15, Cys48, Cys102 and Cys170), and a 4X Cys-less (Cys:Ser) mutant expressed in MCF7 cells surprisingly retained labelling with palmitate. In vitro palmitoylation with purified GSTP1 from E. coli demonstrated that a PAT is not required for palmitoylation and N-ethylmaleimide pre-treatment prevented labelling (suggesting a Cys residue is modified). In contrast with this data, treatment of autopalmitoylated E. coli GSTP1 with NaOH 0.1 M, which should cleave thioester bonds, enhanced palmitoylation. Functional assays demonstrated that palmitate up regulated the catalytic activity of GSTP1 purified from MCF7 cells whereas GSTP1 purified from E. coli was inhibited. This ambiguity could be due to a different post-translational modification profile of GSTP1 between eukaryotic and prokaryotic cells. These results suggest that GSTP1 is modified by palmitate at multiple sites including at least one non-Cys residue and that palmitoylation alters catalytic function. Future work will investigate the influence of palmitoylation on GSTP1 cellular localization and cell signalling, identify the specific amino acid(s) modified by palmitate and further define the differences between E. coli and MCF7. Understanding the basic biology of GSTP1 is important information for the prevention and treatment of many types of cancer. Supported by Canadian Institutes of Health Research, Alberta Cancer Foundation and Alberta Innovates Health Solutions.
**75. Germline copy number variations as genetic susceptibility determinants in Sporadic Breast Cancer**

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Background: Breast cancer (BC) susceptibility has genetic and environmental components. While familial BC is explained by heritable high penetrant mutations in Breast Cancer (BRCA) and other moderate penetrant genes, genetic predisposition to sporadic BC is largely unexplored. Copy number variations (CNVs) are an important class of polymorphisms. CNVs cover larger regions of the genome, may harbor gene regulatory elements or may function through gene dosage. We hypothesize that CNVs explain the heritable basis for sporadic breast cancer risk in populations.

Methods: Whole genome CNV profiles from germline DNA were generated on Affymetrix SNP 6.0 arrays. Quality assurance steps were carried out using Golden Helix SVS. Eigenstrat method was adopted for correcting population stratification. CNV analysis was carried out using Partek genomics suite 6.6 (genomic segmentation algorithm) using HapMap 270 samples to create a reference baseline (diploid copy status). For Stage 1 of the study, we used samples from The Cancer Genome Atlas project (TCGA, 308 BC cases) and Welcome Trust Case Control Consortium (WTCCC, 408 controls). For stage 2 of the study, we used 348 BC cases and 348 controls (in-house). All study subjects are women of European ancestry. Chi-square tests for association were used with a cut-off p-value of 0.05 as significant. The study was approved by institutional ethics board.

Results: In Stage 1 and 2 samples, we detected a total of 224,986 and 260,627 CNVs, respectively and these were tested for association with BC. Statistically significant (p<0.05) associations were observed for structural aberrations (copy gains or copy loss regions). Total aberrations (gain + loss) were 32,466 and 7,827 in the two independent data sets, respectively. We also observed 4,076 CNV aberrations that are common between the samples sets from the two stages of the study and pathways involved in BC are being investigated (Gene Ontology Classifications). We observed 300 CNVs in Stage 1 study showing very high frequency gains in cases only relative to controls. We tested for these CNVs in Stage 2 study and found 29 CNV gains to be statistically significant indicating that the high frequency gains are indeed reproducible. The 29 CNV regions span the RET, CSMD1, KIT, FERD3L, MEGF11 genes and are reported in literature with functional roles in BC etiology.

Conclusion: The concordant CNV signatures from germline DNA in independent data sets reflect reproducibility and robustness of the identified associations. These signatures will be validated using qRT-PCR.

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**76. Characterization and mechanism of EGFL7 and its role in regulating angiogenesis.**

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EGFL7 (or Epidermal growth factor like domain 7) is an angiogenic factor that is secreted by and almost exclusively works on endothelial cells. EGFL7 expression is highly elevated in tumours, including kidney tumours, malignant gliomas, hepatocellular carcinomas, and colon cancers. EGFL7 is comprised of three domains: the Emilin-like (EMI) domain, Epidermal Growth Factor-like (EGF) domain and the Matrilin (Mat) domain. In our studies, we have found that overexpressing EGFL7 (in cancer cell lines HT1080 and MDA-MB231) inhibits angiogenesis in vitro and in vivo. HT1080 cells over expressing EGFL7 formed significantly smaller tumour compared to the wildtype control in nude mice and chicken embryos. The same phenomenon was observed in the MDA MB 231 breast cancer cells. Ea.hy 926 endothelial cells, when exposed to HT1080 overexpressing cells, showed significant reduction in the number of sprouts (angiogenesis) compared to the control cells in the in vitro angiogenesis assay.

Deleting the Emilin-like (EMI) domain from the EGFL7 protein leads to a reverse in this phenotype, suggesting that the EMI domain is the functional domain for the EGFL7 protein. No significant changes were observed when the EGF or the Matrilin domains were deleted.

The exact mechanism in which EGFL7 functions is still unknown but the protein has been implicated to bind Lysyl Oxidase (LoxL2), αVβ3 integrin and Notch receptors (Notch1). Our next aim is to determine the interacting partners for EGFL7 and the specific domains that the interacting partners bind to.
77. Identification of microRNAs as Prognostic Markers for Breast Cancer

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Introduction: Identifying optimal biomarkers for a subset of early stage breast cancer (BC) patients who may be at potential risk for recurrence of the disease is of paramount importance to guide therapeutic decisions. Implementing aggressive therapies long before the disease recurs may improve outcomes. Although ER, PR and HER2 currently provide prognostic value, these alone are not sufficient to identify those at risk since tumor heterogeneity (molecular and histological) and inter-individual variations to treatment response also contribute to treatment failure, leading to poor clinical outcomes (Overall survival, OS and Relapse Free Survival, RFS). MicroRNAs (18-25nt) are small non-coding RNAs, which regulate gene expression by either mRNA degradation or by translational repression and have shown promise as potential prognostic markers. We hypothesize that differential expression levels of miRNAs in tumors contribute to inter-individual variations in response to treatment and eventually outcomes. The objectives of the study are to identify prognostic miRNAs using next generation sequencing (NGS) platform.

Methods: Total RNA extracted from Formalin fixed paraffin embedded tissues of 104 tumor (Luminal A, n=74 and Triple Negative Breast Cancer (TNBC), n=30) samples were subjected to NGS (Illumina Genome Analyzer IIx) and analyzed using Partek Genomics Suite software 6.6. RNAs were filtered for low read counts and only those with a minimum of 10 read counts in at least 90% of the samples were retained (149 miRNAs). Cox’s proportional hazards regression model (SAS9.3) was performed for univariate and multivariate analyses (adjusting for confounders) to identify prognostic miRNAs associated with outcomes. p<0.05 was considered to be statistically significant for all the analyses.

Results: 25 miRNAs and 23 miRNAs were significant in univariate analysis for OS and RFS. hsa-miR-151a-3p (Hazard ratio, HR = 2.68), hsa-miR-181d-5p (HR = 0.313), hsa-miR-27a-3p (HR = 3.229), hsa-miR-363-3p (HR = 0.430) and hsa-miR-99b-5p (HR = 2.664) were found as potential independent prognostic factors for OS. hsa-miR-148b-3p (HR = 0.366), hsa-miR-181d-5p (HR = 0.406), hsa-miR-203a (HR = 0.379), hsa-miR-98-5p (HR = 5.493) and hsa-miR-99b-5p (HR = 3.290) were found as independent prognostic markers for RFS.

Conclusions: Except hsa-miR-27a-3p and hsa-miR-203a, other identified miRNAs have not yet been reported for BC prognostication. However, these miRNAs require further validation to ascertain their role as biomarkers.

78. Covalent inhibition of Ubc13 affects ubiquitin signaling and reveals active site elements important for targeting

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Ubc13 is an E2 ubiquitin conjugating enzyme that functions in nuclear DNA damage signaling and cytoplasmic NF-kB signalling. It has recently been shown to control breast cancer metastasis, as well it shows increased expression in nasopharyngeal carcinoma cells resistant to cisplatin. Upon depletion of Ubc13 in these resistant cells sister chromatid exchange is suppressed, which resensitizes them to cisplatin. Here we present the structures of complexes of Ubc13 with two inhibitors, NSC697923 and BAY 11-7082. These inhibitors have been shown to inhibit DNA damage and NF-kB signalling in human cells. NSC697923 and BAY 11-7082 both inhibit Ubc13 by covalent adduct formation through a Michael addition at the Ubc13 active site cysteine. The adducts of both compounds exploit a binding groove unique to Ubc13. We developed a Ubc13 mutant which resists NSC697923 inhibition and, using this mutant, we show that the inhibition of cellular DNA damage and NF-kB signalling by NSC697923 is largely due to specific Ubc13 inhibition. We propose that unique structural features near the Ubc13 active site could provide a basis for the rational development and design of specific Ubc13 inhibitors.
79. siRNA Therapy in Treatment of Acute Myeloid Leukemia (AML) Targeting CXCR4

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Treatment of acute myeloid leukemia (AML) with current therapies remains a challenge due to difficulties in high relapse rates, the emergence of resistance, and resulting toxic side-effects. Progress in AML drug development has lagged behind both with conventional drugs as well as with RNAi mediated therapies. The RNAi technology mediated by siRNA is a promising treatment method, where silencing expression of key proteins can result in decreased proliferation, increased apoptosis and/or increased chemotherapy drug effectiveness in leukemic cells. Specifically for AML, the CXC chemokine receptor 4 (CXCR4) and its ligand, stromal-cell derived factor-1 (SDF-1), are promising targets as they are associated with resistance to chemotherapy drugs and disease relapse due to their role in leukemic cell migration and anchorage to bone marrow and are also involved in leukemic cell survival [1]. However, development of clinically useful siRNA carriers is needed since siRNA on its own is an incompetent silencing agent. We developed lipid-modified polymers capable of delivering siRNA and silencing desired proteins in AML cells. Lipid-polymer carriers were synthesized with low molecular weight polyethyleneimine (PEI) as the backbone with lipids, caprylic acid, palmitic acid, oleic acid and linoleic acid, substituted onto amine groups [2]. The carriers are self-assembled with siRNA to form complexes at weight ratios (2-8) above the minimum required for complete siRNA binding (0.5). We have previously demonstrated and optimized delivery ability of the carriers in AML cells in vitro. The best performing siRNA carriers were then selected to demonstrate silencing of a reporter gene, GFP, which was characterized by polymers:siRNA ratio formulation, siRNA dose concentrations, and treatment time [3]. Our current focus is on the leukemic therapeutic target, CXCR4. Consistent silencing of CXCR4 was obtained utilizing the caprylic acid substituted PEI carrier and resulted in a decrease in cell proliferation and a small but significant decrease in adherence to bone marrow stromal cells (BMSCs). Although silencing SDF-1 (CXCR4 ligand) produced a similar decrease in proliferation, an enhanced effect was not observed when targeting both simultaneously. We furthermore demonstrated clinically related outcomes, such as effective CXCR4 silencing in the presence of bone marrow stromal cells and improved efficacy of a leukemic chemotherapy drug (cytarabine) when co-treated with CXCR4 siRNA. We conclude that decreasing CXCR4 expression via siRNA is a promising therapeutic option for AML therapy and provides an effective alternative to current approaches in pursuit.


80. The Post-transcriptional Regulation of Pluripotency in Breast Cancer by Hypoxia.

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Hypoxia in the tumor microenvironment promotes cancer growth, metastasis and resistance to treatment. Similarly, Nodal, an embryonic morphogen belonging to the transforming growth factor beta (TGFb) superfamily, has been identified as a factor enhancing metastasis in numerous cancer types. Our recent work has shown that Nodal is up-regulated by hypoxia in cancer. Discordantly, Nodal mRNA levels decrease under hypoxic conditions for the first 16h. Based on this discrepancy we hypothesize that Nodal is regulated by post-transcriptional mechanisms including increased ribosome binding, protein stability and miRNA regulation. Factors such as mTOR, 4E-BP, and eIF-2α, that control global rates of translation can allow for the translational up-regulation of specific transcripts in response to stress. This translational up-regulation is facilitated by increased ribosome binding to targeted mRNAs. Here, we examine post-transcriptional mechanisms by which hypoxia regulates Nodal by i) measuring the activity of the pathways regulating translation; ii) assessing protein stability; and iii) evaluating a method required to measure the amount of ribosome binding to mRNA termed polysome profiling. Nodal’s stability increased in low oxygen. Also the mTOR and the eIF2α pathways are active in hypoxia helping to elucidate another potential mechanism of up-regulation.
Purpose: Cancer cachexia contributes to reduced cancer treatment response rates, physical function, and overall survival through progressive depletion of the body’s energy and protein reserves. Involuntary weight loss (WL) is the cardinal diagnostic criterion for cancer cachexia. Existing definitions of clinically important WL in cancer patients are unclear, heterogeneous, and do not consider current trends towards obesity. We propose a robust classification for WL that is based on contemporary cancer patients, and that evaluates the prognostic significance of WL in patients with initially low, medium, and high body weights.

Methods: Canadian and European cancer patients (n=8160) formed a contemporary, population-based data set. Data were contributed from population-based cohorts, randomized clinical trials, and nutrition risk screening programs, and included: age, sex, cancer diagnosis, cancer stage, performance status (PS), survival, and BMI and %WL. All patients were followed prospectively until death. Data were entered into a multivariate analysis controlling for age, sex, cancer site, stage, and PS. Relationships for BMI and %WL to overall survival were examined to develop a grading system. An independent data set (n=2693) validated the grading system.

Results: Mean overall %WL was -9.7 ± 8.4 and BMI was 24.4 ± 5.1 kg/m2, and both %WL and BMI independently predicted survival (P<0.01). Differences in survival were observed across 5 categories of BMI (<20.0, 20.0-21.9, 22.0-24.9, 25.0-27.9, ≥28.0 kg/m2, P<0.001), and 5 categories of %WL: (-2.5 to -5.9%, -6.0 to -10.9%, -11.0 to -14.9%, ≥15.0%, weight stable (±2.4%), P<0.001). A 5x5 matrix representing the five %WL categories within each of the 5 BMI categories was graded based on median survival and prognostic significance. Weight stable patients with BMI ≥25 (Grade 0) had the longest survival (20.9 months, 95%CI 17.9-23.9), and %WL associated with lowered categories of BMI were related to shorter survival (P<0.001): Grade 1 (14.6 months, 12.9-16.2), Grade 2 (10.8 months, 9.7-11.9), Grade 3 (7.6 months, 7.0-8.2), Grade 4 (4.3 months, 4.1-4.6). Survival discrimination by grade was observed within specific cancers, stages, ages, and PS, and in the independent data set (Grade 0: 25.1 months, Grade 1: 15.7 months, Grade 2: 17.6 months, Grade 3: 11.5 months, Grade 4: 6.9 months, P<0.001).

Conclusion: A robust grading system incorporating the independent prognostic significance of both BMI and %WL was developed. The proposed grading system takes into account the impact of high versus low initial body weight in the risk assessment of cancer patients with WL.

82. Fas Ligand Protein is Expressed by CD8+ Cells Responding to Intraperitoneal Tumor Cells

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CD8+ Cytotoxic T lymphocytes (CTL) can recognize cancerous cells in the body and eliminate them via multiple effector mechanisms, including degranulation of granzymes and perforin, or expression of Fas Ligand (FasL) on the CTL surface. CTL can store FasL intracellularly, and the translocation of these stores to the cell surface is independent of degranulation. Whether the unique stimulus requirements for FasL expression indicates a potential role for FasL in response to tumors is not known. We hypothesize that FasL is expressed in CD8+ cells in response to tumor cells.

C57/B6 mice received adoptive transfers of naïve CD8+ OT-1 cells, which recognize the SIINFEKL peptide on H-2Kb MHC. 24 hours later, mice were injected intraperitoneally with 2.5x10^6 EG.7 lymphoma cells, which are recognized by OT-1 CTL. At 7, 14, and 21 days post-injection, mice were euthanized and lymphocytes in the peritoneum were collected. Cells were stained with fluorescent antibodies against surface and internal antigens prior to analysis by flow cytometry.

At all three time points, significantly more cells expressed Fas Ligand protein in internal stores than only Granzyme B. Stored FasL was present in antigen-specific CD8+ cells as detected by TCR Vα2 Vβ5 staining, as well as in the general population of activated CD8+ cells present in the peritoneum. Stored FasL was expressed in both effector and memory differentiated CD8+ cells. We also detected FasL on the surface of CD8+ cells in the peritoneum, and this surface expression of FasL is significantly greater in effector CD8+ cells compared to memory CD8+ cells.

We have shown that FasL is actively expressed by T cells in a peritoneal lymphoma model. Future experiments will focus on determining the contribution of FasL to clearance of tumor cells by CD8+ cells. This information may indicate future directions for adoptive immunotherapy of cancer.
83. Epigenetic modification of an endothelial specific gene, Von Willebrand Factor (VWF) activates its transcription in some cancer cells

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Background: VWF is an adhesive procoagulant protein that is specifically expressed in endothelial cell (EC) and megakaryocytes. It mediates adhesion of platelets to the damaged EC surfaces. Increased plasma levels of VWF and alterations in the blood clotting system in cancer patients with metastasis progression are reported. Immunohistochemical analysis of an osteosarcoma cell line suggested VWF expression by this cancer cell line. The importance of VWF in thrombosis, suggests a potential role for this protein in cancer metastasis. We hypothesised that a subpopulation of some cancer cells of non-endothelial origin may acquire VWF expression and as a result develop enhanced metastatic potential.

