2nd Annual Cancer Research Symposium

UNIVERSITY OF CALGARY | Alberta Health Services

Arnie Charbonneau Cancer Institute

Friday, February 5th, 2016

MacEwan Conference Centre, University of Calgary

Registration deadline: **Friday, January 18th, 2016**

… and many more!

Keynote Lecture: **David Huntsman**

Professor at University of British Columbia

With talks from:

Sunil Verma
Department Head, Oncology
Medical Director,
Tom Baker Cancer Centre

Lynne Marie Postovit
Associate Professor, Department of Oncology, University of Alberta, Sawin-Baldwin Chair in Ovarian Cancer

Gareth Williams
Assistant Professor,
Robson DNA Science Centre,
University of Calgary

Marco Gallo
Assistant Professor, Clark H. Smith Brain Tumour Centre,
University of Calgary

Corinne Doll
Assistant Professor,
Department of Radiation Oncology, Tom Baker Cancer Centre

Darren Derksen
Assistant Professor,
Department of Chemistry,
University of Calgary

Darren Brenner
Research Scientist, Department of Cancer Epidemiology and Prevention Research, University of Calgary

With poster presentations from all Charbonneau graduate students and post-doctoral fellows.

To register, please contact Carmen at ccoelho@ucalgary.ca

Organized By:
Aaron Goodarzi & Carmen Coelho

Arnie Charbonneau Cancer Institute
Research Symposium

Friday, February 5, 2016
MacEwan Conference & Events Centre, University of Calgary

8:00 – 9:00am	Registration desk at MacEwan Hall Foyer *(Breakfast buffet with morning refreshments in MacEwan Hall A)*
9:00 – 9:10am	Welcome address from **Dr. Greg Cairncross,** Director of the Arnie Charbonneau Cancer Institute
Session 1	Chair: **Dr. Aaron Goodarzi**
9:10 – 9:55am	**KEYNOTE LECTURE** **Dr. David Huntsman, MD** Department of Pathology and Laboratory Medicine, University of British Columbia "*Genomic Pathology has changed our Understanding of Ovarian Cancer: What's next for Patients?*
10:00 – 10:25am	Morning Break *(assortment of squares and gourmet cheese platter)*
10:25 – 10:50am	**Dr. Lynne-Marie Postovit,** Department of Oncology, Cancer Research Institute of Northern Alberta (CRINA), University of Alberta *"Cancer Cell Adaptation: What Doesn't Kill Them Makes Them Stronger"*
10:55 – 11:20am	**Dr. Sunil Verma**, Tom Baker Cancer Centre, University of Calgary *"Personalizing Breast Cancer Treatment: From Biology to Tailored Therapy"*
11:25 – 11:50pm	**Dr. Corinne Doll,** Tom Baker Cancer Centre, University of Calgary *"Exploring mechanisms of treatment resistance in cervical cancer"*
11:55 – 12:45pm	Lunch *(Custom Chef's Lunch Buffet)*
Session 2	Chair: **Dr. Jennifer Chan**
12:45 – 1:10pm	**Dr. Marco Gallo,** Departments of Physiology & Pharmacology, Biochemistry & Molecular Biology, University of Calgary *"Chromatin Architecture Defines Cancer Stem Cell Properties"*
1:15 – 1:40pm	**Dr. Gareth Williams,** Department of Biochemistry & Molecular Biology, University of Calgary *"Structural Insights into the RAD51 Paralog Tumor Suppressors Reveal Molecular Details of Disease Causing Mutations"*

1:45 – 2:10pm	**Dr. Darren Derksen,** Department of Chemistry, University of Calgary *"Natural Products and New Drug Delivery Strategies"*
2:15 – 2:40pm	**Dr. Darren Brenner,** Department of Cancer Epidemiology and Prevention Research, CancerControl Alberta, Alberta Health Services *"Lifestyle in the Prevention of Cancer"*
Session 3	Chair: **Dr. Aaron Goodarzi**
2:45 –3:05pm	Afternoon Break *(fresh and savory snacks)* Poster set-up in MacEwan Hall B
3:05 – 3:30pm	**Dr. Lauren Walker, Psychosocial Oncology, University of Calgary** *"Androgen Deprivation Therapy for Prostate Cancer: Developing a National Survivorship Program"*
3:35 – 4:00pm	**Dr. Marcus Eszlinger,** Department of Oncology, University of Calgary *"Perspectives for Molecular Fine Needle Aspiration Diagnostics of Thyroid Nodules in Calgary"*
4:05 – 5:45pm	Poster Session *(Wine and Cheese Reception – cash bar)*
6:00 – 8:00pm	Conference Banquet *(Pre-Registration Required)*

Arnie Charbonneau Cancer Institute
Research Symposium

Friday, February 5, 2016

Poster Abstracts

01 - SUnSET: An efficient non-radioactive method to determine global protein synthesis

Abhishek Ghosh, Savraj S. Grewal
University of Calgary, Arnie Charbonneau Cancer Institute, Dept. Biochemistry and Molecular Biology, Clark H Smith Brain Tumor Centre, 3330 Hospital Drive NW (HRIC-2A33A), Calgary AB, T2N 4N1

Under favorable conditions protein synthesis is a major determinant of cell, tissue and body growth in animals. In contrast, stress-mediated inhibition of protein synthesis alters metabolism and promotes survival and homeostasis. Therefore, understanding the regulation of global mRNA translation will provide insights about the physiological state of an organism. Here we report a non-radioactive method to monitor and quantify global mRNA translation called "Surface Sensing of Translation (SUnSET)" in *Drosophila*. SUnSET uses limited amounts of Puromycin – an antibiotic and a structural analog of aminoacyl tRNA, incorporated into nascent polypeptides to inhibit translational elongation. In particular, we dissected and exposed *Drosophila* larvae in nutrient rich media with 5μg/ml Puromycin for 40 minutes at room temperature and subsequently, froze the larvae on dry ice for further analyses. Immunoblot was performed on proteins extracted from Puromycin-treated larvae, using an anti-Puromycin antibody. Our results indicated that co-treatment of larvae with cycloheximide – a translational inhibitor completely blocked Puromycin incorporation suggesting that Puromycin labeled nascent polypeptides. We also found that Puromycin incorporation can be visibly detected on an immunoblot with as low as 10μg of protein. Finally, we found that Puromycin incorporation was abolished in starved larvae (a condition known to inhibit mRNA translation) compared to well-fed animals. Altogether, our data suggest that SUnSET can be reliably used in *Drosophila* as well as mammalian tissues or cultured cells to assess global protein synthesis as an alternative to radioactivity based methods or polysome density centrifugation. Overall, SUnSET might have diverse applications in various areas of life science such as developmental biology and cancer biology.

02 - Survival among Stage III NSCLC Patients based on Radiation and Chemotherapy Treatment Options

Adrijana D'Silva, Shannon Otsuka, Harold Lau, Gwyn Bebb
Tom Baker Cancer Centre, Department of Oncology, University of Calgary

Background: The standard treatment for stage III non-small cell lung cancer (NSCLC) patients is combined modality concurrent chemo radiation. However, there is no consensus on the optimal chemotherapy, duration of systemic treatment nor the optimal dose of radiation. Furthermore, there is anecdotal evidence to suggest that many patients are not candidates for this treatment. We set out to examine the uptake and outcome of combined modality chemo radiation for stage III NSCLC at the Tom Baker Cancer Centre over a 12 year period.

Methods: The Glans Look Lung Cancer database was used to identify all stage III NSCLC patients living in southern Alberta seen at the Tom Baker Cancer Centre between January 1, 1999 and June 30, 2012. Patient charts were reviewed, basic demographics, treatment, progression date, and overall survival were reviewed. Median survival (MOS) was compared between different treatment modalities using Kaplan-Meier survival analysis.

Results: We identified 1232 stage III NSCLC patients, median age 69, 55% male, 90% current or former smokers, and MOS of 14.7 months (95% CI, 13.7, 15.8). 74% received radiation therapy (MOS 16.6 months) and approximately two-thirds received palliative radiation (defined as <39 Gy) with MOS of 15.0 months (95% CI, 14.0, 16.0). In contrast, only one third received radical radiation (defined as >40 Gy) with a significantly higher MOS of 24.1 months ($p<0.001$, 95% CI, 21.2, 27.1). 89% patients who received radical radiation did so in combination with chemotherapy with MOS of 28.9 months (95% CI, 26.6, 31.2), whereas only one out of ten received radical radiation alone (MOS =16.6 months; 95% CI, 13.4, 19.8; $p=0.484$). Approximately half the patients treated with concurrent chemotherapy did so in the form of platin-based therapy most commonly using Vinorelbine (85%) MOS of 24.5 months (95% CI, 20.9. 28.2) or Etoposide MOS of 18.9 months (95% CI, 7.6, 30.3).

Conclusion: Although the highest MOS survival in stage 3 NSCLC patients is, as expected, associated with radical radiation, two thirds did not receive guideline recommended treatment for their disease. MOS increases when radical radiation is given in combination with chemotherapy. The regimen choice in the form of platin-based chemotherapy with Vinorelbine offers higher MOS survival compared to Etoposide.

03 - PD-1 blockade and Reovirus enhance the efficiency of oncolytic viral therapy in breast cancer

Ahmed A. Mostafa, Kathy Gratton, Jianrui Liu, Jason Spurrell, Zhong-Qiao Shi, Chandini Thirukkumaran and Don G. Morris.
Department of Oncology, Tom Baker Caner Center, University of Calgary, Calgary, Alberta

Oncolytic viruses such as reovirus (RV) are non-pathogenic viruses, which specifically target and lyse cancer cells due to genetic abnormalities, with no effect on normal cells. Recently, RV has been used in human clinical trials in the form of monotherapy or in combination with chemotherapy against different histological malignancies. The challenge of these trials is the elicitation of anti-viral immune response, which results in viral clearance. Moreover, the uses of immunosuppressive agents have only resulted in modest improvement. Immune checkpoint receptors such as programmed cell death 1 ligand (PDL-1) that is upregulated on the surface of cancer cells, binds to the programmed cell death 1 (PD-1) receptor on the surface of activated cytotoxic T lymphocytes (CTL) and results in inhibition of the antitumor T-cell response. Recently we found that RV treatment resulted in upregulation of PD-L1 on the surface of breast cancer cells lines. In addition, in vivo RV treatment upregulates PD-L1 and PD-1 surface expression in pancreatic and glioblastoma patients. Taken together, these results support that RV in combination with PD-1 has both direct oncolytic and immunotherapeutic activity against breast cancer. We tested the combination of local oncolytic RV therapy with systemic immune checkpoint inhibition in a syngeneic immunocompetent murine breast cancer model. We showed that combination of anti-PD-1 antibody and RV significantly decreased tumour burden and enhanced survival of mice compared to RV ($P < 0.01$) or anti-PD-1 therapy alone. In vitro immune analysis demonstrated that combination of PD-1 and RV improved the ability of CD8 T cells to migrate to tumour cite which was correlated with reduced T regulatory cells. PD-1 blockade also enhanced the antiviral immune response through production of long live effector memory T cells. Therefore, these data will provide new treatment strategies with resultant improved efficacy and safety of our breast cancer patients to be translated into phase I/II clinical trials.

04 - A cell cycle arrest and anti-mitotic spindle activity in human cells treated by extracts prepared from the Canadian prairie plant species *Gaillardia aristata* (Blanket flower).