Method: Several glioma and osteosarcoma cell lines were analysed for expression of VWF mRNA and protein. The VWF chromatin interaction patterns of transacting factors (repressors and activators) that regulate VWF promoter, including histone modifying cofactors, were analyzed by chromatin Immunoprecipitation (ChIP) assays. In vitro cell-cell interaction assays were used to demonstrate the effect of VWF expression in cancer cells adhesion to platelet and EC surfaces. VWF expression in some patient’s glioma samples were determined.

Results: RT-PCR, western blot analyses and immunofluorescent staining showed significant levels of VWF expression in glioma (U251, M016, M049) and an osteosarcoma (Saos2) cell lines. An osteosarcoma cell line (KHOS) that did not express VWF was used as control for various analyses. ChIP assays demonstrated that VWF regulatory transcription factors bind to the VWF promoter in the VWF-expressing cancer cells in a pattern similar to that observed in EC. The epigenetic modification analysis showed that histone modification of the VWF promoter in glioma (U251, M016, M049) and Saos2 is consistent with transcriptional activation of the VWF promoter. In vitro cell-cell interaction analyses showed that cancer cell lines expressing VWF, exhibit increased adhesion to the platelets and an endothelial monolayer under the sheer stress. These Cancer cells also showed increased binding affinity to the purified platelets. Immunofluorescent confocal microscopy analyses of human glioma tumor samples demonstrated VWF expression in some cells of non-endothelial origin in the tumor region, highly suggestive of VWF expression in a subset of glioma cancer cells.

Conclusion: A subpopulation of cancer cells of non-endothelial origin acquire denovo expression of VWF as a result of epigenetic modification of VWF promoter, in association with modified interaction of the promoter with VWF regulatory transacting factors. Acquired VWF expression by cancer cells increase their adhesive properties and may contribute to their metastatic potential.

84. Recognition and comparison of K-Ras and N-Ras oncogenic hot spots for chemical and UV damage

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It has been well established that mutations in K-Ras and N-Ras proto-oncogenes can convert them into active oncogenes. The current molecular cancer research has been focused on determining the key steps by which cellular genes become oncogenes, not on the underlying and fundamental chemical damage mechanism and susceptibility. In this study, we investigate the mutational hot spots of damage present in Ras genes upon exposure to a Ru cis-platin analog and UV damaging agents. Detection of damage is accomplished by a simple, sensitive, mix-and-read assay using an EvaGreen (EG) probe in a 96-well microtiter plate. Our results shows that although there is a high degree of similarity among K-Ras and N-ras genes, they show different responses to particular damaging agents. Our experiments demonstrate that sequences of K-Ras are more prone to Ru and UV damage when compared to N-Ras. Combining the results obtained for both the genes, we observe that the extent of damage increases with an increasing number of G’s for Ru-induced damage and increasing number of T’s for UV-induced damage. We discuss the effect of mutagenic agents on various codons of K-Ras and N-Ras genes, and potential reasons that may lead to differential pattern of damage.
85. Germline Copy Number Variations as Genetic Susceptibility determinants for Cancer Cachexia

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Background: Cancer Cachexia (CC) is a multifactorial syndrome characterized by skeletal muscle depletion with or without fat loss and net loss of weight. Copy number variants (CNVs) are structural changes in the genome. Amplifications and deletions of more than 1 kb and extending up to several million base pairs comprise structural aberrations; CNVs may regulate gene expression through gene dosage or through intergenic cis-regulatory elements. CNVs have gained considerable prominence as susceptibility determinants for various diseases such as Parkinson disease, Autism and several cancer types. Although genetic basis for CC susceptibility were attempted using candidate gene Single Nucleotide Polymorphisms (SNPs), interrogation for determinants at whole genome level remain unexplored.

Hypothesis: CC as a phenotype has a heritable component and that the germline common DNA variants explain individual’s overall genetic susceptibility. Objective: To identify germline CNVs as genetic markers for CC using a Genome-Wide Association Study (GWAS) design.

Methods: Stratification of patients was based on weight loss and C-Reactive Protein (CRP); CRP, an indicator of systemic inflammation positively correlates with weight loss. From a CC genetic study cohort of 1500 cases with documented weight loss and CRP data, we selected a subset of cases for this feasibility study. Oesophagogastric and lung cancer cases with weight loss ≥ 10% and CRP > 10mg/L (n=16, cachectic) and controls with no weight loss and CRP levels < 10mg/L (n=25, weight stable) satisfied the criteria of the extremes of phenotype of CC in the current study design. Affymetrix Genome-wide Human SNP array 6.0 served as a genotyping platform. Partek Genomics suite 6.6 was used for analysis. Functional annotations of genes proximal to CNVs were identified using DAVID Bioinformatics v6.7.

Results: We identified 7,688 CNVs that were statistically significant (p<0.05). We initially examined only those CNVs showing copy gains or copy loss regions of >1 kb (n= 496), of which 171 CNVs were proximal to genes. Gene Ontology identified clusters associated with amino acid catabolic process, muscle development and proteolysis. Further genotyping of larger cohorts confer statistical rigor and confidence in study findings. This is the first genome-wide study targeted towards identifying genetic determinants for CC.

86. Modeling p53 mutant activators: ranking their binding energies, pharmacokinetics and toxicological profiles

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The most mutated protein found in all cancer cells is p53, part of an effective strategy to promote cancer survival and progression. Restoration of the wild-type activity to mutant p53 offers promise to eradicate cancer cells. Several molecules have already been found to activate mutant p53 although their exact mechanism has not yet been fully understood. However, a transiently open pocket has been identified in some p53 mutants. In our study, we docked twelve known activators into the open pocket of p53-R273H to rank the best binders and further understand their mechanism. In addition, we predicted their pharmacokinetics as well as their toxicological profiles in order to assess their pharmaceutical usefulness. Our simulations showed that not all alkylating ligands bind at the same position, probably due to variations in their sizes. We also found that non-alkylating ligands can directly interact with Cys124 and are capable of binding at the same pocket. In addition, our results demonstrate that stictic acid has a great potential as an activator to p53-R273H mutant especially that it has less adverse effects, although its pharmacokinetic properties are poorer compared to the other activators.
Poster Presentations

87. siRNA therapy against cell cycle proteins in breast cancer cells
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Introduction: There are significant limitations (side effects) to all breast cancer chemotherapy regimens, radiation and surgical treatments, which urgently warrant a search for alternative and more effective therapies. Control of breast cancer growth based on RNA interference (RNAi) using small interfering RNA (siRNA) has been a promising approach in recent years. The siRNA-mediated silencing of a unique or over-expressed cell cycle protein that is essential for unregulated cell growth could lead to malignant cell death and, in turn, control tumor growth without affecting the normal tissues.

Methods and Results: To identify the best carrier for siRNA delivery against cell cycle proteins, we screened low molecular weight (2.0 kDa) linoleic acid, caprylic acid and α-linoleic acid substituted polyethylenimines (PEI) with commercially available carriers by inhibition of cell growth assay. The linoleic acid substituted PEI (PEI-LA) has delivered the siRNA most successfully. The uptake of siRNA-carrier complexes was determined by flow cytometry and the up-taken complexes per cell were calculated by confocal microscopy. To explore the potential cell cycle proteins as therapeutic targets, we screened an siRNA library in MDA-MB-231 and MDA-MB-435 cells using PEI-LA as a delivery agent. Out of 169 cell cycle protein targets, the siRNA against cell division cycle protein 20 (CDC20), a recombinase RAD51, and serine-threonine protein kinase CHEK1 diminished the cell growth most significantly in MDA-MB-435 cells. These identified targets with another well-studied cell cycle protein, kinesin spindle protein (KSP), were then evaluated in MDA-MB-435, MDA-MB-231 and MCF7 cells using independently prepared siRNAs. KSP was the most successful target among all identified cell cycle proteins as around 80% MDA-MB-435 cell growth was inhibited by KSP siRNA. However, the significant down-regulation of mRNA transcript of all proteins was found by digital-PCR. The synergistic effect was not seen in the combinational siRNA delivery of cell cycle proteins. We also explored the efficacy of dicer-substrate siRNAs (DsiRNAs) against CDC20, RAD51 and CHEK1. All DsiRNAs decreased the cell growth significantly. However, CDC20 was the most effective DsiRNA since it inhibited MDA-MB-435 and MCF7 cell growth approximately 80%.

Conclusions: The identified cell cycle proteins could be promising targets to treat breast cancer by non-viral RNAi therapy. The presented study has enlightened the importance of cell cycle protein targets in cell survival and breast cancer therapy, and has given the safe and nontoxic delivery system (PEI-LA) for the down-regulation of cell cycle proteins.

88. Polynucleotide kinase/phosphatase, PNKP, as a target for enhanced cancer therapy
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The radioresistance and chemoresistance of tumors are major obstacles that may lead to the failure of cancer therapy. Reducing DNA repair in cancer cells is emerging as a new strategy for improving cancer treatment. One approach is to identify small molecules inhibitors of DNA repair enzymes.

Human polynucleotide kinase/phosphatase (hPNKP), which possesses DNA 5′-kinase and 3′-phosphatase activities, plays an essential role in DNA strand break repair by rendering strand-break termini suitable for DNA polymerases and ligases. Previous work in our lab has shown that reduced expression of PNKP sensitizes cells to ionizing radiation, alkylating agents and the topoisomerase I poison camptothecin. We have developed a rapid fluorescence-based assay to identify small molecule inhibitors of the 3′-phosphatase activity of PNKP. The main components of this assay are two oligonucleotide hairpin probes, PNKP enzyme, and T4 DNA polymerase. T4 DNA polymerase has a powerful 3′-5′ exonuclease activity that digests oligonucleotides that have a 3′-OH terminus, but is blocked by the presence of a 3′-phosphate terminus. We detect the inhibition of PNKP by monitoring the fluorescence signal resulting from the release of 2-aminopurine (2 Ap) embedded in the stem of the hairpin probes. The fluorescence of 2 Ap is highly quenched when it is incorporated in duplex DNA.

As a proof of principle, the novel assay was used to test 200 compounds selected from three chemical libraries of natural derivative compounds, and two chemically related compounds were identified that inhibit the phosphatase activity of hPNKP. Currently, we are examining the mechanism of inhibition by these compounds and their ability to sensitize cancer cells to radio/chemo-therapy.
89. Oncolytic Vaccinia Virus has Significant Activity Against Bladder Cancer

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Objectives: The majority of Bladder Cancers (BCa) are amendable to intravesical therapy and many of these BCa have amplification of ribonucleotide reductase (RR); especially the rate limiting R2 subunit. BCa resistant to gemcitabine (GEM) in particular have over-expression of R2. RR is essential for DNA synthesis in proliferating cells but because the host cellular enzymes that catalyze DNA synthesis are mostly degraded at the end of S-phase, vaccinia virus (VAC) expresses its own set of enzymes including both I4L (large, R1) and F4L (small, R2) subunits of RR. We have shown that virus-encoded RR subunits can associate with their cellular homologs, and that deleting the F4L gene inhibits virus replication and reduces virulence in a mouse model while retaining replication proficiency in BCa cells. We tested these modified VACs (mVACs) for their ability to replicate in and lyse cancer cells while sparing normal cells.

Methods: We developed VACs tagged with the gene encoding mCherry and deletions in F4L, J2R (viral thymidine kinase) or both. Activity of these mVACs were evaluated in a panel of human and rodent BCa or normal bladder cell lines and primary fibroblasts in vitro. We also tested the combination of our mVACs and GEM. Finally, the immunocompetent orthotopic AY27luc rat BCa model were used to assess the VACs in vivo.

Results: Cytotoxicity assays show a high degree of cell killing in infections with mVACs. Highly efficient mVACs replication was seen in our BCa cells while limited replication was seen in normal bladder cells or primary fibroblasts. We found that pre-treatment of cancer cells with GEM causes a significant enhancement in the mVACs induced cell killing, especially in GEM resistance cells. We show that our mVACs selectively replicated in the AY-27 orthotopic BCa model with significant tumor regression or complete ablation with limited or no toxicity.

Conclusions: Our data indicate that the mVACs have retained much of their replication proficiency and cytotoxicity in BCa cells despite deletions of these critical viral replication genes. There is also an enhanced effect between GEM and the mVACs that could have a significant clinical implication. In vivo replication is selective for the cancerous cells and avoids transmission. Further investigation is being conducted on comparing the mVACs to the standard of care, Bacillus Calmette–Guérin (BCG). Additionally, work is assessing virus dissemination and virus-mediated immunological anti-tumor activity in the immunocompetent orthotopic AY-27 rat BCa model.

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90. Development of BRCA1 inhibitor for combinational cancer therapy

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Breast cancer is one of the most common diagnosis cancer in Canada. Mutation in breast cancer type 1 & 2 susceptibility (BRCA1 & 2) gene is known to elevate the risk of breast cancer in human. Interestingly, BRCA1 also play essential role in double strand break (DSB) DNA damage repair by interacting with various protein partners through recognition of a conserved pSer-x-x-Phe(pSxxF) motif in phosphorylation dependent manner using its C terminal (BRCT) domain. Since many cancer therapies employ DNA damaging chemotherapy or ionizing radiation that primarily target to causes DSBs leading to cell cycle arrest or cell death, development of small inhibitor drugs against DSB repair protein such as BRCA1 can effectively enhance the efficacy of current cancer treatment especially for breast and ovarian cancer patient. We have successfully identified a small nonphosphopeptide inhibitor for BRCA1 BRCT using mRNA displaying technique and obtained the structural of this inhibitor in complex with BRCA1 BRCT using X-ray crystallography. Our structure revealed a Glu-x-x-Phe(ExxF) motif replacing the conserved pSxxF motif required for phosphorylation dependent recognition at BRCT binding site. Moreover, it uncovers a unique hydrophobic interactions between N-terminal peptide and BRCT which alter the peptide conformation as well as extra hydrogen bonding with C-terminus of inhibitor peptide. Both interactions are proved to be essential for this phosphorylation independent BRCA BRCT recognition in vitro, and preliminary test of this inhibitor is done in vivo to examine its potential as sensitizer for DNA damage cancer therapy. Together, our study not only provide new structure insight that may help the development of BRCA1 inhibitor but also revealed a mechanism in which ExxF motif may mimic pSxxF motif in a phosphorylation independent recognition.
91. Study Design for Assessing the Impact of a Multimodal Intervention on Energy Metabolism in Cancer Cachexia

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Background: Cancer cachexia is characterized by significant weight loss and metabolic imbalances that cannot be reversed by conventional nutrition support. Cachectic patients frequently, but not universally, present with a negative energy balance as a result of high energy expenditure and suboptimal caloric intake. This scenario exacerbates catabolism, ultimately causing weight loss, which is independently associated with poorer prognosis. To date, few studies have assessed energy metabolism of patients with advanced cancer. Compromised nutritional status and unknown nutritional requirements are a significant unmet medical need in cancer cachexia. Furthermore, the potential impact of novel therapeutic approaches (e.g. multimodal care) on energy metabolism and its consequential change in body composition are unknown.

Objectives: To characterize baseline energy metabolism in patients with advanced non-small cell lung cancer (NSCLC) and its respective changes after a 6-week multimodal approach intervention.

Methods: Twenty patients with non-operable NSCLC participating in a multi-center randomized phase III clinical trial of cancer cachexia (the Multimodal Exercise/Nutrition/Anti-inflammatory treatment for Cachexia [MENAC] trial) will be recruited. Patients in the MENAC trial treatment arm will receive multimodal care encompassing nutrition (ProSure), home-based exercise intervention (strength and aerobic activity) and anti-inflammatory therapy, versus standard care protocols. Assessments will be conducted at baseline, prior to initiation of chemotherapy treatment and 6 weeks post-intervention, after completion of two chemotherapy cycles. Energy metabolism will be assessed in a 24-hour whole body calorimetry unit. Resting metabolic rate, thermic effect of food, exercise energy expenditure, sleep energy expenditure and oxidation of fat and carbohydrate will be obtained. Body composition (dual X-ray absorptiometry) and biochemical measures of glucose, insulin, free fatty acids, triglycerides, cortisol, leptin, ghrelin, and peptide YY will also be collected. These variables will be integrated with outcomes from the MENAC trial such as nutritional status, performance, strength, physical activity, quality of life, treatment response, and survival.

Conclusion: This study will provide a thorough appraisal of energy balance and potential predictors of change associated with multimodal treatment, thereby elucidating important metabolic and nutritional characteristics of patients suffering from cancer cachexia.

92. Resveratrol Derivatives: Inhibitors of NFkB Activity and Potential Therapeutics for Inflammatory Bowel Disease and Cancer

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Resveratrol is a phytoalexin polyphenolic compound found in various plants, including grapes, berries, and peanuts. One striking biological activity of resveratrol is its cancer chemo-preventative ability. In this study we show that representative resveratrol derivatives enhance the survival of 1A+/- mice most probably by inhibiting the activation of NFkB in response to the DSS insult of the colon. (R), (C-11), (C-12) and other derivatives (not shown here) showed promising NFkB inhibitory properties, in HCT-116 and SW 480 cells (not shown here). With further studies characterizing their role in protection against IBD and IBD-associated cancer, resveratrol derivatives might be promising prophylactics and therapeutics against both IBD and cancer.
Inflammatory Bowel Disease (IBD) is a chronic intestinal disease characterized by inflammation of the gastrointestinal (GI) area resulting in abdominal pain, chronic diarrhea, and weight loss. IBD includes Crohn’s disease (CD) and ulcerative colitis (UC) and is highly prevalent in Canada. Over 150 susceptibility genes have been identified for IBD including some on chromosome 3p21 the location of the Ras association domain family protein 1A, RASSF1A (or 1A). 1A is a tumor suppressor gene epigenetically silenced by methylation in numerous human cancers. The epigenetic silencing of 1A has also been detected in inflammatory diseases such as IBD and pancreatitis in addition to colorectal cancer (CRC) to suggest regulation of cellular homeostasis beyond a tumor suppressor gene and an important driver of inflammation-driven cancers.

There is evidence for 1A associations with Toll (TLR) and TLR molecular components in order to restrict NFκB activation. TLR are a class of proteins that play a key role in the innate immune system. They are single, membrane-spanning, non-catalytic receptors usually expressed in sentinel cells such as macrophages and dendritic cells, that recognize structurally conserved molecules derived from microbes. Dextran sodium sulfate (DSS, a chemical inducer of colitis) treatment of Rassf1a-/− or Rassf1a+/− mice resulted in clinical symptoms of human colitis including increased intestinal permeability, enhanced cytokine/chemokine production, elevated NFκB activity, severe colonic epithelial cell injury and < 20% survival. In addition, DSS treatment of Rassf1a-/− mice was associated with an increase of DNA and oxidative damage, abnormal tyrosine phosphorylation of the Hippo transcription factor, YAP and elevated pY-YAP/p73 transcription of apoptotic genes to result in inflammation induced injury. Furthermore, we can observe changes in autophagic biomarkers at the in protein (LC3, p62) and DNA (Atg4c, Atg2b and Laptm5) level. Lastly, 1A can associate with the nucleotide-binding oligomerization domain-containing protein 2 (NOD2), another member of the pathogen pattern recognition receptors and a major player in autophagic signaling. Interestingly, the Rassf1a-/−Nod2-/− mice are resistant to inflammation induced injury models when compared to Rassf1a-/− mice. All of these data suggest that DSS-induced inflammation injury in the Rassf1a-/− mice may be driven by active NOD2 signaling linked to NFκB and autophagy. We have molecular evidence that 1A can restrict NOD2 signaling to regulate abnormal activation states leading to inflammation induced injury. An understanding of how 1A influences autophagic signaling to promote murine colitis will greatly aid in developing pharmacological inhibitors of NOD2 driven autophagy, will allow for a better understanding of inflammation injury and for rational drug design for IBD and IBD-associated CRC.

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94. Development of mobile and web-based applications for safer dispensing of oral chemotherapeutic agents

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With increasing availability and use of oral chemotherapeutics agents, a greater chance of prescribing error arises, especially when the healthcare professionals involved have limited experience with these medications. We have created an innovative solution, for use in the Canadian context, to help non-oncology pharmacists identify problems associated with oral chemotherapy, to achieve timely resolution, and most importantly, avoid patient harm. We constructed a repository to disseminate information to pharmacists related to oral chemotherapy prescribing: “www.oralchemotherapy.ca”. This website features researched, comprehensive, one-page monographs for each oral chemotherapeutic available in Canada, including risk stratification (high, moderate, low) associated with each medication, as well as drug-drug interaction tables. Additionally, we created a mobile version of this data in a dedicated app called AntiC, for use on iPhones and Android phones. In many cases mobile applications fit better in today’s pharmacy workflow; it is designed to work in the absence of a Wi-Fi connection, allowing for access to information at all times. Both www.oralchemotherapy.ca and the AntiC mobile app are powered by the same data source. The website: www.oralchemotherapy.ca was constructed and is now available. Printable pdf versions of the monographs will be accessible in the near future. The AntiC mobile version of this data is currently being tested at the Grey Nuns Community Hospital, Edmonton, Alberta. It is hoped that this improved access to an additional level of assessment for oral chemotherapeutics available in Canada will empower non-oncology pharmacists and other healthcare professionals in the safer dispensing of oral chemotherapeutic agents, and thus improve patient care. We plan to host workshops to educate pharmacists on potential issues they face when dispensing oral chemotherapy, and introduce them to www.oralchemotherapy.ca and the AntiC app as an easy, rapid and concise resource to improve patient safety.
95. Development and evaluation of novel 18F-labelled radiotracers for the molecular imaging of cyclooxygenase-2 by positron emission tomography


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Objectives: Cyclooxygenase-2 (COX-2) is the inducible isoform of the Cyclooxygenase enzyme family. The enzyme is expressed in a variety of disease states including inflammation, pain, neurodegenerative disease and cancer. Inhibitors of COX-2 represent a particularly promising class of compounds for chemoprevention and cancer therapy. The experimental data on the involvement of COX-2 in tumor development and progression, as well as the observed overexpression of COX-2 in a variety of human cancers provide the rationale for targeting COX-2 for molecular imaging.

Methods: A library of three 18F-labelled COX-2 inhibitors was developed and lead structure [18F]Pyricoxib identified. [18F]Pyricoxib underwent radiopharmacological evaluation as a novel PET radiotracer for the molecular imaging of COX-2 expression in a colorectal cancer model. The radiotracer was evaluated in vitro in the COX-2 expressing human colorectal cancer cell line HCA-7 and the COX-2 negative cell line HCT-116. In vivo evaluation was performed in female NIH III mice, carrying HCA-7 and HCT-116 xenografts. In vivo tumor uptake and clearance parameters were evaluated by dynamic PET and biodistribution experiments.

Results: The IC50 value against COX-2 of Pyricoxib was found to be 7 nM, compared to 40 nM for known COX-2 inhibitor celecoxib in the same assay. Radiosynthesis [18F]Pyricoxib from [18F]FBA was accomplished in approximately 90 min with a decay corrected yield of 25%. In vitro uptake of [18F]Pyricoxib in HCA-7 cells was significantly higher than in HCT-116 cells and was in part blocked in the presence of non-radiolabelled Pyricoxib and the known COX-2 inhibitors, celecoxib, rofecoxib and SC58125. The radiotracer was slowly metabolized in mouse plasma, with approximately 60% intact compound 2 h post-injection. Tumor uptake of [18F]Pyricoxib in the COX-2 positive HCA-7 xenografts was significantly higher than in muscle tissue and was reduced when mice were pre-treated with pharmaceutical doses of celecoxib.

Conclusions: The uptake of [18F]Pyricoxib in COX-2 positive HCA-7 xenografts is at least partially due to selective COX-2 binding. [18F]Pyricoxib is a candidate for non-invasive assessment of COX-2 expression in cancer.

Advances in knowledge: [18F]Pyricoxib displayed promising properties as a novel radiotracer for molecular imaging of COX-2 in inflammatory cancer lesions.

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96. Assessing breast cancer cell lines as tumour models by comparison of expression profiles

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Since the discovery of HeLa cells in 1951, cancer cell lines have frequently been used in vitro as tumour models. However, the suitability of these models has come into question as many in vitro phenomena are difficult or impossible to replicate in vivo. Recent transcriptional profiling of a collection of breast cancer cell lines and hundreds of tumours from The Cancer Genome Atlas has enabled a direct bioinformatic comparison of cell lines and tumours. In general, we found the transcriptional characteristics of breast cancer cell lines to mirror those of the tumours. We have identified those basal and luminal cell lines that are most transcriptionally similar to their respective breast tumours. Our comparison of expression profiles revealed pronounced differences between breast cancer cell lines and tumours that could largely be attributed to the absence of stromal and immune components in cell culture. The list of differentially expressed genes was filtered for correlation with stromal and immune signatures uncovering a list of differentially expressed genes more likely to genuinely reflect changes induced by cell culture. A focus on the Wnt pathway — one of the most commonly studied pathways in cell culture — revealed the transcriptional downregulation or absence of several secreted antagonists in culture. This study discovered major transcriptional differences between breast cancer cell lines and tumours while accounting for stromal and immune components not found in typical cancer cell monoculture. The specific differences discovered emphasizes the importance of choosing an appropriate model for each research question.
97. Assessing the role of HIF1α as a target for overcoming drug resistance in breast cancer

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Purpose: The long-term objective of this study is to overcome hypoxia induced drug resistance in breast cancer. For this purpose, we have developed a drug resistant model of MDA-MB-231 cells (a triple negative metastatic breast cancer cell line) through its culture under hypoxic condition. The potential role of hypoxic inducible factor alpha 1 expression as a target for overcoming hypoxia-induced resistance to cisplatin in this cell line was then investigated.

Methods: MDA-MB-231 cells were maintained in RPMI medium (supplemented with 10% FCS, 100 IU/mL penicillin and 100 mg/mL streptomycin) either under normoxia (20% oxygen) or hypoxia (1% oxygen) at 37 °C. Viability of cells were measured by MTT assay and Flow Cytometric analysis using propidium iodide and FITC Annexin V staining for cells which were treated with different concentration of cisplatin as an anticancer drug under normoxia and hypoxia for different length of incubation. Expression level of HIF-1α protein was analyzed by transcription factor assay kit and immunoblotting under normoxic and hypoxic condition for different incubation times. Small interfering RNA (siRNA) techniques were used to knockdown HIF-1α expression in response to hypoxia. The effect of this treatment on the cytotoxicity and apoptosis induction by cisplatin under normoxic and hypoxic condition was assessed.

Results: Significantly higher level of HIF-1α expression was measured under hypoxia comparing to normoxia in MDA-MB-231 cells. Treatment of MDA-MB-231 cells with cisplatin (1-100 µg/mL) under hypoxia led to a higher cellular viability comparing cells treated with cisplatin at similar concentrations under normoxia. Successful knockdown of HIF-1α expression by lipofectamine 2000 or PEO-(PCL-SP) HIF1α-siRNA complexes (the latter is developed in our lab) didn’t translate to lower cellular viability or induction of apoptosis after drug exposure under hypoxic or normoxic condition.

Conclusion: Treatment of cells with cisplatin under hypoxia induced drug resistance and resulted in lower level of cellular toxicity comparing to the cells which were treated under normoxia. Higher expression level of HIF-1α may play a role in hypoxia induced drug resistance.

98. Body composition changes in patients with advanced non-small cell lung cancer receiving Enobosarm

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Introduction: Computerized tomography (CT) is a state-of-the-art technique providing direct assessment of skeletal muscle (SM), a key anabolic component of lean body mass (as measured by other techniques such as DXA). SM loss observed among patients with non-small cell lung cancer (NSCLC) is associated with poorer prognostic outcomes. Here, we investigate the efficacy of an anabolic agent in promoting SM gain in patients with stage III or IV NSCLC.

Methods: SM was assessed by quantitative analysis of CT images from participants enrolled in two Phase 3 trials of enobosarm, a nonsteroidal, selective androgen receptor modulator (SARM). Patients were randomized to enobosarm (3 mg/day) or placebo at initiation of first-line standard platinum doublet chemotherapy (Power1=platinum+taxane or Power2=platinum+non-taxane) and continued through day-147. SM change is reported from baseline to day-84 and day-147.

Results: Enobosarm was associated with significant SM (whole body basis) gains. In power1, the median change in SM from baseline to day-84 differed (p<0.0001) between enobosarm (+0.5 kg, n=103) and placebo patients (-0.3 kg, n=107) and more pronounced at day-147 (+0.1 kg and -0.8 kg; p<0.0001). Similarly, in Power 2, median change in SM from baseline to day-84 differed (p=0.03) between enobosarm (+0.3 kg, n=101) and placebo patients (-0.2 kg, n=99) and was again more pronounced at day-147 (+0.5 kg and -0.3 kg; p=0.003). SM gain ≥1 kg at day-84 was associated with longer median survival in the enobosarm arm (+2.6 months, p=0.04).

Conclusions: Enobosarm promoted SM gain, and SM gain was associated with prolonged survival. CT analysis can be used as an opportunistic tool to assess body composition in clinical trials.
**99. Regulation of Calpastatin Gene Expression by Nuclear Factor I in Malignant Glioma**

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Background: Malignant gliomas (MG) are the most common type of adult brain tumours. These deadly tumours are difficult to treat in part because of the inherently infiltrative nature of the tumour cells. Nuclear factor I (NFI), a family of four transcription factors (NFIA, NFIB, NFIC, and NFIX), has been implicated in the regulation of genes involved in MG cell migration. One of the putative NFI target genes, CAST, encodes calpastatin, an endogenous inhibitor of calpains (calcium-dependent, non-lysosomal cysteine proteases).

Objective: CAST was selected for further analysis because calpains regulate the function of calcineurin, a phosphatase that dephosphorylates and activates NFI. Upregulation of calpain in mammalian cells has been shown to be associated with increased MG infiltration. The main objective of this project is to study the regulation of the CAST gene by NFI and its effects on MG cell migration.

Methods: A bioinformatics approach was used to identify putative NFI binding sites upstream and within the CAST gene. Physical interaction between NFI and the putative NFI binding sites were confirmed by gel shift assays. We also used RT-PCR to examine changes in total levels of CAST transcripts in response to different combination of NFI knockdowns. The effects of NFI overexpression or depletion on calpastatin levels were examined by western blotting. MG cell migration was evaluated by live cell imaging, in the presence or absence of Calpain Inhibitor I (ALLN).

Results: I have authenticated the NFI binding sites upstream of intron 3 in the CAST gene and shown that these sites are bound by NFIC and X. NFI depletion, specifically NFIC and X, by RNAi results in upregulation of CAST RNA levels. Intriguingly, overexpression of individual NFIs leads to a decrease in calpastatin protein levels in T98 MG cells in which NFI is hyperphosphorylated and inactive. NFI knockdown did not alter calpastatin protein levels in these cells. As expected, overexpression of NFI in U251 MG cells in which NFI is hypophosphorylated and active, did not change calpastatin expression; however, NFI depletion resulted in increased calpastatin levels. U251 cells exhibits a dose-dependent reduction in migratory capability in response to ALLN.

Conclusion: NFI members, specifically NFIC and X, regulate CAST gene expression. Changes in CAST RNA and protein levels in response to NFI depletion and overexpression suggest a regulatory loop between NFI and the calpain/calpastatin pathway. This regulatory loop may result in increased NFI transcriptional activity, thereby increasing cell migration in MG tumours. Calpain antagonists including ALLN may therefore represent a novel class of chemotherapeutic agents for MG treatment.

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**100. The tumor suppressor STAT1 is phosphorylated and down-regulated by the oncogenic tyrosine kinase NPM-ALK in ALK-positive anaplastic large cell lymphoma**

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The tumorigenicity of most cases of ALK-positive anaplastic large cell lymphoma (ALK+ALCL) is driven by the oncogenic fusion protein NPM-ALK in a STAT3-dependent manner. Since it has been shown that STAT3 can be inhibited by STAT1 in some experimental models, we hypothesized that the STAT1 signaling pathway is defective in ALK+ALCL, thereby leaving the STAT3 signaling unchecked. Compared to normal T-cells, ALK+ALCL tumors consistently expressed a low level of STAT1. Inhibition of the ubiquitin-proteasome pathway appreciably increased STAT1 expression in ALK+ALCL cells. Furthermore, we found evidence that NPM-ALK suppresses STAT1 expression, as it bound to, phosphorylated STAT1 and promoted its degradation in a STAT3-dependent manner. If restored, STAT1 is functionally intact in ALK+ALCL cells, as it induced apoptosis/cell-cycle arrest, and sensitized the cells to doxorubicin-induced apoptosis. STAT1 interfered with the STAT3 signaling, by decreasing STAT3 transcriptional activity/DNA binding and its homodimerization. The importance of the STAT1/STAT3 functional interaction was further highlighted by the observation that siRNA knockdown of STAT1 significantly decreased apoptosis induced by STAT3 inhibition. To conclude, STAT1 is a tumor suppressor in ALK+ALCL. Phosphorylation and down-regulation of STAT1 by NPM-ALK represent yet another mechanism by which this oncogenic tyrosine kinase promotes tumorigenesis.
101. siRNA Therapy in Treatment of Chronic Myeloid Leukemia (CML) Targeting BCR-ABL


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Introduction: Chronic Myeloid Leukemia (CML) is initialized at the hematopoietic stem cells after a chromosomal translocation (BCR-ABL fusion oncogene) which causes uncontrolled myeloid cell expansion and accumulation in the blood system. Drug resistance and insensitivity to current leukemia treatments call for development of new treatments. To control the expression of BCR-ABL or other aberrant genes, synthetic small interfering RNA (siRNA) can be delivered into cells to interact with target mRNA and silence protein expression. The aim of this project is to deliver siRNA moieties that reach the BCR-ABL mRNA of CML cells with the use of a novel polymeric carrier, which consists of low molecular weight polyethylenimine (PEI) grafted with linoleic acid (LA) (PEI1.2-αLA), and to evaluate its potential in the reduction of CML cell and tumor growth.

Methods: Decrease in green mean fluorescence (protein silencing) of PEI1.2-αLA was assessed by flow cytometry and compared with commercial reagents using GFP-expressing K562 cells. Cell viability changes after BCR-ABL siRNA delivery were assessed by MTT experiments. CML tumors grown in mice were injected in their vicinity with PEI1.2-αLA/siRNA complexes 3 times every 72 h, and tumor volume was measured every 3 days. Tumors were processed for BCR-ABL mRNA quantification by PCR.