Alessandra Bosco, Kernéis, Sophie, and Golsteyn, Roy M.
Cancer Cell Laboratory, University of Lethbridge, Department of Biological Sciences, Lethbridge, AB, Canada.

We have launched the Prairie to Pharmacy Program in which we investigate endemic plant species from the prairie ecological zone for anti-cancer activities. Several plant species within this zone produce secondary metabolites as part of an ecological survival strategy. These compounds can be a valuable source of chemicals that have biological activities.
The plant species *Gaillardia aristata*, locally known as the “blanket flower”, is endemic to the prairie ecological zone. It has antioxidant and anti-inflammatory activities and was reported to have cytotoxic effects, although little is known about its anti-cancer properties. We prepared extracts of the aerial parts (flower heads, stems and leaves) of *G. aristata* and tested them by phenotypic assays using HT-29 (human colon cancer) cells. The most potent activity was found in a leaf extract, named PP-050E, which was toxic to HT-29 cells at an IC50 concentration of 44 µg/mL. By flow cytometry analysis, HT-29 cells treated by an ethanolic leaf extract arrested at the S and G2-M phases of the cell cycle. These results were consistent with observations of morphology in cells treated by PP-050E. By 24 hours, approximately 50% of the cells were rounded in three treated cell lines, HT-29, SH-SY5Y, and RPE-1. We confirmed that the rounded HT-29 cells were arrested in mitosis by immunofluorescence microscopy and staining with anti-phosphohistone H3 antibodies and DAPI. Analysis by tubulin staining revealed that the cells arrested in metaphase and then acquired defective mitotic spindles as the arrest continued. During this arrest, cells acquired gamma histone H2AX foci. In experiments performed in parallel, we confirmed that the mitotic arrest was distinct from that of the tubulin toxins, Paclitaxel or Nocodazole. The mitotic arrest activity in PP-050E could be isolated to a small number of peak fractions column chromatography indicating the possibility to isolate the bioactive compound/s.
We are collaborating with Dr. Raymond Andersen (University of British Columbia, Vancouver) to isolate the active compound/s by biology-guided fractionation. Several other prairie plant species, such as *Thermopsis rhombifolia*, have shown different anti-cancer activities in preliminary tests.

05 - The role of Artemis in ICL repair

Alexander Anikin and Aaron A. Goodarzi. Robson DNA Science Centre, Southern Alberta Cancer Research Institute, Departments of Biochemistry & Molecular Biology, Cumming School of Medicine, University of Calgary, Calgary, Alberta, Canada. T2N 4N1

Cellular DNA is highly susceptible to chemical modifications from a variety of both endogenous and exogenous agents. The lesions which arise from the associated chemical reactions are typically in the form of base or phosphate backbone modifications that occur on one strand of the DNA backbone: for example, a Reactive oxygen species (ROS) induced Single strand break (SSBR). However, certain agents, such as some of the earliest and most widely-used chemotherapeutic agents pioneered by the nitrogen mustards which can produce usually- irreversible covalent adducts with DNA bases on either the same DNA strand forming Intra strand crosslinks, can be readily removed by the nucleotide excision repair (NER) pathway. They can also be bypassed by some DNA polymerases. Both mechanisms make this type of lesion less toxic. However, when such covalent adducts connect two nucleotide residues from opposite DNA strands, highly toxic interstrand crosslinks (ICLs) are formed. Such lesions act as a barrier to essential metabolic processes such as transcription and replication, and are much more complex in nature for cellular repair pathways to deal with. It has now been revealed that the metallo-β-lactamase (MBL) family members SNM1A, SNM1B (Apollo) and Artemis, digest down the residual cross-linked oligonucleotides, producing a gapped intermediate structure which then allows for gap filling by TLS and the restoration of an intact template strand and thus enabling the completion of DNA replication. We have developed a system for detecting drug induced ICL formation. I have validated DSB repair deficiencies in Artemis-mutated patient primary fibroblasts compared to normal and will be looking at the repair kinetics following ICL inducing agents. I will present my ongoing studies exploring the role of Artemis in the repair of ICL induced DNA damage.

06 - Influence of Antioxidant and Oxidant Properties of Diet on Leukocyte Telomere Length

Alexis T. Mickle,[1,2] Darren R. Brenner,[1,2,3] Tara Beattie,[3,4,5,] Tyler Williamson,[2,6,7] Christine M. Friedenreich[1,2,3]
[1]Department of Cancer Epidemiology and Prevention Research, CancerControl Alberta, Alberta Health Services [2]Department of Community Health Sciences, Cumming School of Medicine, University of Calgary [3]Department of Oncology, Cumming School of Medicine, University of Calgary [4]Department of Biochemistry and Molecular Biology, University of Calgary [5]Arnie Charbonneau Cancer Institute [6]O'Brien Institute of Public Health [7]Alberta Children's Hospital Research Institute

Background: Shorter telomeres have been implicated in the etiology of several human cancers. Telomeres form the ends of human chromatids, where they protect DNA from genomic instability, prevent end-to-end fusion, and limit the replicative capabilities of the cell. Telomeric attrition rates are affected by environmental and lifestyle factors. Shorter telomeres and genomic instability are precursors to carcinogenesis, and are directly influenced by oxidants, antioxidants and chronic inflammation. Specific dietary nutrients may directly influence telomeric DNA by acting either in an antioxidant capacity or as an oxidant. Previous literature suggests that diets low in specific nutrients and antioxidants, or containing processing chemicals may negatively influence telomeric length leading to an increased risk for many cancers; however the literature is inconclusive and lacking in some areas.

Objectives: To determine the association between telomere length and consumption of: total antioxidants, omega-3 fatty acids, processed meat, non-diet soft drinks and alcohol.

Methods: A cross-sectional analysis within the Alberta Physical Activity and Breast Cancer Prevention (ALPHA) Trial will be conducted. Participants were women aged 50-74, postmenopausal and previously inactive. Diet date was self-reported. Telomere length was measured using quantitative polymerase chain reaction (qPCR). Multivariate linear regression analyses will examine the relation between relative telomere length (T/S ratio), modeled as a continuous linear variable, and dietary measures of interest. Potential effect modification and confounding by other breast cancer risk factors will also be examined.

Conclusion: The protective effect of physical activity and diet in cancer etiology have been well established, however the molecular mechanisms whereby these effects occur are currently unclear. Elucidating these molecular processes will lead to increasing understanding on how these lifestyle factors could influence cancer risk. Ultimately, this research will be of relevance for updating dietary guidelines for both the prevention and treatment of cancer.

07 - Immunotherapeutic and Antitumour Effects of Oncolytic Virus and Sunitinib Treatment in Neuroblastoma

Andrea Rakic, Paul Beaudry, and Douglas Mahoney
University of Calgary, Calgary, AB, Canada

Background: Neuroblastoma (NB) is the most common extra-cranial solid tumour of infancy and current treatments for high-risk NB have minimally improved patient survival. Therapies that promote immune-system attack on NB cells show potential but the tumour microenvironment (TME) is a fundamental obstacle in generating antitumour immune responses. To enhance antitumour responses, TME immunosuppressive cells such as myeloid derived suppressor cells (MDSCs) can be eliminated using sunitinib (Su), a receptor tyrosine kinase inhibitor. Therefore, an effective NB treatment may be to deplete MDSCs using Su while simultaneously eliciting an antitumour immune response using the oncolytic virus Vesicular Stomatitis Virus (VSV). Objectives: Determine the antitumour efficacy of VSV Delta M51 in combination with Su in a syngeneic NB mouse model.

Methods: The murine NB cell line Neuro-2a response to the combination therapy was explored via cytotoxicity assays, viral production, and fluorescence microscopy. Bioluminescence Imaging was used to determine tumour and splenic VSV productivity in A/J mice. Flow cytometry was used to detect splenic and tumour immune cell levels. Tumour growth rate and survival determined efficacy of combination therapy *in vivo*.

Results: The combination of VSV and Su demonstrated significantly increased efficacy than with either treatment alone both *in vitro* and *in vivo*. Su also enhanced the oncolytic capacity of VSV as the two agents synergistically increased cytotoxicity *in vitro* and the combination therapy improved survival responses and decreased tumour growth rate *in vivo*. The mechanism for the observed increased antitumour efficacy was explored. Su did not appear to increase viral productivity with regard to NB cell infection either *in vitro* or *in vivo*. Variation in splenic infection exists among higher VSV titres *in vivo*, suggesting that some differences in systemic immune response to the tumour may exist. Splenic myeloid cell depletion was observed in Su treated mice.

Conclusion: Combination therapy may be synergistic and the mechanism does not involve increased VSV production. Further studies will determine if the improved outcomes *in vivo* are due to increased T cell infiltration.

08 - Establishment of Biospecimen Processing Laboratory to Support Large Population Cancer Studies

Angela Chan[1], Danielle Simonot[2], Paula Robson[3], Randy Johnston[2,4] and Nigel Brockton[5]
[1]Translational Laboratories, Tom Baker Cancer Centre, Alberta Health Services, Calgary, Alberta, Canada. [2]Alberta Cancer Research Biobank, Tom Baker Cancer Centre, Alberta Health Services, Calgary, Alberta, Canada. [3]Alberta Tomorrow Project, Alberta Health Services, Calgary, Alberta, Canada. [4]Arnie Charbonneau Cancer Institute and Biochemistry and Molecular Biology, University of Calgary, Calgary, Alberta. [5]Cancer Epidemiology and Prevention Research, Alberta Health Services, Calgary, Alberta, Canada.

With the increasing scale of cancer research studies, including population cancer prevention and biomarker studies, comes increased demand for laboratories capable of large-scale bioprocessing and extraction of nucleic acids. To provide consistent and economical bioprocessing of samples, laboratory automation is required to accommodate the number of samples banked by these ambitious studies. In many cases, the lack of lab automation is the bottleneck and limitation for carrying out such large population study and downstream applications.
The Alberta Cancer Research Biobank's (ACRB) Biospecimen Processing Laboratory (BPL) was established to provide a research-focused facility for the processing of specimens from critical cancer research studies in a high-throughput manner. BPL fulfils the niche between the recruitment, collection and storage focus of the ACRB and the analytical focus of the Translational Laboratories. The main functions of BPL included: biospecimen processing (urine and blood), blood fractionation, nucleic acid extraction and quantification, and tissue microarray (TMA) construction. Since the establishment of the BPL, several projects have been processed using this facility including the Alberta Tomorrow Project, Cancer Epidemiology and Prevention Research studies and other studies from various tumor groups. To be presented are methods used for blood fractionation, TMA construction and the DNA extraction methods and results of one of those studies, the Alberta Tomorrow Project.
The BPL is constantly engaging in improvements and quality assurance. In 2015, the BPL participated in proficiency testing offered by the Integrated Biobank of Luxembourg (IBBL). The lab was tested for DNA Quantification and Purity and DNA Extraction Efficiency from Whole Blood. The lab received the highest score of proficiency for both tests. In summary, the establishment of the BPL had enhanced the feasibility and quality of research requiring large number of biospecimens and productivity of cancer research in Alberta.

09 - The epigenetic regulator ING3 acts as an oncoprotein and Androgen Receptor co-activator

Arash Nabbi, Urszula Lucja McClurg, Olivier Binda and Karl Riabowol
Arnie Charbonneau Cancer Institute & Department of Biochemistry & Molecular Biology, Faculty of Medicine, University of Calgary, Calgary, Alberta, CanadaNewcastle Cancer Centre at the Northern Institute for Cancer Research, Newcastle University, Newcastle upon Tyne, England

Prostate cancer is the most common malignancy among men. Androgen receptor (AR) plays a key role in the progression of this disease. Many proteins including lysine acetyltransferases (KATs) have been reported to be involved in activation of the AR pathway, thereby affecting prostate cancer progression. INhibitor of Growth proteins target histone acetyltransferase or histone deacetylase complexes to the histone H3K4me3 mark, affecting chromatin structure and are frequently altered in cancers. ING3 is the third member of this family, which is an essential member of TIP60 KAT complex.

We find that ING3 potentiates androgen effects, activating expression of AR-regulated genes and ARE-driven reporters. ING3 increases AR-TIP60 interaction, promoting AR acetylation and nuclear translocation. Knockdown of ING3 inhibits prostate cancer cell growth, establishing ING3 as a positive regulator of growth in the prostate and a novel prognostic factor in prostate cancer. *Ex vivo* studies on human benign prostate hyperplasia tissues demonstrate that the expression of ING3 stimulates proliferation of normal cells. Moreover, gene expression survey and biochemical analysis demonstrate that ING3 induces proliferation by controlling an intricate network of cell cycle genes by associating with the $H3K4^{me3}$ mark at transcriptional start sites. Finally, we find that ING3 levels are higher in aggressive PCs, with high levels of ING3 predicting shorter patient survival. Analysis with other predictive factors shows that addition of ING3 levels provides more accurate prognosis in primary prostate cancer. Our study has identified the mechanism by which ING3 promotes prostate cancer progression and indicate its potential as a novel prognostic biomarker.

10 - Investigating *PIK3CA* mutational status on cisplatin and radiation sensitivity in cervical cancer cell lines: implications for PI3K inhibitor therapy

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The phosphatidylinositol-3 kinase (PI3K)/AKT/ mTOR signaling pathway is activated in many different human cancers. Activation is frequently mediated by "hotspot" mutations including E542K, E545K and H1047R in the *PIK3CA* gene, which encodes the catalytic subunit of PI3Kα. We previously reported *PIK3CA* mutation in patients with early stage (IB/II) cervical cancer was associated with poor survival (McIntyre *et al.* Gynecol Oncol. 2013, 128(3): 409-14). The purpose of this study was to determine whether *PIK3CA* mutation renders cervical cancer cells more resistant to conventional therapy (cisplatin chemotherapy and/or radiation), and whether PI3K inhibition enhances cell kill and/or reverse this phenotype. Here, we report that the cervical cancer cell line CaSki, which expresses the *PIK3CA*-E545K mutation, is more resistant to cisplatin than cervical cancer cells with wild-type *PIK3CA*. To validate our findings, we depleted endogenous *PIK3CA* from HeLa cells using shRNA and stably expressed either shRNA resistant wild-type *PIK3CA* or *PIK3CA*-E545K. Cells expressing *PIK3CA*-E545K were more resistant to cisplatin and cisplatin plus ionizing radiation than cells expressing either wild-type *PIK3CA* or lacking *PIK3CA*. The *PIK3CA*-E545K mutation resulted in constitutive *PIK3CA* pathway activation, cisplatin resistance and increased cell migration in cervical cancer cells that was reversed by inhibition of the PI3K pathway using the small molecule inhibitor GDC-0941/Pictilisib. Together, our results suggest that inhibition of the PI3K pathway in cervical cancer patients with the PIK3CA-E545K activating mutation may have therapeutic benefit.

11 - IL-33 orchestrates the brain tumor microenvironment to promote glioma progression

Astrid De Boeck[1,2], Xueqing Lun[1,2], Xiaoguang Hao[1,2], Bo Young Ahn[1,2], Mana Alshehri[1,2], Yoaqing Shen[8], Ngoc Ha Dang[1,2], Xiuling Wang[1,2], Jennifer King [1,2], Manoj Mishra[4,6] ,Janet Wang[4,6] ,Susobhan Sarkar[4,6], Marco A. Marra[8], Steven JM Jones[8], Jennifer Chan[1,2,9], J. Gregory Cairncross[1,2,6], Paul Kubes[3,7], V. Wee Yong[1,4,6], Stephen M. Robbins[1,2,5*] Donna L. Senger[1,2,5*]
Arnie Charbonneau Cancer Institute[1], Clark H. Smith Brain Tumor Centre[2], Snyder Institute for Chronic Diseases[3], Hotchkiss Brain Institute[4], Departments of Oncology[5], Clinical Neurosciences[6], Physiology and Pharmacology[7], Pathology and Laboratory Medicine[9], University of Calgary, Calgary, Alberta, Canada. Michael Smith Genome Sciences Centre, British Columbia Cancer Agency, Department of Medical Genetics[8], University of British Columbia, Vancouver, BC, Canada.
* S.M. Robbins and D.L. Senger share senior authorship

Despite a deeper understanding of the genomic landscape of human glioblastoma there has been minimal improvement in overall patient survival in the past three decades. Macrophage infiltration is a common feature of GBM and an inverse correlation between tumor-associated macrophage (TAM) infiltration and GBM prognosis has been reported. Inflammatory TAM are master regulators of GBM progression through networks of cytokines and growth factors. Using genetically and phenotypically diverse patient-derived Brain Tumour Initiating Cell (BTIC) intracranial mouse models we identified a number of secreted factors within the tumor microenvironment that were derived from both the glioma cells and stromal cells. Among these factors, IL-33, IP-10 and MCP-1 were expressed in a series of BTIC xenografts that were abundantly infiltrated with TAM. Here we focused on the role of IL-33 a recently identified member of the IL-1 cytokine family that has received considerable attention based on its ability to act on a wide range of innate and adaptive immune cells including the microglial and macrophage populations. In this study we found that IL-33 is expressed in a subset of glioma patient specimens and that ectopic expression of IL-33 results in concomitant regulation of a number of cytokines and significant enhancement of tumor progression. Moreover, we found that release of IL-33 from the BTICs into the tumor microenvironment facilitated the recruitment and activation of microglia providing a favourable environment for tumor progression. This study provides the first evidence that IL-33 is a major orchestrator of the brain tumour microenvironment and contributes to tumourigenesis and possible therapeutic resistance. Altering therapeutic emphasis from the cancer cell to one of altering or targeting the normal host environment provides a paradigm shift for therapeutic treatment of patients with GBM.

12 - THE SUMOYLATION PATHWAY AS A REGULATOR OF BREAST CANCER INVASION AND METASTASIS

Ayan Chanda[1], Shorafidinkhuja Dadakhujaev[1], Angela Chan[2], Elizabeth Kornaga[2], Emeka Enwere[2], Lili Deng [1], Don Morris[2,3], Shirin Bonni [1].
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[3]Department of Oncology, Alberta Health Services, Calgary, Canada.

Metastasis is the major cause of cancer-related morbidity and mortality. The fundamental development process of epithelial-mesenchymal transition (EMT) can be triggered in cancer to promote invasion and metastasis of epithelial-cell derived tumors including breast cancer. EMT is potently induced by the secreted protein, transforming growth factor beta (TGFβ) with relevance to cancer. Thus, unravelling the mechanisms that regulate TGFβ-induced EMT should add valuable insights into how cancer metastasis can be controlled. PIAS1, a SUMO E3 ligase (an enzyme that promotes sumoylation of substrates), acts via its substrate SnoN to inhibit TGFβ-induced EMT in untransformed mammary epithelial cells (Netherton and Bonni, PLoS One, 2010). More recently PIAS1 was shown to act in a SUMO E3 ligase-dependent manner, to suppress TGFβ-mediated breast cancer cell invasive growth in a three-dimensional (3D) system and the metastasis potential in a xenograft breast cancer model (Dadakhujaev et al, Oncoscience, 2014). The current study focused on testing whether: 1) SnoN sumoylation mediates the ability of the SUMO E3 ligase PIAS1 to suppress breast cancer invasive growth and metastasis; and 2) the abundance and localization of PIAS1 can serve as a potential biomarker in breast cancer. Experiments addressing parts of point 1 have shown that expression of a SUMO loss of function SnoN but not wild type SnoN significantly decreased [by 90%, $p<0.01$] PIAS1 suppression of the invasive growth of 3D-breast cancer cell-derived multicellular structures by TGFβ. Other experiments showed that TGFβ reduced both the protein half-life of PIAS1 [from 6.42 to 2.77 hours, $p<0.01$] and the proportion of sumoylated SnoN [by 60%, $p<0.001$] in breast cancer cells. Together, these findings suggest that PIAS1 acts via sumoylated SnoN to suppress the invasive growth of breast cancer cells. Using a tissue microarray analysis to address point 2, PIAS1 protein levels and nuclear localisation were found to correlate positively with breast cancer patients' Disease Specific Overall Survival (DSOS). Notably, patients with any PIAS1 tumor expression were less likely to have a DSOS event [Hazard Ratio (HR)=0.38, 95% Confidence Interval (CI): 0.17-0.84, $p=0.016$]. Interestingly, patients who had higher PIAS1 tumor nuclei expression, relative to the tumor cytoplasm, had a more favorable DSOS, when adjusting for tumor size and lymph node status [HR=0.46, 95% CI: 0.22-0.95, $p=0.035$]. In conclusion, our current findings suggest that the PIAS1-SnoN sumoylation axis may suppress breast cancer metastasis, and that PIAS1 level and localization may be used in the future as a biomarker in breast cancer treatment.

13 – Reversible Phosphorylation of ORC2 RVxF Motif Controls PP1 Binding

Brooke Rackel, Isha Nasa, Greg Moorhead
Biological Sciences, University of Calgary

Origin recognition complex 2 (ORC2) is a subunit of the ORC complex which functions to initiate eukaryotic chromosome replication by binding to the replication origins at many places along the DNA strands. The ORC2 protein interacts with Protein phosphatase 1 (PP1) as a regulatory subunit via the conserved RVxF motif. PP1 is a serine/threonine protein phosphatase which serves to control many different cellular processes and is regulated by bonding different regulatory subunits, such as ORC2, which then target the phosphatase towards the substrate. Here we show that reversible phosphorylation of the serine residue within the KSVSF sequence of ORC2 controls ORC2 and PP1 interactions throughout the cell cycle.

14 - Large chr2 Deletions Associated with IDH Mutant Status Result in Loss of IDH Mutant Phenotype

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IDH mutation is the initial event driving gliomagenesis, however, its lasting contribution to later stages remains unclear. Here, we confirm that IDH mutant Brain Tumor Initiating Cells (BTICs) are refractory to *in vitro* culture conditions. We further show that large chr2 deletions are systematically and exclusively detected in BTICs derived from IDH mutant gliomas and result in the loss of either the IDH1 wild type or mutant allele. Importantly, we show that this phenomenon arises *in vivo* based on the detection of a mosaic chr2 deletion in a recurrent sGBM tumor sample. Finally, supporting the premise that this phenomenon is clinically relevant, we confirm *using* an $IDH1^{R132S}$ serial xenograft model, that the loss of the IDH mutation is also selected *in vivo*. Our results suggest not only that IDH mutation is dispensable at later stages of gliomagenesis but also that its loss may lead to more aggressive, therapy resistant recurrences.