Results: Based on flow cytometry results, commercial reagents show a silencing of 80% while PEI1.2-αLA shows a silencing of 54% without inducing as much cell death as commercial reagents (PEI25 and Turbofect). In cell viability assay and using PEI1.2-αLA, control siRNA treatment shows an initial toxicity but cells recovered from it. In contrast, BCR-ABL siRNA treatment induces cell growth arrest for at least 4 days (p < 0.01). SiRNA treatment of tumors grown in mice shows that GFP siRNA treatment has a slight decrease in tumor volumes after day 10, suggesting a degree of toxicity; conversely, BCR-ABL siRNA treatment was effective in reducing tumor volumes starting from day 3. ddPCR supports this effect by revealing a reduction of the BCR-ABL mRNA expression.

Conclusion: With the aim of balancing out effective transfection with lower cytotoxicity, PEI1.2 was grafted with specific lipid moiety and degree modification. PEI1.2-αLA polymer showed similar effect to PEI25 in terms of transfection but milder effect on the cell numbers. Decrease of BCR-ABL mRNA by siRNA delivery shows a functional effect in restraining cell proliferation in vitro and in vivo. These data demonstrate the potential of PEI1.2-αLA polymer to effectively deliver siRNA and for therapeutic use for CML treatment.

102. The activation of matrix metalloproteinease-2 by endogenous reactive oxygen/nitrogen species

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Matrix metalloprotease-2 (MMP-2), along with other MMPs, is up-regulated in cancer cells and has been implicated in metastasis. Cancer cells have increased oxidative stress. In vitro, MMP-2 can be directly activated by low concentrations of reactive oxygen/nitrogen species (RONS) via post-translational modifications, with higher concentrations inactivating it. However, this has yet to be demonstrated in a living system. Here, we investigated whether endogenous RONS produced by pharmaceutical inhibition of ubiquinol-cytochrome c reductase with antimycin could increase MMP-2 activity in cultured cells. We exposed proliferative cell lines (HT1080 fibrosarcoma and H9c2 cardiomyoblasts) and minimally-proliferating primary cells (neonatal rat cardiomyocytes - NRVMs) to increasing concentrations of antimycin (0.01-1, 0.3-3.2 and 1-100 μM for NRVMs, H9c2 and HT1080 respectively) in serum-free media for short (0.5 hrs) or long (6 hrs) intervals to measure MMP-2 activity due to post-translational modification or translational up-regulation, respectively. Antimycin treatment did not affect viability or cell growth, but did increase oxidative stress in a dose- and time-dependent fashion, as demonstrated by decreased aconitase activity. MMP-2 activity (measured by gelatin zymography) was measured in cell lysates and conditioned media. Proliferating HT1080 and H9c2 cells exhibited increased intracellular MMP-2 activity at low, but not high concentrations of antimycin. In contrast, NRVMs, despite a similar proportional decrease in aconitase activity (i.e., increase in oxidative stress), exhibited a marked decrease in lysate MMP-2 activity. The activity of secreted MMP-2 was not consistently affected by antimycin treatment in any cell line. We conclude that MMP-2 activity can be affected by endogenous oxidative stress in vivo in cultured cells, but the directionality of this effect is modulated by proliferative status or cell type.
**Poster Presentations**

### 103. Understanding exercise behavior in hematologic cancer survivors

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**Background:** Regular exercise prevents and reverses treatment-related comorbidities in cancer survivors. However, many survivors are not active enough to benefit from exercise’s protective effects (Mishra et al., 2012). To address this disparity, we must better understand cancer survivors’ exercise motivation. The theoretical exercise motivation model most commonly used for cancer survivors is the theory of planned behaviour (TPB; Ajzen, 1991). Despite its numerous applications across cancer survivor groups, no studies have used the TPB to predict exercise levels in hematologic cancer survivors.

Recent theoretical extensions of the TPB explore how exercise intentions translate into regular participation (Rhodes & De Bruijn, 2013). Several behavioural constructs help explain this intention-exercise relationship in healthy populations but no studies have focused on cancer survivors. Furthermore, no studies have modeled cancer symptomologies as determinants of exercise participation, and none have accounted for motivational differences involved in aerobic versus strength exercise participation.

**Objectives:**
1. Predict exercise levels using the TPB-model for hematologic cancer survivor groups
2. Explore the utility of behavioural constructs in explaining the intention-exercise relationships for hematologic cancer survivors
3. Model survivors’ cancer symptomologies as exercise determinants
4. Quantify all relationships separately for aerobic and strength exercise

**Methods:** Objectives will be pursued through a cross-sectional survey-based study design. Stratified by cancer type, 2100 surveys will be mailed to hematologic cancer survivors (i.e., leukemia, Hodgkin lymphoma, & non-Hodgkin lymphoma). Questionnaires will capture TPB, intention-exercise behavioural construct, cancer symptoms, and aerobic and strength exercise measures. Structural equation modeling will assess the effectiveness of motivational frameworks in predicting exercise levels.

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### 104. ERK promotes STAT1 protein ubiquitination and degradation independent of STAT1 phosphorylation in esophageal squamous carcinoma cells

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We recently reported that STAT1 is a tumor suppressor in esophageal squamous cell carcinoma (ESCC); and low STAT1 expression significantly correlates with a worse clinical outcome. In this study, we investigated the mechanism(s) that are responsible for the down-regulation of STAT1 in ESCC. We found evidence that STAT1 in ESCC cells is down-regulated via the ubiquitin-proteosome—mediated degradation, since MG132 treatment of two cell lines (EC1 and KYSE150) substantially increased STAT1 expression in a time- and dose-dependent manner. This degradation of STAT1 is independent of the phosphorylation status of STAT1, since mutation of 701 and 727 (two known important phosphorylation sites of STAT1) did not affect this process. We also found that the extracellular regulated protein kinases (ERK) signaling promotes the proteosomal degradation of STAT1, since pharmacologic inhibition of this pathway resulted in a substantial increase in STAT1, whereas the expression of constitutively active ERK further reduced the STAT1 protein level. Furthermore, ERK significantly reduces the production of IFN-γ, a potent activator of STAT1. While MG132 can effectively induce growth inhibition and apoptosis of ESCC cells, this biological effect was shown to be STAT1-dependent. In parallel with our in-vitro study results, we found that the expression of ERK/pERK inversely correlates with that of STAT1 in a cohort of ESCC tumors (n=121). Importantly, in the 72 patients with follow-up data, those with low ERK survived significantly longer than the others (p=0.04), especially those with ERKlow/STAT1high (p=0.001). To conclude, ERK is an effective negative regulator of STAT1 signaling in ESCC, by promoting its proteosomal degradation and activation by IFN-γ. Our data suggests that inhibition of ERK and/or restoration of STAT1 expression are useful therapeutic strategies for ESCC.
105. Synthesis and evaluation of 2-amino-5-(4-[18F]fluorophenyl)pent-4-ynoic acid ([18F]FPhPA): A novel 18F-labeled amino acid for oncologic PET imaging

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Introduction: 18F-labeled amino acids are important PET radiotracers for oncologic imaging. This study describes synthesis and radiopharmacological evaluation of 2-amino-5-(4-[18F]fluorophenyl)pent-4-ynoic acid ([18F]FPhPA) as novel amino acid radiotracer for oncologic imaging.

Methods: [18F]FPhPA was prepared using Pd-mediated SONOGASHIRA cross-coupling reaction between 4-[18F]fluoriodobenzene ([18F]FIB) and propargylglycine. Radiopharmacological profile of [18F]FPhPA was evaluated in comparison with O-(2-[18F]fluoroethyl)-L-tyrosine ([18F]FET) using murine breast cancer cell line EMT6. Radiopharmacological evaluation of [18F]FPhPA involved cellular uptake studies, radiotracer uptake competitive inhibition experiments, and small animal PET imaging.

Results: [18F]FPhPA was prepared in 42±10% decay-corrected radiochemical yield with high radiochemical purity >95% after semi-preparative HPLC purification. Cellular uptake of L-[18F]FPhPA reached a maximum of 58 ± 14 % ID/mg protein at 90 min. Lower uptake was observed for racemic and D-[18F]FPhPA.

Radiotracer uptake inhibition studies by synthetic and naturally occurring amino acids suggested that Na+-dependent system ASC, especially ASCT2, and Na+-independent system L are important amino acid transporters for [18F]FPhPA uptake into EMT6 cells. Small animal PET studies demonstrated similar high tumor uptake of [18F]FPhPA in EMT6 tumor-bearing mice compared to [18F]FET reaching a maximum standardized uptake value (SUV) of 1.35 after 60 min p.i.. Muscle uptake of [18F]FPhPA was higher (SUV30min = 0.65) compared to [18F]FET (SUV30min = 0.40), whereas [18F]FPhPA showed rapid uptake and clearance from the brain compared to [18F]FET.

Conclusion: L-[18F]FPhPA is the first 18F-labeled amino acid prepared through Pd-mediated cross-coupling reaction. L-[18F]FPhPA displayed promising properties as novel amino acid radiotracer for molecular imaging of system ASC and system L amino acid transporters in cancer.

106. Characterization of cell death induced by the cyanine dye D112: a potentially selective anti-cancer compound

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Chemotherapeutic drugs that are used in anti-cancer treatments often cause the death of both cancerous and noncancerous cells. This non-selective toxicity is the root cause of untoward side effects that limits the effectiveness of therapy. In order to improve chemotherapeutic options for cancer patients, there is a need to identify novel compounds with higher discrimination for cancer cells. In the past, methine dyes that increase the sensitivity of photographic emulsions have been investigated for anti-cancer properties. In the 1970’s, Kodak Laboratories initiated a screen of approximately 7000 dye structural variants for selective toxicity. Among these, D112 was identified as the most promising compound with elevated toxicity against a colon cancer cell line in comparison to a non-transformed cell line. Despite those initial promising studies, and probably as a result of changing company priorities, no further work on D112 was conducted. Therefore, we decided to characterize the mechanism of D112-induced toxicity. We identified that in response to D112 treatment, the T-cell leukemia cell line Jurkat showed caspase activation, mitochondrial depolarization, and phosphatidylserine externalization, all of which are hallmarks of apoptosis. Chemical inhibition of caspase enzymatic activity and blockade of the mitochondrial apoptotic pathway through Bcl-2 expression inhibited D112-induced apoptosis. To gain insight into the molecular mechanism of D112 induced mitochondrial dysfunction, we analyzed the intracellular localization of D112, and found that D112 associated with mitochondria. Importantly, we found that D112 was a more effective apoptotic agent against multiple transformed versus non-transformed cell lines, confirming selective cytotoxic properties. Results from this work identify D112 as a potentially relevant clinical drug warranting further investigation.
107. Synthesis exploration and evaluation of Pivaloyl AZA tosylate for the clinical manufacture of [18F]FAZA: A globally used PET imaging agent for tumor hypoxia

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Clinical advancement of a short-lived radiopharmaceutical for non-invasive molecular imaging of cancer (and other) diseases, besides its targeted accrual in the cancer cells, depends also on the feasibility of the manufacturing process. An automated synthesis process that leads to minimal secondary products can offer a simplified purification e.g., solid phase purification, which can afford the imaging agent in optimal radiochemical yield with high purity. 1-a-D-(5-deoxy-5-[18F]fluoroarabinofuranosyl)-2-nitroimidazole ([18F]FAZA), a positron-emitting azomycin a-nucleoside-based hypoxia-selective radiopharmaceutical developed in our group, has successfully demonstrated its clinical superiority over other agents. ([18F]FAZA provides superior image contrast in comparison to [18F]F-MISO because it is more rapidly cleared from blood. Patients studies with [18F]FAZA in a variety of tumors have shown its usefulness in tumor staging, hypoxia mapping and in establishing correlation between hypoxia levels and treatment response, thereby demonstrating its value in assessing hypoxia as a part of a therapeutic protocol. The clinical manufacture of [18F]FAZA requires a reliable, fully automated synthesis procedure with reproducibility and simplified purification technique, which would lead to clinically acceptable ready-to-inject radiopharmaceutical consistent reproducibility and high yields. The current manufacture process uses diacetyl AZA tosylate (Tosyl AZA) as the radiofluorination precursor that affords [18F]FAZA in 5-29% recovered radiochemical yields (rRCY). Being thermally unstable during radiofluorination, this precursor leads to the formation of secondary chemical and radiochemical products that result in extensive and lengthy purification process and consequently to low RCYs.

Current study is an attempt to synthesize Pivaloyl AZA tosylate (PiTAZA), a novel radiofluorination precursor for clinical manufacture of [18F]-FAZA, and its pursuit in developing a fully automated GMP-compliant synthesis that can offer a simple cartridge based purification of the labeled product.

Synthesis development included the optimization of critical reaction parameters e.g., labeling temperature, reaction time and the amount of NaOH (deprotective agent). [18F]FAZA was prepared by nucleophilic radiofluorination of PiTAZA precursor using no-carrier added 18F. Labeled mixture was purified first by an anion exchange cartridge that retained unreacted radiofluoride, followed by trapping of the labeled product on a C-18 reversed phase cartridge where it was purified. Subsequent hydrolysis of protective groups (pivaloyl groups) using 0.8ml of 0.5N NaOH at 50 ºC for 5 min afforded [18F]-FAZA. Terminal purification of this product using solid phase SCX cartridges afforded pure [18F]FAZA in 56 min (radiochemical purity,>94%) and decay-corrected RCY, 19.2%).

108. Role of PTEN in synthetic lethality with PNKP

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Phosphatase and tensin homologue deleted on chromosome 10 (PTEN) is a tumor suppressor that encodes a dual function phosphatase. We demonstrated previously that inhibition of the phosphatase activity of the DNA repair protein polynucleotide kinase/phosphatase (PNKP) causes synthetic lethality (SL) in PTEN-deficient tumor cells. However, the main function of PTEN in generating SL with PNKP is still under investigation. In the current study, we confirmed the SL using pharmacological inhibitors of PTEN and PNKP. In addition, we detected an increased background level of DNA double strand breaks in PTEN-null cells, which accumulate in the presence of the inhibitor of PNKP. The combined loss of PNKP and PTEN slowed-down DNA double strand breaks repair and sensitized tumor cells to radiation. Moreover, the role of PTEN lipid and protein phosphatase functions as well as the role of nuclear PTEN in generating SL with PNKP was investigated using PTEN-mutant tumor cells lacking lipid or total phosphatase functions or mutant cells lacking nuclear PTEN. Our results demonstrated that the loss of PTEN cytoplasmic protein phosphatase activity is the main function of PTEN responsible for SL with PNKP. Additional evidence for the role of PTEN cytoplasmic activity was provided by the observation that SL partnerships exist between PNKP and two PTEN-modulatory proteins that act on cytoplasmic PTEN, namely, MAST2 and MAGI3. Our data support the role of PTEN in SL with PNKP and suggests an essential role of PTEN cytoplasmic protein phosphatase function in generating PTEN/PNKP SL.

Financial Support: This work was supported by Canadian Institute of Health Research (CIHR). El Gendy, MA is the recipient of CIHR and the Alberta Innovates-Health Solutions (AIHS) Post-PhD Fellowships.
109. **Inhibition of the Na+/H+ exchanger (NHE1) increases susceptibility to paclitaxel in invasive breast cancer cells**

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The pH gradient in normal cells is tightly controlled by the activity of various pH regulatory membrane proteins including the Na+/H+ exchanger, NHE1. NHE1 becomes constitutively active in a neoplastic milieu, dysregulating pH homeostasis and altering the survival, differentiation, and proliferation of cells, thus causing them to become tumorigenic. In breast cancer cells, cytoplasmic alkalinization as a result of NHE1 hyper-activity results in an acidic tumor microenvironment that facilitates aggressive cellular proliferation, migration, and invasion leading to tumor metastasis. The pathophysiological role of NHE1 in tumor progression with regards to ion flux is evident, however, the manipulation of pH in and around tumor cells has only recently been considered as a strategy in augmenting cancer therapy. Here, we studied the effect of NHE1 inhibitors EMD87580 ((2-methyl)-4,5-di-(methylsulfonyl)-benzoyl)-guanidine) and HMA (5-(N-N-hexamethylene)-amiloride), either alone or in combination with paclitaxel (Taxol), on NHE1 exchanger activity, cell viability, proliferation, migration, and invasive potential in a highly invasive triple-negative breast cancer cell line, MDA MB 231. We found that cells treated with EMD or HMA in combination with paclitaxel at low doses (0.1nM to 1nM), were significantly more susceptible to cell death than cells treated with paclitaxel or inhibitors alone. While no discernible differences between treatments were observed in cell proliferation rates, a significant reduction in the rate of migration and invasion was observed in cells treated with paclitaxel with either EMD or HMA. To highlight the importance of NHE1 function, we also generated an NHE1- knockout cell line for comparison with the parental MDA-MB-231 cells that endogenously express NHE1. The NHE1 knockout cell line showed no demonstrable Na+/H+ exchange activity, and much lower rates of migration and invasion compared to wild-type cells. Our results demonstrate, for the first time, that inhibition of NHE1 potentially increases the susceptibility of invasive breast cancer cells to paclitaxel-mediated cell death at doses much lower than the established IC50 of paclitaxel in MDA MB 231 cells. Since pH regulation appears to play an integral role in the switch from a normal to a neoplastic and metastatic phenotype, these data lend further credence to the importance of NHE1 as a potential target in breast cancer chemotherapy.

SRA and LF are supported by the Canadian Breast Cancer Foundation and the Women and Children’s Health Research Institute.