15 - Effect of small molecule inhibitors of PARP and PNKP on ATM-deficient colorectal cancer

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Colorectal cancer is the third most common cancer worldwide, with an estimated 24400 new cases diagnosed in Canada in 2014. Standard treatment of colorectal cancer includes surgery, chemotherapy and radiation therapy. Recently, DNA damage response pathways have emerged as potential therapy for many types of cancers (1). Among the many proteins that are involved in DNA damage response and repair pathways, ATM (Ataxia Telangiectasia Mutated) plays an important role in maintaining genome stability (2). Interestingly, recent studies have shown that ATM is either mutated or deleted in nearly 20% of colorectal cancers, and among which over 50% contain p53 mutations (3). Here, we propose that colorectal cancer containing ATM mutation/deletion or whose ATM activity is inhibited by ATM inhibitors will be sensitive to small molecule inhibitors of poly-ADP ribose polymerase (PARP) and polynucleotide kinase/phosphatase (PNKP). In this study, we show that colorectal cancer cell lines without p53 are more sensitive to ionizing radiation or PARP inhibitor treatments when compared to wild type cell lines. Moreover, cells without p53 are highly sensitive to the combined treatment of PARP inhibitor and ATM inhibitor. We are also characterizing colorectal cancer cells with stable knock down of ATM and testing their sensitivity to PARP and PNKP inhibitors. This study will expand our understanding on the sensitivity of colorectal cancer containing ATM and/or p53 mutations to inhibitors of PARP and PNKP, and promote future applications of small molecule inhibitors for the treatment of ATM-deficient colorectal cancer.

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16 - How active are older family caregivers to cancer patients? Motivations, barriers, and predictors of physical activity.

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Family caregivers (FCs) experience detriments to their physical and emotional health and have difficulty maintaining healthy behaviors such as physical activity (PA). Despite three decades of research, many questions remain, including understanding PA behaviors of older (60+) FCs to cancer patients. Older persons are at increased risk for health problems and participate less in PA. The purpose of this study was to examine the current levels, barriers and motivations, and predictors of PA, in older FCs to cancer patients.

Methods: The data for this study are from a larger survey of FCs aged 60+, recruited at a hospital-based cancer facility. FCs were included if their care recipient had breast, prostate, or colorectal cancer. The Godin leisure time exercise questionnaire (GLTEQ) was used to measure PA levels. Analyses were conducted using descriptive statistics and standard multiple regression to explore predictors of PA.

Results: 132 surveys were returned. The majority of participants were female (59.1 %), provided 21 caregiving hours/week, and were 70 yrs old (sd 7.43). 46.2% were achieving PA levels high enough to confer health benefits. The most commonly reported barriers to PA were painful joints (23.3%), lack of energy (20.2%), lack of interest (20.2%) and adverse weather (31.8%). Motivations to PA were, liking rewards of exercise (42.6%), wanting to be fit (41.9%), and to improve health (38.8%) and fitness (36.4%). Caregiving demands were reported as barriers but not as commonly as expected. PA levels were significantly correlated with state anxiety r=-.25, trait anxiety r= -.26, the Physical Component Summary (PCS) of the SF-36 r=.36, age r=-.24, sleep quality r=-.26, depression r=-.25, and caregiving hours per week r=-.26 (all p's < .01). Regression indicated PA levels were predicted by age β=-.26 (p.02), PCS β=.313 (p=.001) and caregiving hours β=-.24 (p=.003), accounting for 26% of the variance (adjusted R^2=.26).

Conclusions: Findings were consistent with other research on barriers and motivations to PA in older persons. PA levels may also be impacted by the caregiver role. Older FCs to cancer patients may benefit from interventions to maintain or improve PA levels.

17 - Head and Neck Cancer Patients' Physical Activity Preferences and Barriers Before and After Participation in the ENHANCE Intervention

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Purpose: The purpose of this mixed-methods study was to examine physical activity (PA) barriers and preferences of head and neck cancer (HNC) survivors in relation to treatment status and PA experience. The secondary objective was to examine correlates of PA barriers.

Methodology: Participants (n=22) completed self-report questionnaires on demographic and medical information, PA levels, and PA barriers and preferences. A subset of participants completed semi-structured interviews (n=17). All participants had previously participated in the ENHANCE PA trial during, or immediately after, treatment. All quantitative data was examined descriptively and interviews were transcribed and coded for thematic analysis.

Results: Before ENHANCE PA participation, lack of interest and time were the primary exercise barriers. After PA participation, there was a significant decrease in the barriers of lack of interest (p=0.008), exercise not a priority (p=0.039) and exercise not in routine (p=0.004), however fatigue was a significant barrier. Number of barriers experienced was negatively correlated with age, QOL and minutes of resistance exercise training performed per week. After PA participation, significant increases were found in preference for engaging in PA at a cancer centre (p=0..031) and with other cancer survivors (p=0.016). Interviews revealed that interest in engaging in PA with other cancer survivors stemmed from the social accountability, knowledge exchange and solidarity experienced.

Significance: By investigating participant perspectives after PA participation, this study identifies key factors for effective HNC PA program design. Educating patients on the reason and potential benefits of PA programs may serve to increase both initial PA adoption and longer term adherence.

18 - The Evaluation of the Human Polynucleotide Kinase Phosphatase's Role in Non-Homologous End Joining and DNA Repair

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Ionizing radiation (IR) therapy is the predominant form of treatment for a wide variety of cancers, as IR induces numerous variations of genomic lesions which can reduce cell proliferation and growth. The most cytotoxic of these DNA lesions is the double strand break (DSB). A DSB occurs when single stranded breaks (SSBs) occur in close proximity to one another on opposite DNA strands and may result in chromosomal breakage. Importantly, IR-induced DNA strand breaks frequently contain non-ligatible 5'-hydroxyl and/or 3'-phosphate groups. Human polynucleotide kinase/phosphatase (PNKP) exhibits 5' DNA kinase and 3' DNA phosphatase activities, making it ideal for converting "dirty" DSB ends to compatible ends prior to ligation. The major pathway for the repair of IR induced DSBs in human cells is non-homologous end joining (NHEJ). PNKP interacts with the NHEJ scaffolding protein XRCC4 in a phosphorylation-dependent manner (Koch et al, EMBO J, 2004), suggesting a mechanism by which PNKP is recruited to IR-induced DSBs, however, its precise role in NHEJ remains enigmatic. The current work examines the interaction between PNKP and XRCC4 and the DSB repair kinetics of PNKP-deficient cells. Additionally, live cell imaging is currently underway to assess PNKP's recruitment/retention kinetics in vivo. PNKP's interaction with XRCC4 if influenced by the extent of DNA damage caused, and by the inhibition of key DNA damage repair kinases. DSBR kinetics are assessed through immunofluorescence staining for canonical DSB foci markers. DSBs were quantified in a PNKP isogenic background, and have demonstrated that PNKP deficient cells exhibit a late DSB repair defect in a cell cycle dependent manner. Importantly, this phenotype is rescued with the stable re-incorporation of PNKP into knockdown cells. The underlying mechanism by which PNKP knockdown affects DSBR kinetics and localization to DNA damage sites *in vivo* is currently under investigation.

19 - DNA double-strand break repair in different neural cell lineages

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Background: Cranial radiotherapy (CRT) is an important and effective treatment for brain cancers and high-risk leukemias, but is strongly associated with treatment-related neurocognitive decline that worsens the younger a patient is treated. Ionizing radiation (IR) causes DNA double strand breaks (DSBs), which are repaired by two main pathways critical for proper neurodevelopment, homologous recombination (HR) or non-homologous end joining (NHEJ). While previous studies have implicated impaired neurogenesis, decreased brain white matter and neuroinflammation as consequences of CRT, few studies have investigated DSB repair proficiency, pathway choice and cellular responses to CRT in the developing mammalian brain at the pediatric stage. I hypothesize that cells of distinct neural lineages display differential IR sensitivity over time, and these differences may be due to the varied engagement of DNA repair signaling.

Results: To examine the DSB repair kinetics and proficiency amongst different neural cell lineages, mouse neural stem cells, and astrocytes, oligodendrocytes and neurons terminally differentiated from mouse neural stem cells are exposed to ionizing γ-radiation. DSB repair over time is then examined and quantified by immunofluorescence microscopy for γH2AX and 53BP1 (surrogate markers of DSBs). Here, I show that we are able to successful differentiate mouse neural stem cells into the three distinct neural lineages, and that DSB repair kinetics differ between terminally differentiated astrocytes and mouse neural stem cells. I also show that between the two cell types, DSBs are repaired with differing degrees of γH2AX and 53BP1 involvement.

Conclusions: Overall, further investigation is required to elucidate the cellular mechanism underlying the differences in kinetics and signaling. These data, however, suggest there are intrinsic differences in repair kinetics and signaling between different neural cell lineages.

20 - DOT1L Epigenetically Regulates an Epithelial Mesenchymal Transition-Like Process in Glioblastoma Multiforme

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Glioblastoma multiforme (GBM) is a devastating disease, which represents 60-70% of all malignant gliomas and is one of the most lethal human cancers. The poor prognosis associated with GBM is thought to be a result of rapid tumor progression, molecular heterogeneity and diffuse invasion of the brain tissue. Epithelial-Mesenchymal Transition (EMT) plays an important role in controlling critical morphogenetic steps during normal embryonic developmental processes and is characterized by downregulation of E-cadherin, loss of cell adhesion and increased cell motility. Research has also shown that EMT is linked to cancer cell invasion, metastasis and the generation of stem-like cells that contribute to therapeutic resistance. Disrupter of telomeric silencing-1-like (DOT1L) is a class I-like S-adenosyl-L-methionine (SAM)-binding methyltransferase that catalyses the methylation of histone H3 on lysine-79 (H3K79me). It has been shown that DOT1L is crucial for tumor development and may regulate EMT transcription factors. Interestingly, recent studies have shown that STAT3 is an important regulator of DOT1L regulation in colorectal cancer. Our lab has previously shown that the JAK2/STAT3 pathway is upregulated in GBM and essential to brain tumor initiating cells (BTICs) growth and tumorigenic properties. We are thus testing the hypothesis that DOT1L is directly regulated by STAT3 and promotes an EMT-like process in GBM BTICs. To test whether STAT3 regulates DOT1L in GBM BTICs, we performed quantitative polymerase chain reaction (qPCR) analysis and western blot analysis on BTICs treated with a small molecule JAK/STAT inhibitor (R333). Results showed that STAT3 inhibition decreased RNA and protein expression of DOT1L. We also observe decreased levels of dimethylated H3K79 (H3K79me2), for which DOT1L is the only known methyltransferase. Furthermore, activation of STAT3 in BTICs via Oncostatin M (OSM) treatment showed increased DOT1L RNA and protein expression as well as increased levels of H3K79me2. Preliminary chromatin immunoprecipitation (ChIP) experiments suggest direct STAT3 regulation of DOT1L. Treatment with the DOT1L inhibitor EPZ-5676 *in vitro* led to prominent decreases in H3K79me2 levels and decreased BTIC viability. Additionally, preliminary results show that DOT1L inhibition decreases RNA expression of EMT transcription factors such as SNAIL, SLUG and TWIST and increases expression of E-cadherin. Ongoing studies are aimed at studying *in vitro* invasion and migration potential of BTICs after DOT1L inhibition and confirming DOT1L epigenetic regulation of EMT transcription factors with H3K79me2 ChIP experiments. Future studies will continue to elucidate the role of DOT1L in BTICs, GBM progression and the potential therapeutic implications.

21 - XRCC4/DNA Ligase IV induces conformational changes in Polynucleotide Kinase/Phosphatase and increases kinase repair activity on double-stranded DNA substrates

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If unrepaired 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 prevent 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 tight binding between XRCC4/Lig IV and PNKP both in the presence and absence of CK2-phosphorylation of XRCC4. We also show by activity assays that both CK2-phosphorylated and non-phosphorylated-XRCC4/Lig IV contribute to an increase in PNKP kinase activity on double-stranded DNA substrates. We determined a low-resolution ensemble structure of the purified phosphorylated- XRCC4/Lig IV/PNKP ternary complex by Small-angle X-ray scattering (SAXS) experiments. The SAXS structure suggests a secondary interaction between the PNKP and XRCC4/Lig IV that is independent of the PNKP-FHA domain and of XRCC4 CK2- phosphorylation. We further used hydrogen-deuterium exchange (HDX) experiments to probe potential PNKP secondary interaction sites with XRCC4/Lig IV and identified a candidate interaction site within a clinically significant loop in the PNKP phosphatase domain. This functionally related secondary interaction appears 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.

22 - The major IR-induced Artemis substrate is a DSB with an inter-strand cross-linked terminus

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The integrity of our DNA is constantly under threat from prevalent environmental mutagens, including ionizing radiation (IR). One of the most dangerous types of DNA damage caused by IR exposure is the DNA double strand break (DSB), whose failed repair can kill a cell or whose erroneous repair can lead to chromosomal translocations, deletions or amplifications, driving genomic instability – the fundamental hallmark of all cancers. The majority of DSBs are repaired quickly and without error due to mechanisms that rapidly detect and repair DNA damage. However, there are additional, complex damages that preclude simple re-ligation of DNA ends. The Artemis nuclease is necessary for the repair of a subset of IR-induced DSBs associated with heterochromatin, and humans lacking Artemis are hugely sensitive to radiation. However, the precise nature of the IR-induced DSB-associated lesion that is processed by Artemis is not known. We hypothesize that the in vivo substrate of Artemis during DNA damage repair represents a complex structure at slowly-repaired, persisting DSB ends. We will present evidence that Artemis functions to cleave 'pseudo-hairpin' ended DSBs generated following radiation exposure, formed by the conflagration of a 2'-deoxycytidine interstrand crosslink with a DSB at the same locale.

23 - The role of tRNA synthesis in the control of hypoxia survival

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Oxygen availability is an important regulator of growth and survival in all animals. When oxygen is abundant, growth is promoted, but when oxygen is scarce, metabolic processes are altered to limit growth and promote survival. One mechanism by which this metabolic change may occur is through a decrease in protein synthesis. We previously identified tRNA synthesis as a regulator of mRNA translation. We hypothesize that animals restrict translation and protein synthesis to cope with hypoxia. I found that tRNAs are significantly reduced following hypoxia in *Drosophila* larvae. tRNA synthesis is regulated by Pol III, which we found is negatively regulated by Maf1 under starvation. I hypothesize that Maf1 represses Pol III under hypoxia, causing a decrease in protein synthesis and increased survival. I will test if Maf1 is required for tRNA suppression in hypoxia with RNAi and CRISPR/Cas9, and then determine if this suppression is necessary for organismal physiology and survival.

24 - The mitotic phosphoproteome of DNA-PKcs

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Cancer progression requires genomic instability, a primary source of which is DNA damage. Our cells possess mechanisms to repair broken DNA, including the repair of DNA double strand breaks (DSBs). The primary pathway in higher eukaryotic cells for the repair of DSBs is nonhomologous end-joining (NHEJ). The DNA-dependent protein kinase catalytic subunit (DNA-PKcs) is known to phosphorylate itself and other repair factors during the process of NHEJ. Another cause of genomic instability is aberrant cell division, or the misregulation of mitosis. An intricate network of phosphorylation events achieves proper coordination of mitosis, with a number of key protein kinases known to orchestrate the process. Recently, we have shown that DNA-PKcs is also active during normal mitosis, and loss of the protein or its kinase functions results in mitotic aberrations. Here we present a study into the DNA-PKcs phosphoproteome during mitosis, using SILAC. We compared three HeLa cell samples; asynchronous, mitotic, and mitotic with DNA-PKcs inhibitor NU7441, and analysed the phosphorylation status of the peptides detected by mass-spectrometry. We identified 610 mitotic phosphorylation events that are significantly decreased after the addition of DNA-PKcs inhibitor during mitosis. Analysis of these hits implicates kinase networks and biological processes controlled by DNA-PKcs. Validation of new potential targets of DNA-PKcs is in progress.

25 – Reasons for Underuse of Adjuvant Chemotherapy in Elderly Patients with Stage III Colon Cancer

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Objectives: Our aims were to characterize adjuvant chemotherapy (AC) use among elderly patients (EPs) with stage III colon cancer (CC) and to identify potential reasons for under-treatment.

Methods: Descriptive statistics were used to summarize treatment patterns in young patients (YPs) aged <70 vs. EPs ≥70 years. Multivariate Cox regression models were constructed to evaluate the associations between AC and cancer-specific (CSS), disease-free (DFS), and overall survival (OS) and to determine whether these were modified by age.

Results: We identified 810 patients: 51% men, 52% YPs, and 74% received AC. Compared to YPs, EPs were less likely to receive AC (57% vs. 91%, $p<0.01$). Frequent reasons for non-treatment included age, comorbidities and perceived minimal benefit from AC. Among AC-treated individuals, EPs were less likely to receive FOLFOX (32% vs. 74%, $p<0.01$) because of advanced age, comorbidities, and patient preference. Once AC was given, EPs had similar rates of treatment discontinuations (34% vs. 26%, $p>0.05$) and dose reductions (63% vs. 61%, $p>0.05$) as YPs. Reasons for treatment interruptions included side effects, progressive disease, and patient choice. Receipt of either FOLFOX or capecitabine was correlated with improved CSS, DFS and OS when compared to surgery alone; this effect was not modified by age.

Conclusions: EPs with stage III CC frequently received either no AC or capecitabine monotherapy due to advanced age and comorbidities. The effect of AC on survival was similar across age groups, with comparable side effects and rates of treatment modifications. AC should not be withheld because of advanced age alone.

26 - Study Design and Parameters for MATCH: Mindfulness and Tai Chi/Qigong for Cancer Health

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Background: As more people survive cancer, the importance of research on effective interventions for improving quality of life (QOL) amongst survivors is growing. Two interventions with a substantial evidence-base are Mindfulness-Based Cancer Recovery (MBCR) and Tai chi/Qigong (TCQ). However, these interventions have never been directly compared.

Objectives: (1) To compare MBCR and TCQ to each other and a waitlist control condition using an innovative, randomized, preference-based comparative effectiveness trial (CET) design that takes into account potential moderating factors that might predict differential response. (2) To investigate the impacts of MBCR and TCQ on a range of biological outcomes including immune processes, blood pressure, heart rate variability, hormones, cellular aging, and gene expression.

Methods: The study design is a preference-based multi-site randomized CET incorporating two Canadian sites (Calgary, AB and Toronto, ON). Participants ($N_{total} = 400$; $n_{group} = 100$) with a preference for either MBCR or TCQ will get their preferred intervention; while those without a preference will be randomized into either of the two interventions. Within the preference and non-preference groups, participants will also be randomized into immediate intervention groups or a wait-list control. Outcome measures to be assessed pre- and post-intervention and a 6-month follow up will include psychological outcomes (mood, stress, perseveration, mindfulness, spirituality, post-traumatic growth), QOL, symptoms of survivorship (fatigue, sleep), an exploratory analyses of biomarkers (cortisol slopes, cytokines, blood pressure/heart rate variability, telomere length), and health economic measures.

Hypotheses: We theorize that both MBCR and TCQ will improve QOL amongst survivors relative to treatment as usual, particularly if patients have a strong preference for a particular intervention. We hypothesize that while both interventions will improve QOL relative to controls, it may be via different routes. MBCR may be superior to TCQ on measures related to stress and mood. Conversely, TCQ may be superior to MBCR in improvement of physical and functional measures.

27 - Defining the role of ING5 in stemness maintenance of brain tumor initiating cells

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Recent studies of INhibitor of Growth family members (INGs1-5) have implicated functions in apoptosis, senescence, cell differentiation, DNA repair and cancer biology. Some evidence has indicated that INGs3-5 function in stem cell biology and cell reprogramming. We investigated the regulatory roles of ING proteins in a subset of stem-like cells resident in glioblastoma tissues (brain tumor initiating cells or BTICs). We characterized the dynamics of ING5 during *in vitro* BTIC differentiation and observed an immediate decrease of ING5 expression as cells differentiated. Subcellular localization showed ING5 predominantly localized in the nuclei of undifferentiated sphere cells. Transient overexpression and knockdown experiments indicated that ING5 enhanced sphere formation ability and retained stem cell properties under differentiation induction. Sphere assay and differentiation experiments in stable shRNA cell lines also showed the stemness maintenance effect of ING5 on BTICs. Interestingly, ING5 appears to influence cell fate decision by modulating frequency of symmetric and asymmetric division. To investigate the molecular mechanism of ING5, we conducted transcriptome assay in shRNA cell lines. Pathway analysis showed that genes regulated by ING5 were enriched for gonad development, particularly spermatogenesis and folliculogenesis, and several hormone transduction pathways which might activate growth signaling. We will test which pathway is responsible for ING5 functions by blocking several major mitogen pathways using kinase inhibitors. The aim of this project is to acquire an understanding about ING5 in stemness regulation and provide insight into targeting and eliminating stem cell features in BTICs to benefit brain tumor treatment.

28 - Development of a metabolomic biomarker for prognostication of colorectal cancer liver metastasis

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Background. Colorectal liver metastases (CRLM) are treatable by surgical resection and chemotherapy in a subpopulation of patients. Patient selection is enhanced by identifying individuals at high risk of recurrence and truncated survival. Here we introduce a high-throughput metabolomic approach that allows for pre-surgery risk estimation, and therefore, more informed surgical case selection.
Methods. Blood samples were collected prospectively from CRLM patients from 2004 to 2012. Sera from 95 resectable CRLM patients were analyzed using gas chromatography-mass spectrometry (GC-MS). Risk estimation models were generated through a novel combination of multivariate statistical analysis methods on mass spectrometry data and survival datasets.
Results. By application of a population based multivariate approach, a single continuous recurrence risk score was calculated for each patient. A multivariate Cox proportional hazards model built on the metabolomic risk score and clinical factors enabled stratification of patients by a scaled recurrence risk score.
Conclusion. A metabolomics-based biomarker for prognosis was generated for patients with CRLM. In combination with clinical factors, the biomarker enabled stratification of risk of recurrence. Such an approach may enhance patient selection, and assignment of CRLM patients to the most beneficial treatment option. Statistical methods developed in this study can be used for constructing prognostic and predictive biomarkers, where a high-dimensional genomic or metabolomic dataset is available.