110. **CRABP1: A novel triple-negative breast cancer marker implicated for retinoic acid resistance**

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Retinoic acid (RA) is an anti-proliferative agent shown to act as a potent inhibitor of tumour growth in preclinical models. However, clinical trials designed to test the efficacy of RA as an adjuvant for the treatment of solid cancers have been disappointing, primarily due to intrinsic and/or acquired RA resistance. Although the mechanism underlying RA resistance in breast cancer is not known, ER-negative and triple-negative breast cancer cells have been reported to be more resistant to RA than ER-positive cells. Two RA binding proteins, FABP5 and CRABP2, have previously been shown to play an important role in determining RA response in breast cancer cells. Here, we identify CRABP1 as a third RA binding protein involved in mediating breast cancer cell response to RA. CRABP1 is preferentially expressed in triple-negative breast cancers, with elevated levels of cytoplasmic CRABP1 associated with poor patient prognosis. In contrast, CRABP2 is expressed at significantly higher levels in ER-positive compared to ER-negative breast cancers, with elevated levels of nuclear CRABP2 significantly associated with a better prognosis. We demonstrate that CRABP1 expression inhibits RA signalling in breast cancer cells by sequestering RA in the cytoplasm. We also show that CRABP1 exerts an inhibitory effect on RA action through modulation of critical downstream effectors of RA signalling such as CRABP2, cyclin D1 and AP2A, while enhancing the expression of RBP7, a cellular retinol-binding protein that facilitates retinol metabolism. Our results indicate that CRABP1 is an adverse factor in triple-negative breast cancer and a potent inhibitor of RA activity in breast cancer cells. We propose that CRABP1 may serve as a predictive biomarker for RA therapy and a target for overcoming RA resistance in ER-negative and triple-negative breast cancers.
Poster Presentations

111. Energy Metabolism and Requirements in the Cancer Patient: Your Guess is NOT as Good as Mine!

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The nutritional care and management of cancer patients includes provision of adequate nutritional support to ensure that they attain and maintain a desirable body weight. In order to improve nutritional status and avoid negative outcomes associated with over- or underfeeding, it is imperative that accurate assessments of their nutritional requirements are obtained. Unfortunately, weight loss and malnutrition occur commonly in patients with cancer and are often thought to be associated with disturbances in energy metabolism caused by the tumour. The ability to accurately determine the energy requirements of these patients is therefore essential for the provision of optimal nutrition support. In a clinical setting, energy requirements are often estimated from population equations (best guess scenario) however, recent literature has shown that these equations may underestimate energy requirements in cancer patients. One way to determine energy requirements is by quantifying energy expenditure. Despite the accuracy of indirect calorimetry, this method has been rarely used in cancer populations, and offers a novel insight into the energy metabolism of cancer patients.

A Whole Body Calorimetry Unit is a non-invasive sophisticated research suite that measures energy expenditure, substrate oxidation, and energy balance for individuals using respiratory gas exchange information (i.e., CO2 production and O2 consumption). This method is the only way to truly measure an individual’s energy requirements. Energy expenditure and substrate oxidation are calculated every minute from measurements of oxygen (O2) and carbon dioxide (CO2) in the air samples that are continuously removed from the unit. This allows for calculation of energy expended in kilocalories (kcal) for a given time period (such as during resting, exercise or sleep), as well as total energy expenditure (kcal/day). The assessment of respiratory quotient (CO2/O2) provides an indication of how specific energy substrates (carbohydrate or fat) are being utilized in the body. These variables can then be integrated with nutrient intake to determine energy balance.

A better understanding of the characteristics of the utilization and metabolism of the major fuel sources in cancer patients will take the “guestimation” out of energy requirement calculations. Furthermore, using more accurate measures of energy expenditure has the potential to improve diagnostic tests for assessing body energy balance and requirements during the cancer trajectory and under the differing modalities of therapy.

112. Biomarkers as predictors of response to cancer treatment: Breast cancer example

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Breast cancer treatment has experienced several changes in the last decades due to the innovation of specific prognostic and predictive biomarkers that facilitate the application of more personalized therapies to different molecular sub-groups. Presently, more women are being treated with neoadjuvant (preoperative) therapy which involves chemotherapy or endocrine agents before surgery, for earlier-stage operable breast carcinoma. Following this mode of pre-operative systemic treatment could improve the surgical option and make inoperable tumors operable. It can also increase the breast conservation rate. Another key benefit of neoadjuvant therapy is monitoring response to the treatment. The good response to neoadjuvant therapy with complete pathological response (pCR) is a surrogate marker for overall survival.

Objective: The aim of this research was to demonstrate the gene expression changes with response to treatment. This information can be helpful to check the sensitivity of drug after short period of therapy. Specifically, to built a model to interrogate the association between neoadjuvant chemotherapy response (pCR in this case) and gene expression modules, recapitulating important biological processes such as proliferation, immune, stroma and “druggable”oncogenic pathways in different breast cancer subtypes.

Data Sources: We collected publicly available gene expression data based on the review of selected literature on breast carcinoma after neoadjuvant therapy with the clinical and pathologic characteristics.

Results: We demonstrated different biological processes and pathways are associated with pCR in different BC subtypes.
113. Unraveling the role of DEAD box 1 in early mouse development and RNA degradation

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Background: DEAD box proteins are RNA unwinding proteins implicated in all aspects of RNA metabolism. DEAD box 1 (DDX1) was identified by differential screening of a retinoblastoma cDNA library and was subsequently found to be over-expressed in a subset of neuroblastoma and retinoblastoma tumours and cell lines. Recently, we have shown that elevated levels of cytoplasmic DDX1 in breast cancer is an indicator of poor prognosis.

Objectives: (1) To examine the role of cytoplasmic DDX1 during development with the goal of understanding why elevated levels of cytoplasmic DDX1 are associated with poor prognosis. (2) To investigate the mechanism of action of DDX1 using biochemical assays designed to examine the degradation of RNA.

Design, Methods: We are investigating the role of DDX1 in vivo using a DDX1 knock-out mouse model. Embryos from wild-type mice and from heterozygous matings are immunostained with anti-DDX1 antibodies and genotyped by PCR. For biochemical analyses, we are using native and truncated DDX1 proteins, an RNA degradation assay and radioactively labeled single-strand RNA. Products are run on native acrylamide gels.

Results: Ddx1-/- mice die pre-implantation between the 8-cell and blastocyst stages. Immunostaining of pre-implantation embryos shows cytoplasmic localization for DDX1. In these embryos, DDX1 forms granules which transition through development from multiple small granules per cell (oocyte) to one large aggregate per cell located adjacent to the nucleus (blastocyst). Ribonuclease A treatment abolishes DDX1 granules, suggesting that cytoplasmic DDX1 granules are RNA dependent. Based on our RNA degradation assays, we have found that DDX1 is a ribonuclease that degrades RNA in a magnesium-dependent but energy-independent manner. By mutational analysis we have found that the unconserved carboxy-terminal region of DDX1 is critical for RNA degradation. We are currently identifying the key amino acids required for this activity. We are also analyzing the products of RNA degradation in order to elucidate the mechanism that DDX1 uses to degrade RNA.

Conclusions: DDX1 is found in RNA containing granules that are established during oogenesis. As large amounts of RNA are stored during oogenesis, we are proposing that DDX1 is associated with storage, protection and/or turnover of RNA during early embryonic development. However, as development progresses, DDX1 may subsequently play a role in the degradation of maternal RNA. Together, these studies suggest a role for DDX1 in the maternal RNA to zygotic RNA transition.

114. Application of a new multicomponent reaction to the inhibition of a human DNA repair enzyme with a library of polysubstituted piperidines

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The human polynucleotide kinase/phosphatase (hPNKP) is a dual action class of enzyme having the main function of repairing damaged DNA at both the 5'/3’–termini. Cancer cell depleted of hPNKP presented a lowered rate of survival after being exposed to ionizing radiation, thus opening a new battlefield against cancer. An efficient method developed around a multicomponent reaction (MCR) enabled our group to create a library of polysubstituted piperidines to be tested against hPNKP in a fluorescence base assay. A12B4C3 was marked as our lead compound effectively inhibiting the hPNKP (allosteric inhibitor). The core of the library design evolved around tandem reactions consisting of a Diels-Alder cycloaddition between a boronoazadiene and maleimide followed by a stereospecific alkylation with an aldehyde. Commercial N–substituted maleimides are either rare or expensive for the purpose of a library design. This drawback led us to create our own set of maleimides through various reactions like imidation, Mitsunobu and Chan–Lam coupling. A new high-throughput screening based on a Universal Molecular Beacon was adapted to monitor the real time phosphatase activity of hPNKP. With a number of new maleimides in hand and the new biological assay we hope that A12B4C3 successor will now be within our reach.
**Poster Presentations**

115. Study of NHP-Drug Adverse Reactions (SONAR) Focus on Cancer

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**Purpose:** To investigate the rates of adverse event(s) (AE) associated with natural health product (NHP) use, prescription drug use and concurrent NHP-drug use in individuals with cancer.

**Background:** We know that nearly 73% of Canadians use natural health products (NHPs), such as vitamins and herbs etc, and more than half take NHPs along with their prescription medicines. NHP use in oncology patients puts them at greater risk for NHP adverse events and makes them vulnerable to NHP-drug interactions, since commonly used NHPs such as ginseng and echinacea stimulate immunity and may interfere with chemotherapy.

**Methods:** This is an ongoing 3 year study where participating oncologists from Centers in Alberta and across Canada are asking subjects with Cancer about (i) chemotherapy and prescription drug use, ii) NHP use and iii) experiences of AEs during last one month.

**Results:** So far the data from the Cross Cancer Institute in Edmonton shows that of the patients coming in the GU and Breast clinics 45% patients reported taking NHPs only while 35% reported taking NHPs and drugs concurrently. Of these, 20% patients in GU clinics while 16% patients in Breast clinics reported an AE respectively. Compared with prescription drug use, patients reporting concurrent NHP-prescription drug use were twice more likely to experience an AE in Breast clinics while the ratio was equal in GU clinics.

**Conclusion:** Nearly half of cancer patients coming to the centers take NHPs and those patients who take NHPs and drugs concurrently are at a greater risk of experiencing an AE than those taking prescription drugs only. Active surveillance provides a valuable means of detecting such AEs and can be incorporated into the medical histories obtained by clinicians. Also since each cancer type may react differently to NHPs and rate of AE as seen here, hence the need to study as diverse clinics as possible.

At the end of the study we hope to learn which combinations can be taken together safely and which combinations can cause serious harm.

Also this study will provide drug and safety information that will be valuable for all clinicians, patients, their families as well as regulatory bodies such as Health Canada.

We have developed a community based NHP-drug interaction grid previously and will make an oncology specific grid from our study to prevent harms by avoiding products that are not safe to take together.

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116. Protein Expression Analysis Using Multiplex Quantitative Immunofluorescence

Enwere E

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Immunohistochemistry has been used for decades as a means to determine protein expression and localization in normal and diseased tissue. Its use as a screening tool has been limited, however, by the low throughput and procedural complexity. Here we present a workflow which allows for concurrent immunostaining, data acquisition and analysis of up to four protein targets in several hundred tissue samples. The procedure hinges on the combination of many samples into a tissue microarray (TMA), whereupon hundreds of samples can be stained concurrently on a single slide. Automated fluorescent immunostaining and whole-slide scanning reduce procedural variability, permitting single-cell or tissue-scale study of protein expression and relocalization. We demonstrate a protocol for TMA production, staining, and data analysis of four markers (including a nuclear marker) in formalin-fixed, paraffin-embedded (FFPE) human normal and cancerous tissue. We further demonstrate the use of image analysis software to quantify biomarker expression, and associate these data with various clinical indicators such as progression-free survival or overall survival.
Prostate Cancer is one of the most common cancers in American men. Most men with prostate cancer can make a normal life, many never know that they have cancer, and most of them die of other causes.

If the disease is detected in early stage, the tumor can be treated, and the chances for survival are very high (most of the prostate cancer patients are diagnosed in an early stage). But, if the tumor has already started spreading through the bones, lymph nodes or lungs, it is not curable.

Screening can help finding early stage cancers, when it is easier to be cured. Regarding prostate cancer, prostate-specific antigen (PSA) blood test is used frequently to find early the disease. Prostate cancer can also be tested by digital rectal exam (DRE), in which physician test the prostate gland manually.

However, no-one of these test are 100% accurate. If the results of either one are abnormal, further testing may be done to confirm if there is a cancer. Either the PSA test or the DRE can have false results; false positive and false negative. In both cases the consequences are devastating. A false positive imply that men will have a prostate biopsy (with the intrinsic risk of infection, pain and bleeding) when it was not necessary. Whereas, a false-negative will give some men the false feeling that they are healthy but they actually have a cancer. Furthermore, the cancer will be probably detected in a later stage.

On the other hand, some prostate cancers grow so slowly that they would probably never cause the death, or even caused any symptoms. But, sometimes, these men may have to be treated with surgery or radiation. Both treatments have side effects, such as urinary, sexual and/or bowel effects which may affect patient’s quality life.

The main objective of our project is to develop an early detection test, with which men will be able to know (with a high percentage of accuracy) if they have a tumor or not. Moreover our platform can deliver radiotherapy selectively to the tumor in case it is necessary. For that purpose Tobacco Mosaic Viruses (TMV) will function as scaffolds to which will be anchored radiolabeled agents and tumor specific peptides for targeting. In vitro and in vivo methods will be performed to confirm the integrity and the target specificity of the probes and to give away the progression of the tumor. Additionally, ex vivo methods will be carried out in order to move these nanoparticle formulations towards clinical translation.
119. Fusogenic liposomes: a novel therapeutic strategy to efficiently target and destroy prostate cancer

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Prostate cancer is the most common malignancy in North American men. While patients have benefited from advances in androgen deprivation therapy, prostate cancer ultimately evolves to a metastatic or castrate-resistant state that makes it the second-leading cause of cancer mortality in males. In the past 3 years, new chemotherapeutic drugs with efficacy in advanced disease have been introduced, yet their efficacy is limited by dose-limiting toxicities and side effects. The reason for this is primarily suboptimal biodistribution - they do not target tumors directly. To overcome these limitations, we have developed a novel drug encapsulation system for efficient and specific delivery of chemotherapeutic agents to prostate tumors. The reptilian reovirus-derived fusion-associated small transmembrane (FAST) protein (p14) significantly increases the fusion of liposomes to cell membranes by virtue of its N-terminal fusion peptide motif. In this study, we combine these fusogenic FAST liposomes with peptides targeted to gastrin-releasing peptide receptors (GRPR) to improve the delivery of chemotherapeutic payloads specifically to prostate cancer cells while sparing normal tissue.

A fusion protein containing p14 and a C-terminal bombesin peptide was produced in a baculovirus expression system and incorporated into liposomes. The targeting and fusogenic properties of the p14-bombesin protein-containing liposomes were confirmed using flow cytometry. These experiments demonstrated improved delivery to cancer cells in vitro (PC3), compared to non-tumoral cells (BPH). The specificity of targeted fusogenic liposomes was confirmed by knockdown of GRPR and by blocking with free bombesin peptides.

As a further proof of principle, we utilized a commercial formulation of Doxil and modified the formulation to incorporate our p14-bombesin fusion protein. This formulation showed significantly enhanced cancer-specific cytotoxicity in vitro, decreasing the IC50 of Doxorubicin in PC3 and DU145 by 2 fold. The biocompatibility of liposomes including p14 and p14-bombesin has been validated in a rat model, and we are currently testing the anti-tumor effect of doxorubicin delivered with targeting and fusogenic liposomes in a tumor-bearing mice model.

Taken together, these studies demonstrate that molecular-targeted fusogenic liposomes are a promising platform for improving the efficacy of chemotherapies and should show enhanced activity in advanced and metastatic prostate cancers.

120. The Alberta Proteome Platform: Recent Developments and Opportunities.

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Our recent analytical developments in whole tumor proteomics have initiated the pilot studies of tumors from the Canadian Breast Cancer Foundation Tumor bank. These studies are quantitatively characterizing the tumor proteomes from patients that had varied response to treatment. The objective of these three independent studies on estrogen receptor positive breast cancer, triple negative breast cancer and non-small cell lung cancer are to evaluate whether deep proteome coverage of the tumors is sufficient for sub-typing any tumor type according to clinical outcomes.

The concepts and technical challenges of these investigations will be presented in the context of the current capabilities of the Alberta Proteomics and Mass Spectrometry Facility, the Alberta Proteome Platform and research opportunities for investigators at the University of Alberta.
121. TP53 mutations and codon 72 polymorphism in inflammatory bowel disease and colorectal cancer

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Introduction: Colorectal cancer (CRC) is the third most commonly diagnosed cancer in Canada. According to Canadian Cancer Society, 24,400 Canadians will be diagnosed with colorectal cancer and 9,300 will die from it in 2014. It has been known that patients with inflammatory bowel disease (IBD) are at increased risk for colorectal cancer with greater mortality.

The TP53 tumor suppressor gene encodes a transcription factor that regulates expression of genes involved in numerous biological processes. Mutations in TP53 gene reported in 50% CRC and those affect mainly five ‘hotspot’ codons that include 175, 245, 248, 273 and 282. A single nucleotide polymorphism (SNP) in the p53 gene resulting in the presence of either Arginine (Arg) or Proline (Pro) or both Arg/Pro at codon 72 was shown to alter p53 tumor-suppressor properties. This SNP has been investigated as a risk factor for numerous cancers, including CRC. However, no studies have been performed in IBD patients.

Methods: In this study we analyzed 4 most prevalent in CRC p53 mutations (175, 273, 248 and 282) and codon 72 polymorphism in IBD. Genotyping of p53 was performed by sequencing and RFLP analysis of genomic DNA extracted from peripheral blood. The study included 232 Crohn’s disease (CD) patients, 329 ulcerative colitis (UC) patients and 61 non-IBD controls. Analysis of p53 mutations was performed using genomic DNA extracted from 44 biopsies.

Results: The most frequent p53 genotype in IBD patients was Arg/Arg occurring in 54-60% of cases (and only 32% of controls). Arg/Pro was the most prevalent genotype in controls (54%) and less common in patients (35-40%). Pro/Pro frequency was not significantly different between controls and IBD patients. We didn’t detect any TP53 mutations in IBD samples; however in 2 out of 5 CRC patients we detected mutations 175 and 248.

Conclusions: Our data suggest that the p53 72Arg/Arg genotype is associated with increased risk of IBD and possibly IBD-related CRC. Pathogenic TP53 mutations most probably arise later in cancer development.

Funding Sources: AI-HS, WCHRI and the Stollery Children’s Hospital/Hair Massacure Donation Fund

122. Selective targeting of human neuraminidase enzymes for cancer therapeutics

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The human neuraminidase enzymes (hNEU) are rapidly emerging as important mediators of physiological pathways. Members of the hNEU family have been implicated in tumorigenesis, cancer metastasis, inflammation, cell adhesion, and insulin signaling. Importantly, several of these enzymes are known to be upregulated in certain cancers, suggesting they could be an important target for development of new therapies. There are four known isoenzymes in the hNEU family, which vary both in their subcellular location and their preference for glycolipid (NEU3, NEU4) or glycoprotein (NEU1, NEU2, NEU4) substrates. Our group has been working to develop an understanding of the active site topologies among the hNEU isoenzymes, with the goal of designing specific inhibitors of each isoenzyme. In cell adhesion, our findings suggest that the enzyme is altering integrin function through changes in the glycolipid composition of the plasma membrane. Cell migration experiments as well as biophysical studies of integrins in live cells using single molecule diffusion measurements have been used to prove this system. Recent collaborative work has tested newly discovered inhibitors selective for the NEU4 enzyme in glioblastoma cells. A selective NEU4 inhibitor was able to suppress the stem cell markers found in glioblastoma cells grown in spheroid culture. These studies confirm an important role for NEU4 in glioblastoma, and our results with model systems suggest that hNEU inhibitors could be the basis for design of anti-adhesive therapeutics.

References
123. Chromothripsis in the germline. Should we care?

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Chromothripsis is a recently described phenomenon of a local apparent shattering event in the genome and subsequent reassembly, observed in 2-3% of cancers and particularly in bone cancer (25%) (Stephens et al, 2011). It has also been identified in the germline of a subset of individuals with congenital developmental disorders (Kloosterman et al, 2011). Chromothripsis appears to be more common than previously thought as it is found constitutionally in about 20% of the cases of patients with balanced rearrangements (Chiang et al, 2012). While the understanding of the complex chromosomal rearrangement (CCR) resulting from chromothripsis has started to be unveiled (Kloosterman et al, 2012), the relation to developmental problems and long term consequences for the patients’ health, in particular susceptibility to neoplasms, are unknown. We are interested in investigating paediatric patients with incidental findings of chromothripsis, their clinical phenotypes and explore potential risks for cancer susceptibility. A group of two local patients with germline chromothripsis have been already identified. PATIENT 1 was evaluated at 7 years of age for short stature, relative macrocephaly (OFC 75-90%), mild global developmental delay, and an apparently paracentric inversion 13q21-q31. A microarray showed a CCR within chromosome 13q involving 7 copy number imbalances. Five duplications (size 216 kb-5.2 Mb) and two deletions (size 496 kb-5.2 Mb) were identified, and may suggest potential predisposition to cancer and neurobehavioral problems. PATIENT 2 presented at 21 months of age for query Neurofibromatosis type 1 (NF1). No other NF1 signs were seen aside from 15 cafe-au-lait macules, but there were subtle dysmorphic features, normal growth parameters, significant global developmental delay, and a diagnosis of autism. A microarray identified a 1.15 Mb interstitial deletion that partially overlapped the critical region for Potocki-Schaffer syndrome, and a full karyotype revealed at least 8 apparently copy-neutral breaks involving chromosomes 9, 10, and 11. Pair-end next generation sequencing (NGS) studies were undertaken and results suggest a larger number of structural variations than initially revealed by conventional methods. We are planning to screen further patients with apparent balanced chromosomal anomalies to identify further patients with chromothripsis, evaluate their CCR by deep sequencing and explore cancer susceptibility risk.

124. Altered mitotic checkpoint protein expression and kinetochore localization: mechanism of checkpoint defect and aneuploidy in melanoma

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The RZZ complex composed of Roughdeal, Zeste-White10, Zwilch, and accessory proteins Zwint-1 and Spindly, is an essential component of the mitotic checkpoint. The mitotic checkpoint is a failsafe mechanism to ensure accurate chromosome segregation during mitosis. Kinetochore localization of many mitotic checkpoint proteins is essential for function, because the kinetochore is the site of mitotic checkpoint regulation as well as spindle microtubule interaction. It has been postulated that chromosome instability, seen in many cancers, is due to defects in the mitotic checkpoint and perhaps somatic mutations in mitotic checkpoint genes. Surprisingly, somatic mutation is exceedingly rare in cancers, but over-expression of mitotic checkpoint components are more frequently seen in some but not all cancers. It is still not clear how overexpression of mitotic checkpoint genes would relate to defects in mitotic checkpoint as suggested by chromosome instability in cancer cells; therefore, the understanding of the alterations of the mitotic checkpoint in cancer is fundamental to the exploration of chromosome instability and aneuploidy as therapeutic targets of cancers.

Based on public microarray gene expression data, we found that a component of the RZZ complex, Zwilch, is overexpressed in malignant melanoma versus benign nevi and normal melanocytes. We found that kinetochore localization of Zwilch and other components of the RZZ complex are defective during prometaphase (when the mitotic checkpoint is active) in a panel of melanoma cell lines. While the consequences of overexpression of Zwilch is not known, defective kinetochore localization of mitotic checkpoint proteins is expected to cause a weakened checkpoint. My laboratory has mapped the kinetochore localization domain of Zwilch to two regions, one closer to the N-terminus and the other closer to the C-terminus. Based on structural data, the two domains are on two opposite faces of the Zwilch molecule. We hypothesize that the domains might be involved in protein-protein interactions for the assembly of the RZZ complex. Since the kinetochore localization of the RZZ complex subunits are interdependent on each other, we further hypothesize that overexpression of Zwilch interferes with the normal assembly of the RZZ complex and their kinetochore localization and thus the mitotic checkpoint. We propose to 1) confirm and investigate the expression and kinetochore localization of the RZZ complex and accessory proteins in melanoma; 2) investigate the relationship of defective kinetochore localization to checkpoint function in melanoma cells; 3) investigate the consequences of overexpression of Zwilch in melanoma; and 4) investigate the molecular mechanism(s) behind overexpression and defective kinetochore localization. Our goal is to understand how altered expression of Zwilch and defective kinetochore localization of the RZZ complex might affect the efficacy of the mitotic checkpoint in melanoma cells.
125. Exploring a Novel Wee1 inhibitor pathway to enhance mitotic catastrophe in cancer cells

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The mitotic checkpoint ensures the fidelity of chromosome segregation in mitosis by inhibiting the Anaphase Promoting Complexes/Cyclosome, a E3 ubiquitin ligase that targets Cyclin B1 for degradation and thus inactivates Cdk1-Cyclin B1 to promote anaphase onset. Wee1 phosphorylates Y15 on Cdk1 to suppress its activity at G2/M. Wee1 functions in the G2/M DNA damage checkpoint by arresting cells in G2 to allow repair before mitotic entry. Wee1 is upregulated in glioblastoma, osteosarcoma, seminoma, colon cancer and breast cancer justifying Wee1 as a target for cancer therapy. A Wee1 inhibitor, MK-1775, is currently undergoing phase I and II clinical trials on multiple solid tumors in combination with radiation as well as chemotherapy drugs such as Irinotecan, Paclitaxel, Docetaxel, Gemcitabine, Carboplatin, Cisplatin, Temozolomide and Pemetrexed. MK-1775 works synergistically with other drugs in cancer treatment by overriding the G2/M DNA damage checkpoint to promote mitotic catastrophe (MC), which is a form of cell death either during or shortly after an aberrant mitosis. MC usually begins after prolonged mitotic arrest caused by premature entry or failed mitosis. Although MC has been recognized as a major mode of cell death in tumor cells after radiation or chemotherapy, the molecular mechanism that lead to cell death is still unclear. Mitotic arrest sustained by high Cdk1-Cyclin B activity has been implicated to promote MC.

Our data indicate that cancer cells initiate aberrant mitosis with prolonged metaphase arrest and delayed Cyclin B1 degradation with correlated lack of Y15 phosphorylation prior to MC. By inhibiting both Wee1 and Chk1, a positive regulator of Wee1, we and others detect more MC, implying an increased synergistic lethality. Wee1 is inactivated and degraded upon mitotic entry, but a small pool of wee1 persists until mitotic exit. We propose that Wee1 inhibits Cdk1-Cyclin B complexes at the end of metaphase to allow anaphase onset.

Objectives:

- Define the role of Wee1 in metaphase to anaphase transition
- Screen and verify synthetic lethality of all known tumor suppressors and kinases with Wee1 inhibitor

Significance: Wee1 is upregulated in a variety of cancers justifying Wee1 as a target for cancer therapy. Because of the role of Wee1 on the G2/M DNA damage checkpoint, it is commonly believed that MK-1775 works synergistically with other drugs in cancer treatment by overriding this checkpoint. Our research focuses on the unrecognized role of Wee1 in promoting metaphase to anaphase transition prior to mitotic catastrophe. Exploring this pathway will lead to identifying synergistic targets to enhance killing of cancer cells by MC.

126. Glia Maturation Factor beta (GMFβ) promotes glial and neuronal tumor cell differentiation

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Introduction: GMFβ was identified as a factor promoting glial cell process outgrowth in vitro and is predicted to be a member of the actin depolymerization factor (ADF) family. GMFβ is highly expressed in the nervous system, with cytoplasmic expression in neurons and glia. We sought to understand the role of GMFβ in CNS development and in gliomas.

Methods: Anti-peptide antibodies to GMFβ were generated. Co-immunoprecipitations (co-IP) were performed with actin antibodies. Glioma cells were treated with cytochalasin D to depolymerize actin or with colchicine to disrupt microtubules. Cis-retinoic acid (RA) was used to promote neurite outgrowth. Phosphorylation status of GMFβ was ascertained using Western blots.

Results: Co-IP experiments confirmed GMFβ:actin complexes. Subcellular localization of GMFβ only changed with cytochalasin D. In primary embryonic forebrain cultures and RA treated cells, GMFβ localized to axons and growth cones. Transfection of wild-type GMFβ but not a C-terminal deletion mutant promoted process outgrowth. Phosphorylated GMFβ (pGMFβ) expression was found in adult brain and low grade gliomas, but not in embryonic brain or glioblastoma.

Conclusions: GMFβ binds directly to the actin cytoskeleton and is an ADF. GMFβ’s phosphorylated form is highly expressed in the differentiated nervous system and low grade gliomas. Future studies will determine whether GMFβ or pGMFβ expression correlates with patient survival. Using the GMFβ knockout mouse, the role of GMFβ in glioma tumor invasion and signaling will be addressed in vivo.
127. Comparison of revised Edmonton Classification System for Cancer Pain (ECS-CP) features across diverse settings

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Aim: Assess the predictive validity of revised definitions for neuropathic and incident pain in the ECS-CP and additional variables not presently included, in a pilot sample of 300 palliative patients in the Edmonton Zone Palliative Care Program (EZPCP) in Edmonton, AB Canada: Test an internet multisite data collection system; Compare the achievement of personalized stable pain control goals to the standard definition of stable pain control used in previous studies of the ECS-CP.

Hypothesis: Patients with less problematic pain features (as classified by the ECS-CP); lower pain intensity and depression scores; and absence of a smoking history will require a shorter time to achieve stable pain control; require less complicated analgesic regimens; and use lower opioid doses than patients with more complex pain syndromes. We hypothesized that frequencies of pain classification features would vary across sites and location of care, with the tertiary palliative care unit (TPCU) having more complex pain features than other acute settings.

Methods: 300 advanced cancer patients were recruited from 3 palliative care sites: Royal Alexandra Hospital (RAH), n=100; University of Alberta Hospital (UAH), n=100; Grey Nuns Hospital, Tertiary Palliative Care Unit (TPCU), n=100. A physician/palliative care consultant completed the revised ECS-CP on initial assessment, weekly follow up (as required) and on final assessment. Additional information included: patient demographics; patient-generated symptom assessments; opioid and adjuvant analgesics; other pain control methods and a Personalized Pain Goal (PPG). PPG defined as: what the patient considered a suitable level of pain. Data were directly entered into web-based data form and analyzed using SPSS. Informed consent not obtained from patients, as only clinical data routinely documented in all services was collected. Patients finished study when reached standard study and PPG stable pain control, died or discharged. Stable Pain defined as for 3 consecutive days (Figure 2): <3 PRN doses and ≤3/10 pain score or ≤PPG if cognitively intact <3 PRN doses only if cognitively impaired.

Discussion: The variability in pain classification features across sites does demonstrate the increased complexity of pain syndromes in the TPCU; The lessons learned with the internet data collection system will be very useful in future national and international studies; The PPG data validate the previous stable pain control definition for the majority of patients. However, the significant number of patients with highly variable pain control expectations reinforce the need to integrate this marker into individual clinical care.

128. Epidemiology of Adult Post-Transplant Lymphoproliferative Disorder Following Solid Organ Transplant in a Canadian Transplant Centre

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Post-transplant lymphoproliferative disorder (PTLD) is a consequence of organ transplantation with a high risk of mortality and graft loss. We retrospectively analyzed records of all patients who received a solid organ transplant (SOT) at the University of Alberta between 1984 and 2013 (n=4453); 1201 (27%) of patients received heart or lung transplant, 2107 (47%) kidney or pancreas transplant, 1145 (26%) liver or multivisceral transplant. PTLD developed in 109 patients, including 44 (40%) cases that occurred less than 2 years after transplant (early PTLD) and 65 (60%) cases occurred over 1 year after transplant (late PTLD). The yearly incidence rate was bimodal, with the largest peaks in incidence occurring at year 1 and a smaller peak at year 8. PTLD continued to remain a long-term risk, with new cases of PTLD occurring over 20 years post-transplant. Multivariate cox regression analyses were performed to determine risk factors for PTLD. Epstein-Barr virus (EBV)-seronegativity in the donor was determined to be an independent risk factor for PTLD (HR 9.2, p=0.008) and all subtypes of PTLD including early (HR=16.4, p=0.000), late (HR=6.6, p=0.000), diffuse large B cell lymphoma (DLBCL) (HR=9.2, p=0.000), EBER-positive (HR=17.4, p=0.000), EBER-negative (HR=5.4, p=0.000). Compared to patients with liver, small bowel or multivisceral transplants, patients with heart or lung transplant were at higher risk of PTLD (HR=2.13, p=0.008), late PTLD (HR=3.1, p=0.002), and DLBCL (HR=5.0, p=0.01), and patients with kidney or pancreas transplants were at lower risk of early PTLD (HR=0.25, p=0.02). Patients age 18-40 at transplant were at lower risk of late PTLD than those over 55 (HR=0.42, p=0.01). Patients who developed PTLD had a significantly shorter overall survival than those who did not (HR=1.45 (CI 1.14-1.85), p=0.003). In conclusion, incidence rates of PTLD in our population was highest in the 1st and 8th years post-transplant, and patients recipients with negative EBV serology and heart or lung transplants had excess risk.
Cancer is the leading cause of premature death in Canada and is associated with significant disability prior to mortality. Death normally occurs after extensive medical treatments that add not only to the various burdens of patients and their families but also to the financial burden of governments. The high costs of health care and demographic pressures of an aging population contribute to the perception of an impending health care crisis, and many taxpayers are concerned about the possibility of unsustainable growth in the health care budget. Policy makers have assumed that expanding palliative care programs will help to reduce costs by shifting care from institutions to the community. Cost savings however depend largely on untested assumptions regarding the relationships between health care expenditures, age and time to death. These relationships and other consequences of de-institutionalization are not well understood.

The observatory describes a virtual research and education laboratory supporting clinicians, administrators and policy makers to measure cost and performance associated with the financing and delivery of health care and social services. Our research focus include the effects of health reform and technological change on health and economic outcomes of dying patients and their families. We aim to enhance the understanding of palliative and end-of-life care for terminally ill cancer patients. Specifically:

1. improve data warehousing and enhance clinical care of dying patients through standardization and application of information technology to symptom assessment, prognostication and resource allocation.
2. improve access and continuity of care provided to dying patients by enabling the patient, family, family physician and regional palliative care program to share assessments and care plans throughout the illness trajectory.
3. enhance performance measurement of care provided to dying patients by facilitating quality improvement initiatives (ie clinical practice guidelines, balanced scorecard indicators, annual reports and atlases plus comparative research studies).