29 - Altered Chromatin Remodeling as a Potential Driver of Genomic Instability in Cancer

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The protein and DNA complex known as chromatin is a dynamic structure, adapting to alter the spatial arrangement of genetic material within the nucleus in response to a variety of factors and conditions. In recent years, a vital role for the regulation of chromatin has emerged within the DNA damage response field. The integrity of this network is vital to the maintenance of genomic stability and its mis-regulation is a foundational characteristic of most cancers. DNA double Strand Breaks (DSBs) represent one of the most serious threats to genomic stability and, as such, arrays of proteins have evolved to enable cells to repair these lesions. We are interested in the relevance of chromatin remodeling to DSB repair, which has been revealed to be particularly important within complex chromatin environments such as heterochromatin, or regions undergoing energetic transactions such as transcription or DNA replication. Our interest lies in what function Chromodomain, helicase, DNA-binding (CHD)-class chromatin remodeling enzymes have in maintaining genomic stability. We primarily study CHD5, a member of this protein class which has been shown to be down regulated and/or deleted in a wide range of cancers. We investigate the importance of CHD5 in DNA repair, and can demonstrate its dynamic regulation in response to DNA damage. We will present our ongoing work on regulation of CHD5 by DNA Damage Response signaling pathways and in particular the novel involvement for CHD5 enzyme in the mammalian response to DSBs caused by ionizing radiation exposure.

30 - Clinical Characteristics of Survival Outliers in Stage IV Non-Small Cell Lung Cancer Patients

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Background: Lung cancer is the leading cause of cancer deaths among men and women in Canada. Many lung cancer patients are diagnosed at advanced stages of disease, which is associated with poor survival outcomes. The mean survival of stage IV non-small cell lung cancer (NSCLC) patients is typically less than 12 months; however, there appears to be a small subset of patients with advanced disease that live substantially longer than the norm. Our study aims to determine whether certain clinical characteristics correlate with longer survival in stage IV NSCLC patients, in order to help ascertain why some patients live longer than expected.

Methods: Data on 1803 stage IV NSCLC patients (1291 adenocarcinoma, 512 squamous cell carcinoma) from 1999-2011 were extracted from the Glans Look Lung Cancer database. Clinical characteristics such as age, gender, ethnicity, smoking history, histology, molecular testing, metastatic disease, treatments, and socioeconomic factors were compared between survival outliers and patients with average survival. Survival outliers were defined as those patients who lived greater than 5 years, or greater than 2 standard deviations from mean survival (adenocarcinoma, 39.82 months; squamous cell carcinoma, 47.45 months).

Results: Out of 1291 adenocarcinoma patients, there were 19 patients who lived longer than 5 years from time of diagnosis. When defining survival outliers as 2 standard deviations from mean survival, 58 patients were identified in the outlier group. Preliminary data suggest that patients in the survival outlier group have a smaller smoking pack year history and lower metastatic disease burden at diagnosis. Upon further characterization of sites of metastatic disease, there are a smaller percentage of patients with liver metastases in the survival outlier group compared with patients in the average survival group.
Conclusion: Further statistical analysis is ongoing to determine the clinical significance of various clinical characteristics with respect to survival. The present study will help us better understand the importance of various clinical parameters and their association with survival, in hopes of improving outcomes for lung cancer patients in the future.

31 - CXCR4 expression is associated with poor survival in early, resected NSCLC.

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Background: CXCR4, a G protein coupled chemokine receptor, and its ligand, stromal cell derived factor-1 (SDF-1), play a critical role in organ specific tumor metastasis. In vitro, CXCR4 expression has been shown to correlate with migration, invasion and adhesion in various cancer cell lines including lung, breast and colon, among others. In clinical studies, patients whose tumors exhibit high CXCR4 expression tend to have a poorer clinical outcome. We previously demonstrated that high expression of CXCR4 by quantitative IHC in a cohort of 170 stage IV NSCLC specimens was associated with significantly decreased overall survival, particularly in the female patients. We subsequently investigated whether CXCR4 also conferred a poorer prognosis in our early stage NSCLC patients with resected disease, to validate our previous findings.

Methodology: Demographic details, clinical variables and outcome data were gathered on patients diagnosed at the Tom Baker Cancer Centre (TBCC) from 2003 to 2006. Formalin-fixed paraffin embedded tumor specimens were obtained from those patients diagnosed with resected stage I, II or III NSCLC and tissue micro arrays (TMAs) were generated. CXCR4 expression in NSCLC cells was analyzed by immunohistochemistry using anti CXCR4 mAb and the HistoRx PM-2000 platform, then correlated with clinical outcome. Statistical analysis was performed using the Kaplan-Meier method, multivariate analysis and a multi-state model to account for the competing risks of disease free and overall survival.

Results: Of 1502 patients diagnosed with NSCLC at the TBCC in 2003-2006, 166 had resected stage I (63%), II (30.7%) or III (9.6%) disease. 37.3% of the patients received adjuvant treatment after their surgery. 46.4% of the patients were still alive at the time of analysis. The mean CXCR4 AQUA scores were significantly lower for the early stage patients than those obtained for the advanced stage IV patients (1715.90 vs 2512.44 $p < 0.0001$). High CXCR4 expression was associated with worse overall survival ($p = 0.026$) but had no significant effect on disease free survival after resection ($p = 0.376$). Subgroup analysis showed no significant differences between genders in the association between high CXCR4 expression and clinical outcome.

Conclusions: CXCR4 is expressed in early stage resected NSCLC tumors and appears to increase significantly from stage I-III to stage IV NSCLC. High CXCR4 expression is associated with significantly poorer overall survival in early stage resected patients, validating our previous findings in stage IV NSCLC using the same method. CXCR4 does not seem to be associated with disease free survival in this cohort of patients, nor does there seem to be any association between gender and the effect of CXCR4 on poor outcome unlike that seen in our stage IV NSCLC patients.

32 – The effect of ATM on the mobility of broken chromosome ends and revealing a role for the nuclear architecture in repairing site-specific double-strand breaks.

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The organization of the genome and architecture of the nucleus play important roles in regulating several metabolic processes and are emerging as critical parameters in regulating DNA repair and genome instability. To monitor the repair of DNA damage in a native environment, we are introducing a site-specific double strand break (DSB) at defined loci in the mammalian genome coupled to DNA labels that flank the break. To overcome the side effects of the currently used DNA labels, we have developed a new non-intrusive method for labeling DNA loci in mammalian cells. It is based on a short DNA sequence (1 Kb) carrying bacterial nucleation sites that recruit and spread specific proteins coupled to fluorophores, respectively called ANCH and OR. Two label variants; ANCH3-OR3GFP and ANCH4-OR4GFP form stable fluorescent foci on each side of the inducible ISceI break site.
This system allows us to visualize the dynamic movement of the break ends during repair by tracking the foci in live cells with time-lapsed fluorescence microscopy. We are investigating the kinetics at a break in the different stages of repair including 1. free ends, 2. bridging/resection, and 3. ligation in different chromatin environments throughout the genome and in various repair deficient backgrounds. Our preliminary results after monitoring one side of the break in osteosarcoma cells with a stable integration of the system, showed an important increase in its displacement immediately following the break, most probably reflecting the more mobile free broken DNA end preceding the binding of the bridging/processing factors. Inhibiting the activity of the Ataxia telangiectasia mutated (ATM) kinase showed a persistent mobility of the broken ends. Measurements from different genomic loci in normal and pathological contexts will provide a better understanding about how repair efficiency impinges on genomic instability.

33 - Reversible phosphorylation of the RVSF motif in PP1 regulatory proteins controls PP1 docking during the cell cycle.

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Regulation of the cell division cycle is critical for the maintenance of genomic integrity at the cellular level. Reversible protein phosphorylation is a prevalent regulatory post-translational modification that is catalyzed by protein kinases and phosphatases in the cell. PP1 is a major serine/threonine phosphatase in eukaryotes responsible for one-third of all dephosphorylations and gains specificity through regulatory subunits that recruit PP1 to unique protein targets. The interactions between the regulatory subunits and the catalytic PP1 subunit occur via the well-characterized RVxF motif. Mapping of the phospho-proteome during the cell cycle shows increased net phosphorylation of proteins during mitosis including the RV(S/T)F motif from regulatory proteins of PP1. We speculate this reversible phosphorylation of the RV(S/T)F motif might play a general role in association/dissociation of PP1 from its regulatory proteins during the cell cycle and be crucial to maintain phosphorylation of PP1 targets during this event. Our results from *in-vitro* PP1 overlays confirm preferential PP1-binding with dephosphorylated RV(S/T)F peptides over phosphorylated RVp(S/T)F peptides. Mitosis-specific phosphorylation of the RVSF motifs has been shown by immunofluorescence studies using a pan-RVSF phospho-antibody. This phosphorylation has also been confirmed by enrichment of phospho-RVSF containing proteins in mitotic immunoprecipitation experiments as identified by mass spectrometry. We have identified Aurora B as the potential kinase that plays a role in the phosphorylation of RVS/TF motifs, thereby regulating the docking of PP1 to its regulatory proteins in cell cycle.

34 - Dose dependent ATM phosphorylation by platin therapy in non-small cell lung cancer

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Platinum-based antineoplastic therapies (platins) are a first line treatment for non-small cell lung cancer (NSCLC) that generate DNA breaks and stimulate DNA damage response pathways. A key mediator of the DNA damage response is ataxia telangiectasia mutated (ATM), an activator of downstream targets involved in DNA repair, cell cycle arrest, and apoptosis. We hypothesize that platin exposure may invoke ATM signaling and that tumours deficient in ATM may be innately sensitivity to platin therapies. Six NSCLC cell lines were assessed for the presence of ATM and were treated with varying concentrations of cisplatin, carboplatin, or oxaliplatin for 18 hours. ATM activation was determined by assessment of phosphorylated-ATM protein levels by western blot. ATM-proficient cell lines (H226, H460 and H522) demonstrated a dose and time-dependent increase in phosphorylated-ATM in response to platin exposure. This data suggests that an ATM-mediated signaling response is induced by platin therapy, however ATM's predictive capability to platin sensitivity still remains unclear. ATM-deficient tumours may respond better to platin based therapies due to this impaired DNA damage response pathway.

35 - Does Deep Inspiration Breath Hold (DIBH) produce a clinically meaningful reduction in ipsilateral lung dose during loco-regional radiation therapy for women with right-sided breast cancer?

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Background: Deep inspiration breath hold (DIBH) has become standard practice to reduce cardiac toxicity during radiation therapy (RT) for patients with left-sided breast cancer. However, the role of DIBH during loco-regional RT for patients with right-sided breast cancer is undefined. Inclusion of the internal mammary chain (IMC) lymph nodes during loco-regional RT improves survival by a modest amount, but many oncologists drop the IMC nodes from treatment if the volume of lung receiving 20 Gy (V20) exceeds 30%.

Purpose: To determine whether DIBH produced a clinically meaningful reduction in the volume of lung receiving a high dose of RT in comparison to delivery of loco-regional RT with free breathing (FB) for women with right-sided breast cancer.

Materials & Methods: Thirty female patients with Stages 0-I left-sided breast cancer and who had both DIBH and FB CT scans as part of standard care at the Tom Baker Cancer Centre were identified. The right-sided IMC nodes were contoured according to ESTRO guidelines on DIBH and FB scans with care taken to ensure comparability between scans. A four-field, modified-wide tangent RT plan was developed on each scan to include the right breast and full regional nodes, including a minimum dose of 80% to the IMC volume. The junction between the supraclavicular and tangent fields was at the inferior extent of the ossified medial clavicle. Treatment plans were calculated in Eclipse using the Acuros algorithm version 11. FB and DIBH plan metrics were compared using Wilcoxon-signed rank testing.

Results: IMC coverage was equivalent between DIBH and FB plans; V80 was 100% on both plans and D100 was 39.2 and 39.5 Gy for DIBH and FB, respectively. Twenty-one patients (70%) had ≥5% reduction in ipsilateral lung V20 with DIBH compared to FB. The average ipsilateral lung V20 decreased by 7.8% (range: 0 to 20%; $p<0.0001$) and the mean lung dose decreased by 3.4 Gy with DIBH (range: -0.2 to 9.1; $p<0.0001$). There was a mean reduction of 42.3 cc (range: 0 to 178.9; $p<0.0001$) in the volume of liver receiving 50% of the prescription dose. The differences in mean heart doses were statistically significant, but not likely clinically significant: MHD was 0.88 Gy (range: 0.67 to 1.27) and 1.00 Gy (range: 0.75 to 1.48) ($p<0.0001$) for DIBH and FB, respectively.

Conclusions: DIBH reduced mean ipsilateral lung V20 by 7.8% and mean lung dose by 3.4 Gy. For some patients, the volume of liver receiving a potentially toxic dose could also be reduced with DIBH. DIBH should be used as a treatment strategy to reduce V20 right lung without compromising IMC or supraclavicular nodal coverage for patients with right-sided breast cancer during loco-regional RT if the V20 on FB approaches or exceeds 30%.

36 - THE IMPACT OF LIGHT THERAPY ON DIURNAL CORTISOL RHYTHMS IN CANCER SURVIVORS WITH FATIGUE

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BACKGROUND: Fatigue is a common and distressing symptom that can last for months or years in up to one-third of survivors. Despite its prevalence, the nature and mechanisms are poorly understood and the available treatments may not provide relief. Light therapy may target the potential dysregulation in cortisol rhythms experienced by some survivors with fatigue. The aim was to: 1) evaluate the impact of a light therapy intervention on diurnal cortisol slopes in cancer survivors with fatigue; 2) examine the association between changes in fatigue and cortisol slopes.

METHODS: This was a 5-week blinded randomized controlled trial. Subjects were recruited from Calgary and included men and women who met criteria for fatigue and had completed cancer treatment. Participants were provided with one of two types of Litebook treatment devices that produced either bright white light (treatment) or dim red light (active control). The devices were used daily for 30 minutes upon waking for one month. Participants provided four saliva samples a day for a period of three days both at baseline and during the final week of light use.

RESULTS: A total of 30 participants were randomly assigned to receive either white (n=15) or red light (n=17). Analyses revealed an increase in the steepness of cortisol slopes from baseline to post-intervention for both groups, $F(1,28)=6.61$, $p=.016$, and a decrease in fatigue scores over time for both groups, $F(1,28)=38.35$, $p< 001$. No group or interaction effects were detected. Cortisol slopes and fatigue scores were not associated, $\beta=.09$, $t(28)=.48$, $p=.635$.

CONCLUSIONS: Analysis of the preliminary data suggested that both groups showed improvements on both fatigue scores and cortisol slopes; however the sample size was too small and there was insufficient power to reach conclusions about treatment efficacy. Data from a second cohort is being collected to examine these outcomes on a larger scale.

37 - Thiaporphyrin-Mediated Photocatalysis Using Red Light

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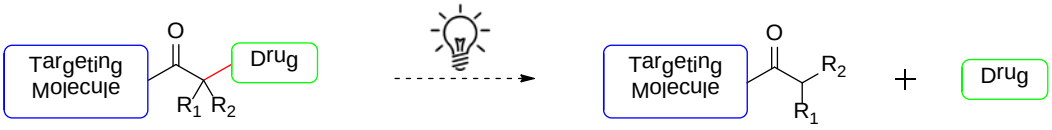
Over the past several years, there has been growing interest in visible light photoredox catalysis and its application in organic synthesis. Most methodologies and applications in literature use photocatalysts that absorb in the blue to green region of the visible spectrum. However, the Derksen group is interested in using red light to bring the photocatalyst to its excited state. Using low energy red light, we propose a potential drug delivery application where the light would cleave drug conjugates of their therapeutic cargo to enable drug binding to a target receptor with high selectivity. The prodrug would be designed to be inactive in circulation throughout the body, and cleaved upon irradiation at a specific site. This presentation describes preliminary work on catalyst and prodrug/linker development.



38 - Changes in DNA double strand break (DSB) repair during cellular aging

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Cellular Senescence is a permanent cell cycle arrest induced by telomere shortening or prematurely due to severe stress. Exposure of cells to DNA damaging agents leads to the recognition of the DNA ends as double strand breaks (DSBs). Normally, cells have very efficient and accurate mechanisms to repair DSBs. However, an increased number of DSBs have been found in replicative senescent cells as well as prematurely aged cells. It is unclear whether these DSBs originated from endogenous or exogenous damage and why these DSBs persist. Since some studies suggested that even one un-repaired break can trigger senescence, the goal of my project is to understand the underlying mechanisms of why and how DNA repair is altered in senescent cells.
One objective of my project is to compare protein levels of repair proteins and key players of DSB signaling in young and quiescent vs. senescent cells. The initial screen of protein levels revealed that factors of initial DSB signaling and certain DSB repair factors are increased in senescent cells. In contrast, other repair factors show decreased protein levels.
To analyze the capability of senescent cells to repair exogenous DNA damage, DSB repair kinetics after ionizing radiation was investigated. Generally, DSBs induced by 1-4 Gray of ionizing radiation, such as γ-irradiation, are repaired within 48 hours. Young and senescent primary human fibroblasts were irradiated and repair kinetics monitored using the universal DSB marker γH2AX (H2AX phosphorylated at S139). Preliminary results suggest that ~10-15% of induced DSB are not repaired after 48 hours in senescent cells, compared to ~1% in young or quiescent cells. These results indicate that despite repair proteins being abundant in senescent cells, the repair pathways might be compromised or certain key players downregulated or inactivated, which leads to the accumulation of un-repaired DSBs in senescent cells.

39 - A pre-clinical combinatorial strategy to target the JAK2/STAT3 and EGFR pathways in Glioblastoma Multiforme

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Glioblastoma multiforme (GBM), characterized by an aggressive clinical course, therapeutic resistance and striking molecular heterogeneity, remains an incurable disease. A large number of GBMs have EGFR alterations, and despite poor clinical translation to date, EGFR inhibition remains of significant therapeutic relevance. Recent evidence, from our group and others, indicates that the JAK2/STAT3 pathway is an important mediator of tumor cell survival, growth, and invasion in GBM. Inhibition of this pathway holds great promise as a therapeutic strategy. Interestingly, EGFR inhibition leads to activation of survival-signalling pathways such as STAT3, thus diminishing the effectiveness of EGFR inhibition. We investigated the efficacy of a novel JAK2 inhibitor, pacritinib, in brain tumor initiating cell (BTIC) lines to evaluate its potential use in the treatment of GBM patients. In a Phase III study of patients with myelofibrosis, pacritinib demonstrated manageable toxicity and statistically improved patient spleen volume and reported outcomes. Pacritinib administration resulted in on-target JAK2/STAT3 inhibition at 1-2μM and dramatically reduced BTIC proliferation, regardless of endogenous MGMT promoter methylation or EGFR, PTEN, and TP53 mutational status. Pacritinib prolonged survival, over Temozolomide alone, in studies of orthotopically xenografted NOD-SCID mice. We tested the hypothesis that concurrent inhibition of JAK2/STAT3 and EGFR signalling would be an effective and clinically relevant therapeutic strategy for GBM. We examined the effectiveness of combinatorial therapy *in vitro* on BTICs using pacritinib in combination with four clinically approved EGFR inhibitors (afatinib, erlotinib, lapatinib, and AZD9291). We confirmed that EGFR inhibition increased levels of activated STAT3 in BTICs. Combinatorial treatment of BTICs with pacritinib and EGFR inhibitors showed striking responses, with lowered IC50s and decreased BTIC viability. The combinatorial actions of EGFR and STAT3 inhibition were particularly effective in BTIC lines with EGFR activating and vIII mutations and on-target activity was demonstrated with reduced phospho-EGFR, phospho-STAT3 and downstream effectors of both pathways. *In vivo* pharmacokinetic and pharmacodynamic studies demonstrated that pacritinib and the EGFR inhibitors afatinib and AZD9291 penetrate the brain and have on-target activity. Ongoing *in vivo* studies using orthotopic xenograft BTIC models will determine whether combinatorial inhibition of these two pathways will provide a survival benefit. Further studies are aimed at investigating whether combinatorial inhibition of other pro-oncogenic pathways, using the BTIC model both *in vitro* and *in vivo*, may be effective strategies in GBM.

40 - MATCH measurement protocol: Blood pressure and heart rate variability

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While advances in early cancer detection and treatment have increased rates of survivorship, there remain many late and long-term effects of cancer and its treatment that can disrupt overall quality of life. While Mindfulness-Based Cancer Recovery (MBCR) and Tai chi/Qigong (TCQ) are two interventions used to improve quality of life and alleviate adverse side effects associated with cancer survivorship, they have never been directly compared. This presentation outlines the protocol for a study designed to assess the autonomic specificity associated with participating in a typical MBCR or TCQ program, as part of a larger preference-based randomized controlled trial. Inclusion criteria are completion of primary cancer treatments, and current experience of significant distress. Participants' clinic blood pressure (BP), high frequency heart rate variability (HF-HRV), and baroreflex sensitivity will be measured following an intake appointment and immediately post-intervention. BP will be calculated based on the average of five measures taken with an automated office measurement device (BpTRU). Interbeat interval will be assessed during a 5-minute rest period using a 3-lead EKG attached to a Biopac MP36 data acquisition system and HF-HRV will be calculated using the spectral decomposition method. Baroreflex sensitivity will be assessed by measuring HF-HRV response to a postural (supine to standing) challenge. Clarifying the similarities and differences of participating in these 2 popular behavioural interventions on autonomic function might lead to a better understanding of mechanisms involved in symptom improvement as well as a better rationale for treatment selection.