Community based scholarship integrates clinical, education, research, leadership and administrative best practices through extensive consultation with our regional palliative care stakeholders. Specific projects include: advance care planning and goals of care, end of life pathways, symptom prevalence, performance measurement and conservative care for end stage renal patients. We propose to reinforce and enhance an internationally recognized model of regionally integrated, coordinated and comprehensive palliative care program through the continued development and support of a provincial framework. Our findings are expected to facilitate efficient planning in the health care system, to improve predictions of the remaining length and quality of life for patients, to enable better communication of clinicians with patients and their families, to improve the allocation of health care resources at the end of life, and to contribute to reducing costs within the health care system.

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Poster Presentations

131. Promoting Healthy Cancer Survivorship in Rural Practices

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According to the Canadian Cancer Society, there are approximately 750,000 cancer survivors in Canada. With improvements in the detection and treatment of cancer, this number is continuing to grow every day.

Increasingly, individuals with a history of cancer will come to rely upon care received from their primary care providers to ensure that they meet all of their healthcare needs as the oncology workforce becomes overwhelmed. Many cancer survivors simply don’t receive needed follow-up care. In smaller communities and more rural areas, family practitioners or mid-level providers, represent the source of most frequent contact for patients, including cancer survivors. Evidence has shown that although primary care providers are interested in caring for cancer survivors they express a lack of confidence in doing so.

Our research team received funding from the National Cancer Institute in 2012 to develop and implement a Cancer Survivorship Curriculum (iSURVIVE) for rural Colorado primary care practices. The specific aims for this study are the following:

1. Develop the iSURVIVE curriculum in accordance with the Institute of Medicine recommendations for cancer Survivorship and translate this information for use in primary care practices in rural Colorado by using the expertise and experience of a Cancer Survivorship Scientific Advisory Board (SAB) and a Cancer Survivorship Community Advisory Board (CAB).
2. Recruit practices to participate in the iSURVIVE curriculum within rural practices in Colorado using community-based participatory research principles and the CAB to engage the communities and practices of rural Colorado.
3. Implement the multi-faceted iSURVIVE curriculum that will incorporate (a) four in-person individualized interprofessional small group discussions within practice settings, (b) a series of 12 Webinars by cancer experts, (c) a content-rich website, and (d) password-protected moderated discussion forums.
4. Promote the use of evidence-based guidelines to care for cancer survivors through the delivery of the iSURVIVE curriculum.
5. Evaluate the effectiveness of the iSURVIVE curriculum by assessing practice participants’ cancer survivorship knowledge, self-reported use of team-based cancer survivor care methods within the practice setting, satisfaction with the curriculum, and use of cancer care planning as measured by chart review.

The target sample size is 54 of 56 medical practices in the High Plains Research Network (mostly rural and frontier counties). To date, 10 practices completed the first in-person session, 4 completed the second session, and 2 completed the third session. Six webinars have been completed. This study, if shown to be effective, may be used for dissemination to rural Alberta practices to implement a Canadian version of the iSURVIVE curriculum.

132. Abnormal cellular signalling in lymphomagenesis

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The overarching goal of my laboratory is to understand the molecular mechanisms regulating cellular signalling pathways, particularly in lymphocytes. Much of our recent work has been directed at understanding how abnormal signalling contributes to lymphomagenesis; specifically in the T cell lymphoma, anaplastic lymphoma kinase-positive, anaplastic large cell lymphoma (ALK+ ALCL) and the classical Hodgkin B cell lymphoma. This includes identifying and characterizing transcriptional targets of the activator protein-1 (AP-1) family transcription factor, JunB, which is highly expressed in both these lymphomas. We primarily use biochemical, cell biological, and molecular biological techniques in our studies, but we also utilize mouse models and patient materials. Another research interest of the laboratory is understanding basic fundamental features of signalling pathways including the role protein-protein interactions and post-translational modifications play in regulating signalling.
Endometrial cancer frequently harbors activating mutations in proteins involved in PI3K signaling pathway, and this underlies the clinical interest in the use of targeted therapy against PIK3CA and/or mTOR in the treatment of patients with endometrial cancer. While clinical trials have demonstrated some efficacy with the use of these targeted agents, there were no apparent correlations between therapeutic response and PI3K pathway mutation status. This suggests that additional factors may be important in determining response to PIK3CA/mTOR inhibitors in endometrial cancer. The Cancer Genome Atlas (TCGA) group recently characterized a large series of endometrial cancers using multi-faceted genomic, transcriptomic and proteomic analyses and they identified four distinct molecular types of endometrial cancer - POLE ultramutated, microsatellite instable hypermutated, copy-number low endometrioid and copy-number high serous-like types. We hypothesize that response to pathway-selective targeted therapy such as PIK3CA/mTOR inhibition depends on both the molecular type (molecular context) and the mutation status of the targeted pathway. To test this hypothesis, we performed a series of comprehensive genomic analyses on 12 established endometrial cancer cell lines to first determine the respective molecular types. The genomic analyses consisted of targeted mutation screen of the full coding regions of 26 endometrial cancer genes (custom Truseq panel from Illumina), microsatellite instability analysis and copy number aberration analyses (Affymetrix SNP 6.0). Of the 12 cell lines, 8 (Hec-1A, Hec-6, Hec59, Hec-108, Hec-116, Hec-151, Hec-265 and RL95-2) showed a high level of microsatellite instability (MSI-H) and frequent mutations in the genes surveyed (a hypermutation genotype) (MSI-H hypermutated molecular type). Two microsatellite-stable cell lines (Hec-88nu and Hec-251) harbored hotspot POLE exonuclease domain mutation (P286R) and possessed a very high number of mutations that exceeded the mutation rate seen in the MSI-H cell lines (POLE ultramutated molecular type). The remaining 2 cell lines (KLE and Hec-50) showed TP53 mutations and a relatively few number of mutations. Copy number analysis revealed a high number of copy number aberrations in their tumor genome (copy number-high serous-like molecular type). In our preliminary drug screen analysis, we selected a cell line from each molecular type (KLE, Hec-251 and Hec-59) and tested their in vitro responses (MTT assay) to a PIK3CA inhibitor (GDC-0941) and dual PIK3CA/mTOR inhibitors (PF-04691502, PF-05212384 and NVP-BEZ235). We found that the PIK3CA/mTOR inhibitors resulted in dose-dependent inhibition on Hec-59 (MSI-H hypermutated with PIK3CA and PTEN mutations) but showed no effects on Hec-251 (POLE-ultramutated with PIK3CA and PTEN mutations) and KLE (copy number-high serous-like without PI3K pathway mutations), even though both Hec-59 and Hec-251 harbor multiple activating PI3K pathway mutations. These preliminary findings underscore the importance of molecular context in predicting response to targeted PIK3K/mTOR inhibition in endometrial cancer.

134. RIPK2 Activation Resulting in NFkB-Related Inflammation in Hodgkin's Lymphoma

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NFkB is one particular inflammatory signaling pathway associated with the abnormal inflammation in Hodgkin's Lymphoma (HL). Normally, NFkB activation drives the transcription of cytokines and chemokines as a normal response to cellular stress or damage. However, on the microscopic level, HL is characterized by an overproduction of cytokines within its inflammatory microenvironment, which may result in malignancy. As a result, it is clinically important to discover the molecular mechanism driving this abnormality. Although it is unknown whether NFkB is the dominant inflammation pathway in HL, there is evidence that NFkB is activated due to activated protein biomarkers involved in this pathway. One of the key biomarkers investigated is RIPK2, a serine/threonine protein kinase, which causes downstream effects towards activation of the NFkB transcription factor. To look for RIPK2 activation, standard Western Blotting was conducted on two HL cell lines, two leukemia cell lines, and a normal B-cell line. Additionally, cytokine levels from these different cell lines were analyzed and compared. Based on the Western Blotting results, RIPK2 is tyrosine phosphorylated in the HL cell lines, but not in the leukemia or normal B-cell line. Furthermore, cytokine analysis reveals that growth factors TGF, GM-CSF, PDGF, as well as cytokines IL-8, I-L6, and IL-15 are elevated in the HL cell lines. Overall, these two results suggest that NFkB may be an important inflammatory pathway in contributing to the pathogenesis of HL. For further analysis, mass spectroscopy was performed on HL cells to look for any other proteins that associate with RIPK2, as these molecules may potentially be important biomarkers in HL tumors. In the future, looking at RIPK2 drug inhibitors to control abnormal inflammation in HL will be an important step towards new HL therapies.
135. Palliative Care Patients’ Perspectives of a French Translation of the Edmonton Symptom Assessment System Revised (ESAS-r)


Division of Palliative Care Medicine, Department of Oncology, University of Alberta

Aims: The Edmonton Symptom Assessment System Revised (ESAS-r) is a nine-item self-report symptom intensity tool developed for palliative care patients, with the option of adding a 10th patient-specific symptom. Each symptom is rated on a scale from 0 (none or best possible) to 10 (worst possible). Due to growing international uptake, the ESAS-r has been translated into different languages. However, there has not been any agreement regarding a single standard process for translation into multiple languages, which also include patients’ perspectives. The purpose of this study was to obtain palliative care patients’ perspectives regarding a French translation of the ESAS-r.

Methods: We developed a French version of the ESAS-r, using a standard translation method, involving both professional translators and bilingual palliative care experts. Fifteen francophone-speaking palliative care patients were recruited from acute care, cancer care and tertiary palliative care sites in two urban centres in Canada. Participants completed the ESAS-r and then reviewed the tool to identify any problems associated with the translation, in the presence of a trained interviewer. Descriptive statistics and thematic analysis were used to analyze the quantitative and qualitative data, respectively.

Results: Most participants were cancer patients (n=14, 93%), with an average age of 72 years (range: 34-83). The two highest rated symptoms were tiredness (Mean=4.8, SD 2.3) and well-being (Mean=4.2, SD 2.8). Based on participants’ concerns, translations for four of the nine symptoms were revised: drowsiness, nausea, lack of appetite and shortness of breath. Concerns expressed for three additional symptoms (depression, anxiety, well-being) were related to overall difficulty rating these symptoms, not specific to the translation. There were no concerns expressed for pain and tiredness.

Conclusion: The findings from this study provide a vital step in the development of a standardized translation protocol, including patient perspectives, which can be applied to other languages.

136. Digital design of a breast prosthesis for post-mastectomy patients

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The Institute for Reconstructive Sciences in Medicine

Introduction: Breast cancer is a highly prevalent and devastating form of cancer for women. For patients who are waiting for reconstructive surgery or who do not wish to undergo additional surgery following a mastectomy, an external breast prosthesis can aid in physical and emotional rehabilitation. Utilizing advanced digital technologies, the Institute for Reconstructive Sciences in Medicine (iRSM) can follow an innovative and efficient procedure for creating custom external breast prostheses. Although applying these technologies would allow for decreased patient contact and increase design flexibility, it is still a novel process that requires refinement.

Objective: A team of four industrial designers and one anaplastologist collaboratively designed a digital mold for an external breast prosthesis using technologies currently available at iRSM. The mold would be 3Dprinted and used to construct a definitive silicone prosthesis. The mold would allow for the fabrication of a prosthetic breast which could be created more efficiently and would be of equal or greater quality compared to breast prostheses fabricated by traditional means.

Methods: The team used a mock patient to design a breast prosthesis mold using computer aided design (CAD) software. Considerations for mold design, material application and user experience were considered in the design process. Once complete, the mold was 3D printed on a polyjet printer, then cleaned, polished & prepared for silicone application. The anaplastologist then packed the mold using standard medical grade prosthetic silicone.

Results and Discussion: Two attempts were made to pack the mold with silicone. The first attempt had errors due to user inexperience. Modifications were made in the second attempt which was overall a success.

Future development is currently underway to improve upon and finalize the design of the breast prosthesis mold and the silicone materials to be used for the final prosthesis.

Conclusion: The Institute for Reconstructive Sciences in Medicine is committed to improving the care of post-mastectomy patients. Although the first attempt to design a breast prosthesis mold was positive, continued development is required to improve upon materials, and optimize prosthesis weight and patient comfort. Future research will include usability testing of the mold, assessment of materials and finally patient application.
137. Collaborative Design for Head and Neck Cancer Patients: A case study on the design process for a Glossectomy Spoon, The Institute for Reconstructive Sciences in Medicine

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The Institute for Reconstructive Sciences in Medicine

Head and neck cancer (HNC) will affect an estimated 4,300 Canadians this year alone and can result in devastating post-treatment functional impairments such as eating and speaking. Eating safely and efficiently following HNC can become a challenge for some patients and is further complicated by recurrences and radiation therapy. The present limited resources available to patients with eating difficulties have left them with few interim options: They can either buy a less than ideal utensil at a very high cost or fabricate an adaptive tool out of existing objects. It is therefore important that assisting HNC patients in their survivorship does not end with conventional restorative and rehabilitative options. Industrial design, a study that focuses on understanding human needs through exploration and prototyping, can help address unique patient needs and ultimately improve their health and quality of life.

Objective: A team of one speech-language pathologist and three industrial designers aimed to collaboratively design a spoon specific to the oral needs of a patient with head and neck cancer and recurrence. The spoon will (1) allow the patient to place the food posteriorly enough to swallow and (2) be aesthetic in design.

Methods: The team contacted a representative patient, who consented to participating in the design process. A semi-structured interview was used to solicit details on the patient’s specific functional problem, their interim solution, and the impact of these on quality of life. Using this information, the design team came up with two concepts considering user experience, functionality, aesthetics, materials and manufacturing methods. Advanced digital technology, including computer aided design software and 3D printing, were used throughout the design process.

Results and Discussion: Two concepts and one iteration were developed for the patient. The design team received positive feedback from the patient on both concepts, although she ultimately selected one spoon as ideal for her needs. Future development is currently underway to finalize this product and make it available for patients with similar functional limitations.

Conclusion: Collaborative design, together with patient perspectives, can be a useful approach to address functional limitations beyond the conventional restorative and rehabilitative treatments. Aesthetic and emotional design may alleviate stigma and shame experienced by patients with eating difficulties. Next steps will assess the safety of the patient’s swallow when the new spoon is used. Future research will include usability testing of this spoon with additional HNC patients as well as other patient groups.


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An accurate model of patient survival time can help in the treatment and care of cancer patients. The common practice of providing survival time estimates based only on population averages for the site and stage of cancer ignores many important individual differences among patients. We apply machine learning techniques to learn the patient-specific survival time distribution based on patient attributes such as blood tests and clinical assessments. When tested on a cohort of more than 2000 cancer patients, our method gives survival time predictions that are much more accurate than popular survival analysis models such as the Cox and Aalen regression models. Our results also show that using patient-specific attributes can reduce the prediction error on survival time by as much as 20% when compared to using cancer site and stage only. Besides, we have built an website that provides this survival distribution prediction service using our model, which can be found at http://pssp.srv.ualberta.ca/.
139. What is the most appropriate time frame for assessing symptoms? A validation study of the Edmonton Symptom Assessment System Revised (ESAS-r) in advanced cancer patients

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Introduction: The Edmonton Symptom Assessment System (ESAS) is a widely used symptom assessment tool developed for advanced cancer patients. In response to recent concerns regarding the ESAS, we developed a revised version, the ESAS-r. The ESAS-r offers distinct advantages over the ESAS, by revising problematic items, while still retaining core elements of the ESAS, including assessing symptoms “now.” Validity evidence is needed to determine an appropriate time frame for assessing symptoms and to compare the tool with similar measures.

Aim: The overall aim of this study was: (1) to gather validity evidence by assessing differences in ESAS-r responses between “now” and “24 hour” assessments; (2) to compare the ESAS-r with a quality of life measure, with symptoms rated “over the past 7 days.”

Methods: 50 cognitively intact advanced cancer patients receiving palliative care services in a tertiary cancer facility completed two versions of the ESAS-r: ESAS-r-A (rating symptoms “now”) and ESAS-r-B (rating symptoms “during the last 24 hours”), as well as the EORTC QLQ-C15-PAL, a quality of life measure designed for palliative care. Correlations were calculated using Spearman’s rho (rs).

Results: Most patients were women, married and outpatients, with an average age=62; The most common cancer was genito-urinary; Half of the patients were receiving radiotherapy; The largest difference in mean scores between the ESAS-r-A and ESAS-r-B were for pain, drowsiness and shortness of breath; Individual item correlations of the ESAS-r-A and ESAS-r-B ranged from .576 (pain) to .858 (anxiety). Total symptom distress scores (SDS) were highly correlated; Item correlations between the QLQ-C15-PAL and the ESAS-r-A and ESAS-r-B ranged from 0.224 (anxiety) to 0.881 (shortness of breath); Correlations between well-being and the QLQ-C15-PAL were relatively low: 0.419 to 0.587; Total SDS scores for ESAS-r-A and ESAS-r-B were moderately correlated with the total score for the QLQ-C15-PAL.

Discussion & Conclusions: Using a time frame of “now” versus “during the last 24 hours” did not substantially influence patients’ responses for most symptoms in this predominant outpatient population, except for pain, drowsiness and shortness of breath, which may have fluctuated more over a 24 hour time period. The differences in time frame between the QLQ-C15-PAL (7 days) vs. the ESAS-r (now, over 24 hours) may have resulted in lower correlations, especially for less stable symptoms. Surprisingly, well-being did not correlate highly with the overall quality of life item or the total quality of life score. The moderately high correlations between the QLQ-C15-PAL and the ESAS-r-A and ESAS-r-B suggest that the ESAS-r is effective for assessing quality of life. Further validity evidence with larger samples of advanced cancer patients, as well as early stage cancer, non-cancer and inpatient populations, is warranted. Qualitative data will be analyzed to gain further insights regarding differences in ratings.

140. Mathematical Modelling of Cancer at the Centre for Mathematical Biology

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Mathematical modelling can be a very useful tool in the understanding of cancer and in the design of treatment strategies. Some of the ways that we use this tool within our lab include modelling glioma spread, cancer cell movement, invasion types, tumour control probability, cancer stem cells and the Bystander effect. In this poster, we give a brief summary of all of these projects, including results and simulations.
Poster Presentations

141. ENCORE (Edmonton Cardio-Oncology REsearch): a novel multidisciplinary clinical and research collaboration


Faculty of Nursing, University of Alberta

Background: Cancer treatment-related cardiac effects produce complex multi-disease patients that require specialist care across several disciplines. Currently, treatment teams are hampered by the lack of evidence for specific biomarkers, frequency and type of monitoring, risk-stratification guidelines, and level 1 evidence for interventions. To address these challenges, we have developed a unique collaborative program with multiple aims:

Specific aims of ENCORE:

• to develop a rapid access clinic for cancer patients (CPTs) to obtain cardio-oncology team assessment, including cardiac imaging and biomarkers;
• to embark on a research program involving multidisciplinary and multimodality approaches to prevention and treatment of cancer therapy-related cardiotoxicity;
• to develop a relational database, or ‘Registry’ to determine patterns of care, imaging and clinical outcomes among CPTs, with associated biospecimens;

Approaches:

• Clinic: In addition to assessment, CPTs with established cardiac disease and “at risk” patients scheduled to receive therapy with cardiotoxic potential receive rapid access to novel diagnostic tests including BNP and advanced cardiac imaging including echocardiography. In addition to standard reports, new post-processing parameters, ie: LV strain, tissue Doppler imaging, and speckle tracking will be analysed to evaluate new techniques.

• Research: we are investigating the effects of standard and targeted cancer therapies from multiple avenues. Using cardiac MRI, MANTICORE examines the effects on LV volumes of standard heart failure pharmacotherapy in patients receiving trastuzumab-based chemotherapy. TITAN examines the impact of an intensive multidisciplinary team intervention on cardiovascular dysfunction and fitness, body composition and symptom intensity in breast and lymphoma patients receiving anthracycline-based chemotherapy. We are also keenly interested in the mechanisms of novel biologics targeting VEGF and PI3K, off-target cardiovascular effects and how to optimize these evolving therapies in multiple patient populations.

• Relational database: CPTs are asked to provide written informed consent for their cancer history, cancer therapy, toxicities, biospecimens, imaging, cardiology interventions and clinical outcomes to be entered in the relational database of the Canadian Breast Cancer Foundation Tumor Bank (CBCF-TB).

Future directions: The mission of ENCORE is to foster and perform multidisciplinary cardio-oncology clinical innovation and research. We expect the ENCORE dataset will be hypothesis generating and identify new avenues of research that will ultimately achieve our goal of predicting, preventing and treating cardio-toxic effects of cancer therapy in CPTs. Collaborations are currently underway to develop provincial approaches.

142. Towards a Field Theoretical Stochastic Model for Description of Tumour Growth

Mondaini L

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We develop a field theory-inspired stochastic model for description of tumour growth based on an analogy with a SI epidemic model, where the susceptible individuals (S) would represent the healthy cells and the infected ones (I), the cancer cells. From this model, we obtain a curve describing the tumour volume as a function of time, which can be compared to available experimental data.
**Poster Presentations**

143. A single nucleotide polymorphism in the coagulation Factor II gene increases the risk of deep vein thrombosis in pediatric cancer patients

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Background: The Factor II gene encodes the zymogen prothrombin, a vitamin K dependent glycoprotein, which is proteolytically cleaved to form thrombin. Thrombin functions as a serine protease converting fibrinogen to fibrin generating a fibrin clot. In Caucasian populations, persons with the G20210A mutation in the Factor II gene resulting in an increase in prothrombin levels, have a 3 fold elevated risk of developing a deep vein thrombosis (DVT). However, only 2-3% of the population is heterozygous for G20210A limiting the clinical utility of this mutation as a prognostic marker for DVT. We hypothesised that there may be other mutations in the Factor II gene that are associated with increased DVT risk in pediatric oncology patients.

Methods: We performed a multicentre cross-Canada case control study. Survivors of childhood cancer who experienced DVT while undergoing treatment for their cancer (cases) were matched with survivors of childhood cancer who did not experience DVT (controls). After obtaining informed consent, DNA samples and plasma were obtained from blood. A total of 235 patients (75 patients with DVT and 160 controls without DVT) were recruited. Subjects were genotyped using tagging single nucleotide polymorphisms (SNPs). An r² of 0.8 for linkage disequilibrium and a minor allele frequency >5% were used as threshold values for SNP selection. Using HapMap, 6 tagging SNPs for Factor II were identified and genotyped by allele specific primer extension. RESULTS: A SNP previously reported to elevate prothrombin levels; rs3136516 (A19911G), was identified as a risk factor for DVT (OR: 2.73; 95% CI: 1.5-4.9, p=0.0008). As increased plasma Factor II levels are associated with increased DVT risk, we measured plasma levels of Factor II in these subjects using a functional assay on a STA Compact Instrument. Patients with the 19911 GG genotype had statistically significantly increased median Factor II plasma levels 113% as compared to 104% ( p=0.004) in the 19911 AA genotype.

Conclusions: We have identified a genetic polymorphism correlating with an increased risk of DVT in pediatric cancer patients. The GG polymorphism is associated with increased plasma levels of Factor II which is the likely mechanism for increased DVT risk. Future research will be aimed at confirming these results in a validation cohort.

144. Can Gamma Delta T Cells Target Breast Cancer Stem Cells?

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Gamma Delta T cells (GDTc) comprise 2-5% of circulating lymphocytes. Immunosurveillance cells that are garnering great interest for their anti-tumoral activity, GDTc recognize antigens directly, without major-histocompatibility-complex (MHC) presentation, enabling rapid response. Among other antigens, GDTc respond to self-molecules signaling cellular stress; recognition and tumour lysis is mediated by the T cell antigen receptor (TCR) and/or the natural killer receptor NKG2D. NKG2D ligands include UL16-binding proteins (ULBP) 1-4 and major histocompatibility-like proteins MICA and MICB, which are often upregulated on transformed cells. Phase I clinical trials have confirmed the safety of GDTc in cancer therapy, either via adoptive transfer or in vivo stimulation with compounds such as the aminobisphosphonate zolendronate. However, we cannot cure cancer if we fail to target cancer stem cells (CSC), the small population of cells responsible for tumor maintenance, resistance to cancer therapies and recurrence of cancer after treatment cessation. Only a few studies thus far have reported the ability of GDTc to target CSC (colon cancer and neuroblastoma). Some studies suggest that most CSC do not express detectable MHC I or natural killer (NK) cell activating ligands, which may enable them to escape adaptive and innate immune surveillance. In general, stem cells have reduced expression of MHC class I as well as NKG2D receptors, governed by epigenetic regulation such as DNA methylation. We are investigating whether immune resistance applies to breast cancer CSC. Our panel of human breast cancer cells shows a range of CSC percentages, defined by the expression of CD44 and concomitant absence of CD24 on the cell surface. MCF-7 and SUM 149 comprise very low levels of CD44+CD24- CSC, whereas MDA-MB 231 and SUM 159 are mainly CD44+CD24-. We will focus specifically on determining the effect of GDTc on CSCs. Interestingly, MDA-MB-231 are much less susceptible to GDTc killing than MCF-7, suggesting an inverse correlation between GDTc cytotoxicity and prevalence of CSCs in breast cancer targets. The bulk population of MDA-MB-231 and MCF-7 express one or a combination of ULBP-2,5,6, ULBP-3 and MICA/B. However, a comparison of their expression levels between CSC- and non-CSC-fractions has yet to be determined. We hypothesize that lower tumour antigen expression on CSCs enables their escape from GDTc killing. Determining the mechanism of action involved in CSC immune tolerance may further aid in developing ways to render CSC more susceptible to the immune system.
Introduction: Research in pharmacogenomics aims to elucidate the contribution of genetic variation to the response to medications used in clinical practice. Once an individual’s genetic profile is determined, the range of adverse reactions, from side effects to potentially lethal reactions, can be predicted. A gene that has been the focus of extensive pharmacogenomic research is the cytochrome P450 enzyme 2D6 (CYP2D6). CYP2D6 is highly polymorphic, thus a wide range of variants are currently known. Identifying its alleles and understanding how they impact phenotype in terms of metabolism is desirable because the CYP2D6 enzyme is involved in the metabolic pathway of up to 50 different drugs currently used in various branches of medicine. Of particular interest to Oncology research is the role CYP2D6 plays metabolizing tamoxifen, and for which there is some evidence that CYP2D6 genotype may predict remission status. This enzyme has four levels of activity: an individual may be classified as a poor, intermediate, extensive or ultrarapid metabolizer. The unusual variants referred to above are complicated variants that may be associated with no active enzyme. This project continues work that has identified individuals that would have been classified by standard techniques as having rapid enzyme activity but in fact appear to have unusual variants that may have no enzyme activity. In this manner, the improvement in technology gained will enable correct identification of a wider range of variants of this enzyme than was previously possible, which can be translated into clinical practice in the form of personalized and hence more efficient medical care. Our objective is to identify precisely which unusual variants are present, in order to correctly deduce the activity of the relevant enzyme for the individual.

Methods: The methodology applied to investigate samples likely carrying CYP2D6 hybrid alleles was the polymerase chain reaction (PCR) approach to identify hybrid alleles of CYP2D6 using the technique described by Kramer et al. (2009) and Black et al. (2011), including agarose gel electrophoresis for fragment delineation.

Results: Preliminary data show the presence of a fragment of the expected length in a sample for which the CYP2D6 TaqMan copy number data were consistent with a CYP2D6 hybrid allele.

Conclusions: Preliminary data are encouraging; further analysis, is in progress.

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Introduction: In response to Accreditation Canada’s recommendation, Covenant Health and the Edmonton Zone Palliative Care Program (EZPCP) have adopted the FAMCARE/FAMCARE-2 as a measure of family caregiver satisfaction of palliative care services. In Covenant Health, the palliative care unit at St. Michael’s Hospital, Lethbridge, was the original pilot site in Alberta for the implementation of the FAMCARE in April 2010. The aim of this study was to describe and evaluate family caregiver satisfaction with tertiary palliative care and hospice services in the Lethbridge palliative care unit between April, 2010 and March, 2014.

Method: The FAMCARE (April 2010-March 2013) and FAMCARE-2 (April 2013-March 2014) was mailed to the identified closest relative of the patient about 1 month after the patient’s death. Between April 2010 and March 2014, there were 571 deaths on the unit; 245 forms were returned. The estimated response rate was 43% based on the assumption that the survey was mailed to the closest relative of every patient who dies on the unit. The tools instruct recipients to think about the care that their family member received and to answer the questions as being very satisfied (VS), satisfied (S), undecided (U), dissatisfied (D), very dissatisfied (VD), or not applicable (N/A). Each item is rated on a five-point Likert scale, ranging from 1 (very satisfied) to 5 (very dissatisfied).

FAMCARE Results: 17/20 items were rated as S or VS by >75% of participants; D or VD replies for each question never exceeded 4%; 3 items with the lowest frequencies of positive responses (see Table 1): Q5 (referral to specialists, 63%), Q14 (time required to make a diagnosis, 70.3%), Q17 (information given about the patient’s tests, 72%); For all 3 items, >19% of respondents left item blank or wrote N/A, & <1.5% were dissatisfied (i.e. D & VD combined); Average subscale scores ranged from 1.3 (availability of care) to 1.5 (physical patient care).

FAMCARE-2 Results: Overall, frequencies of satisfied (S or VS) responses for all 17 FAMCARE-2 items were higher than for FAMCARE; All items were rated as S or VS by >75% of participants; 3 items with the lowest frequencies of positive responses (see Table 3): Q11 (practical assistance, 78.5%), Q3 (information about side effects, 78.6%), Q5 (meetings with palliative care team, 83.9%); Average subscale scores ranged from 1.3 (physical symptoms and comfort, family support, patient psychological care) to 1.4 (provision of information).

Discussion & Conclusions: Despite the generally high level of symptom and psychosocial distress in patients admitted, the reported level of family satisfaction is gratifying; Bear in mind that the FAMCARE/FAMCARE-2 tool may be capturing health experiences prior to the patient being in the unit; The results also point us towards areas in which we can improve; Results show that the FAMCARE-2 questions are generally more applicable than the questions in the FAMCARE survey; Generally a higher level of N/A and blank responses for the FAMCARE - related to the applicability of the questions; In the future, qualitative data will be analyzed to gain further insights into areas of improvement.
### 147. The use of computed tomography for body composition assessment: from cancer to cardiovascular disease

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**Introduction:** Computed tomography (CT) images have been widely used to measure body composition in patients with cancer. In this patient cohort, low muscle mass (sarcopenia) measured by CT is associated with adverse health outcomes including impaired physical function, poor treatment response and shortened survival. However, little is known in regards to the value of CT-assessed body composition in other clinical conditions where CT scans are obtained as part of medical diagnosis/treatment.

Patients scheduled for transcatheter aortic valve replacement (TAVR) undergo a CT scan as part of preoperative evaluation. Our goal was to retrieve these images for a secondary analysis of body composition, comparing their phenotype with that of cancer patients, as previously reported in the literature.

**Methods:** Basic information (gender, age, weight and height) was collected from the medical records of patients referred to a regional hospital in Tallahassee (Florida) for TAVR from September 2012 to 2014. Patients with preoperative CT scans at the third lumbar vertebra were included and the analysis of skeletal muscle cross-sectional area (cm²) was done using the SliceOmatic™ software. Skeletal muscle mass index (SMI) was defined as skeletal muscle cross-sectional area adjusted by height (m²). Obesity was defined as a body mass index (BMI) ≥ 30 kg/m².

**Results:** Twenty-three patients (age 83±6.8 years; BMI 26±4.7 kg/m²) were included in this preliminary analysis. Four patients (17%) were overweight or obese. Men presented with a higher SMI (44.1±11.4 cm²/m²) compared to women (34.7±5.1 cm²/m²), p=0.016. Using previously established gender-specific SMI-cutpoints to define sarcopenia in cancer (52.4 cm²/m² for women, 38.5 cm²/m² for men), 8/10 women (80%) and 9/13 men (69%) were identified as having severe muscle depletion; a greater than 2-fold higher prevalence than typically reported in cohorts of patients with cancer. Notably, the same individuals were identified as sarcopenic when an alternative sarcopenia definition (BMI and gender specific) was used. Patients with sarcopenia had lower SMI compared to non-sarcopenic patients (35.4±5.6 cm²/m² vs. 53.0 ± 9.4 cm²/m² respectively, p<0.001). Three patients (13%) presented with obesity concurrently with severe muscle depletion, a condition known as sarcopenic obesity.

**Conclusion:** Preoperative CT images may be a valuable tool for the assessment of body composition in patients undergoing TAVR. The prevalence of sarcopenia was higher than that observed in patients with cancer, although the mean age of our cohort was also higher. A larger sample size will allow for the development of TAVR patient-specific sarcopenia cutpoints, and its respective association with important prognostic outcomes in this cohort of patients.
148. Accuracy of primary osseointegrated implant installation in fibula free flap maxillary reconstruction using the Alberta Reconstruction Technique (ART)


The Institute for Reconstructive Sciences in Medicine & The Division of Otolaryngology Head and Neck Surgery, Faculty of Medicine and Dentistry, University of Alberta

Background: Head and neck cancer (HNC) patient survival remains a major challenge. Approaches that reduce treatment burden for the patient are critical in improving the quality of life in survivorship of HNC patients. Jaw reconstruction rehabilitation (JRR) may extend over 3-5 years. The Institute for Reconstructive Sciences in Medicine (iRSM) and the Division of Otolaryngology Head and Neck Surgery, University of Alberta have developed a procedure termed the Alberta Reconstruction Technique (ART), which is carried out on cancer patients undergoing primary maxillomandibular reconstruction. The ART procedure was developed to reduce time to completion of JRR to under 1 year. This procedure requires the use of Advanced Digital Technologies in Surgical Design and Simulation. The application of ADT in SDS has expanded rapidly in the field of maxillomandibular reconstruction and has been shown to facilitate surgical challenges and optimize the JRR. The procedure involves collaborative preoperative SDS planning. The surgery is fully guided by cutting and drilling guides and transfer templates; all designed using computer aided design software and manufactured using 3D printing technology.

Objective: To assess the accuracy of positioning of osseointegrated implants in patients undergoing primary implant installation in the leg as part of primary fibula free flap maxillary reconstruction undertaken with the ART using custom CAD designed and 3D printed cutting and drilling guides and transfer templates.

Methods: Post-operative medical CT scans of each patient were obtained and used for comparison of the SDS planned implant positions to the actual implant positions. The X,Y,Z coordinates of each planned and actual implant position were obtained. They were then subtracted from one another to obtain the deviation of the implant positions in millimeters.

Results: In the pilot study, eight patient’s post-operative medical images have been assessed. A total of thirty implants have been compared. The average post operative deviation of the planned implant positions to the actual implant positions are 1.51 mm in the X axes, 1.09 mm in the Y axes and 2.32 mm in the Z axes.

Conclusion: At present, the ART procedure has resulted in a higher than expected accuracy of position of primary installation of osseointegrated implants at the primary maxillary reconstructive surgery. Future research will include the continuation of assessing the accuracy of the ART and translating the outcomes to the clinical and surgical team in order to improve future patient treatment.
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