41 - Regulation of Transforming Growth Factor beta (TGFβ)-induced Epithelial-Mesenchymal Transition (EMT)

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TGFβ is the prototype member of the TGFβ superfamily of cytokines. TGFβ regulates a vast array of cellular processes including cell division and differentiation in diverse types of tissues including epithelia. One cellular process regulated by TGFβ is EMT which has key roles in development. However, EMT can be reactivated by TGFβ in cancer and has implication for promoting tumor cell invasion and metastasis. Identifying mechanisms that regulate TGFβ-induced EMT should help uncover novel players that control cancer progression. We used immunoblotting to screen for specific gene products whose abundance was altered during TGFβ-induced EMT. In particular, we identified the transcription factor TCF7L2 (short for, Transcription Factor-7-Like-2) to be downregulated by TGFβ in mammary epithelial cells undergoing EMT. Further, TCF7L2 downregulation preceded the increase in abundance of the transcription factor SIP1 (that drives EMT) and the decrease in the abundance of the epithelial marker E-cadherin by TGFβ suggesting that TCF7L2 may suppress TGFβ-induced EMT. Three-Dimentional (3D) culture of epithelial cells provides a powerful approach to follow changes in multicellular morphology by processes including EMT. In particular, mammary gland-derived epithelial cells form hollow spherical acini that are disrupted by TGFβ-induced EMT manifesting as filled and disorganized colonies. Using the 3D cell culture approach, we found that downregulating endogenous TCF7L2 via RNAi phenocopied the effect of TGFβ on 3D-mammary epithelial cell-derived acini showing filling and disruption. Conversely, overexpression of TCF7L2 suppressed TGFβ-induced disruption of 3D-mammary epithelial cell-derived acini. Together these data suggest that TCF7L2 suppresses TGFβ-induced EMT in epithelial cells. Future studies will focus on defining the role of TCF7L2 in regulating the invasive and metastatic potential of breast cancer cells. Overall, these studies would provide the foundation for the potential development of novel therapeutic and diagnostic tools for treating breast cancer.

42 - Redundancy between Nej1-Lif1 and the MRX complex preserves genomic stability during Non-homologous end-joining in *Saccharomyces cerevisiae.*

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DNA Double strand breaks (DSBs) are the most deleterious form of damage and can result in genomic instability and cell death if improperly repaired. Non-homologous end-joining (NHEJ) is the major pathway used to repair these lesions in mammalian cells and is highly conserved in budding yeast. In yeast NHEJ, the Mre11-Rad50-Xrs2 (MRX) complex is rapidly recruited to the break site where it holds the loose ends of DNA. This tethering keeps the broken ends in close proximity to promote efficient and accurate repair of the lesion. The tethering of DNA ends by MRX depends on a conserved Cys-X-X-Cys motif within Rad50, which forms a hook in the middle of two coiled-coil arms. The CXXC hook drives interlocking contact between two Rad50 molecules, allowing the formation of a molecular bridge by two different MRX complexes. Lif1-Dnl4 and Nej1 are also recruited to the DSB and are required for the Dnl4 ligase dependent ligation of the DNA ends. Interestingly, the human homologues of Nej1 and Lif1, XLF and XRCC4 respectively, have been shown to form long helical filaments *in vitro* consisting of alternating XLF and XRCC4 homodimers that can bridge DNA ends suggesting a possible second function for Nej1-Lif1 during yeast NHEJ.
We characterize two Nej1 mutants, Nej1-F335A and –V338A that lose interaction with Lif1 and demonstrate that one important outcome of Nej1-Lif1 binding is to prevent large deletions resulting from aberrant NHEJ repair. The increased rates of deletion that arise when the interaction between Nej1 and Lif1 is disrupted is not from defects in tethering DNA ends, but rather Nej1-Lif1 shows functional overlap with Rad50 to counter Sgs1-Dna2 and Exo1 dependent DNA resection at a chronic DSB. The hook domain of Rad50 is important for tethering DSB ends, while the Nej1-Lif1 interaction is critical in preventing resection (likely through protecting the ends). Our work contributes to understanding the balance and interplay of repair factors that are recruited to DSBs.

43 - ATM and other DNA repair mutations in cancer cell lines predict higher mutation rates and genetic instability

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BACKGROUND: Ataxia telangiectasia-mutated (ATM) is a critical first responder in the cell to DNA damage. Individuals lacking ATM are extremely sensitive to DNA-damaging ionizing radiation, and are predisposed to develop cancers. The mechanism for ATM dysfunction in A-T patients, or cancer patients that are ATM-deficient, is unknown. We believe that this may be the result of increased genomic instability within the cancer cells caused by a lack of adequate DNA repair. Given that ATM-deficient cancer cells may have higher genetic instability, we sought to quantify the relationship between ATM mutations and genomic instability, as measured by total somatic mutations.

METHODS: Using data available from the Broad Institute's Cancer Cell Line Encyclopedia (CCLE), we correlated mutations in ATM and other genes involved with the DNA repair response with the total number of mutations annotated in ~900 cancer cell lines. To determine the clinical relevance of the cancer cell line observations, we partnered with the BC Genome Sciences Centre (BCGSC) to perform similar analyses on ~100 whole-genome-sequenced patient samples.

RESULTS: We show that in cell lines across all cancer types, mutations in ATM correlate with a significantly higher number of total mutations. We examined additional genes associated with the DNA-repair response, only mutations in the direct response genes (i.e. ATR, BRCA1&2) appeared to associate with total mutations, whereas p53 and others – while more commonly mutated – did not correlate with higher mutations. In 10 lung cancer patients, one had a truncating mutation and had the second highest number of somatic mutations, and highest among non-smokers.

CONCLUSIONS: We have identified a potential relationship between ATM mutation and total somatic mutations in cancer cell lines and patient tumour genomes, which may be indicative of overall genetic instability in these samples. Analysis of the ATM mutations in cell lines and patient samples clearly shows that there are no specific hotspots for mutation in ATM that correlate with increased total mutations. However, this data may help identify patients that may benefit from targeted or modified therapy options based on ATM-deficiency or higher genetic instability.

44 - Use of a novel anti-inflammatory agent, GML, to inhibit osteosarcoma lung metastasis.

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Currently, the 5-year survival rate for osteosarcoma patients presenting with metastasis to the lungs is less than 30%, identifying a need for novel therapies that inhibit pulmonary metastasis of this pediatric cancer. Our growing understanding of the dynamic relationship between inflammation and cancer has led to the investigation of anti-inflammatory approaches to treating cancer, including metastasis. Specifically, recent studies suggest a role for neutrophils in cancer metastasis, whereby neutrophils facilitate the adhesion of cancer cells to the endothelium, thus promoting extravasation from the vasculature into distant organ sites. However, the role of neutrophils in osteosarcoma lung metastasis and the potential of anti-inflammatory therapeutic approaches have yet to be investigated. In this study, we assessed the potential role of neutrophils and the effects of the novel anti-inflammatory agent, GML, in osteosarcoma lung metastasis. GML (GM1-Targeted, Linoleate-Containing TLR2 Ligand) is an engineered anti-inflammatory ligand that has previously been shown to inhibit neutrophil recruitment in models of chronic inflammatory disease (rheumatoid arthritis and colitis). To study this pediatric cancer, we established an *in vivo* osteosarcoma lung metastatic model. Briefly, 143B human osteosarcoma cells stably expressing luciferase were injected intravenously via the tail vein into CB17 SCID mice. Metastatic burden in the animals was visualized weekly using bioluminescence imaging (Xenogen, IVIS 200) and validated post-sacrifice by histological analysis. We observed an increase in neutrophil levels in the lungs 4 hrs following injection of osteosarcoma cells, and in the absence of neutrophils (using a neutrophil depleting antibody (anti-Gr-1)), there was a reduction in metastasis to the lungs. Consistent with these data, treatment with the anti-inflammatory agent, GML, resulted in reduced osteosarcoma metastatic burden in the lungs. These findings suggest a role for populations of neutrophils in osteosarcoma lung metastasis and provide support for the development of anti-inflammatory approaches for the prevention of osteosarcoma metastasis.

This research is supported by Canadian Cancer Society, Dr. Robert C. Westbury Endowment for Melanoma Research, Canadian Institutes of Health Research, and Kids Cancer Care.

45 - Targeting EZH2 histone methyltransferase as a novel target for therapeutic for the aggressive pediatric brain tumor ATRT

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Central nervous system (CNS) atypical teratoid/rhabdoid tumor (AT/RT) is a rare, aggressive tumor that most often affects young children. The common decisive molecular defect in ATRT has been shown to be a single genetic alteration, i.e., the loss of hSNF5 gene that encodes for a critical subunit of the SWI/SNF complex that modulates chromatin remodeling activities. Therefore, ATRT cells display unregulated cell proliferation due to the dysfunction of an important epigenetic control. Due to the unacceptable toxicity of current chemotherapy on children, safe and effective novel therapies are urgently needed.

Enhancer of zeste homolog 2 (EZH2) is the catalytic subunit of the polycomb repressive complex 2 (PRC2) and is involved in repressing gene expression through methylation of histone H3 on lysine 27 (H3K27) that leads to transcriptional repression of the affected target gene. Genes repressed by the PRC2/EED-EZH2 complex include HOXC8, HOXA9, MYT1, CDKN2A and retinoic acid target genes. Recently the overexpression or activating mutations of EZH2, have been demonstrated in a number of aggressive cancers. Although the mechanisms of EZH2 mediated cancer progression is currently unknown, a potential loss of a critical tumor suppressor gene function has been implicated. Hence, EZH2 presents as a novel target for therapeutics in aggressive malignancies. 3-Deazaneplanocin, DZNep, is a cell-permeable histone methylransferase inhibitor that has been shown to inhibit EZH2 levels and H3K27me3 in cancer cells. In this study, we explored the activity, target modulation and drug combinability of DZNep against ATRT cells.

Cells from two established ATRT cell lines (BT12, BT16) were treated with DZNep at various dilutions and cell growth inhibition was quantified by automated cytometer (Celigo). Next, DZNep was evaluated in drug combination studies with a panel of novel and conventional chemotherapeutic agents for drug synergy. We also explored synergy in treatment schedules where the inhibition of EZH2 was induced at different time points in relation to the treatment of the second agent. Drug combination studies were interpreted using the Chou and Talalay method.

Our initial data indicate that DZNep effectively induced cell death in ATRT cells with IC50 values low micro molar concentrations. Screening of a large panel of chemotherapeutic agents on the other hand failed to find effective drug combinations with the exception of ABT-888 and MK-2206. Interestingly, however, we have found significant synergy when DZNep was treated 24 hours prior to treatment with four other novel therapeutic agents, Pp242, OSI-906, Ramatroban (Bay3405) and BMS-599626. Our studies provide initial proof-of-concept data to further explore a unique combination of epigenetic modification with targeted therapeutics for the treatment for a highly malignant brain tumor in children.

46 - The critical role of iNKT cells in restricting metastatic tumor growth in the liver

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The prognosis of most cancer patients depends on the extent of primary tumor metastasis to distant organs. At the time of diagnosis a substantial percentage of patients with colorectal carcinoma have already developed liver metastases. Many patients which are free of liver metastases can be saved by surgical resection of the primary tumor, however, surgery itself can enhance metastasis development in the liver. Circulating tumor cells in the liver sinusoids interact with various resident immune cells such as Kupffer cells and iNKT cells. This interaction results in weak immunity against tumor cells due to the tumor cell tolerant environment in the liver which promotes the establishment of metastases.

Based on previous observations, the aim of the present study was to investigate the immunomodulatory role of the glycosphingolipid αgalactosylceramide (αgalcer). To assess this question we performed intravital multi-channel spinning disc confocal microscopy to visualize the interaction of iNKT cells and metastasized tumor cells in the liver in vivo as well as in vitro assays.

Repeated αgalcer treatment resulted in a major increase in the number of iNKT cells present in the liver, increased interactions with tumors, and showed significantly diminished tumor growth. Similar results were obtained when mice with established liver metastases received αgalcer. In addition, the survival rate in treated animals was longer as compared to untreated animals. We repeated experiments in NKT cell deficient mice and found that the inhibition of tumor growth is CD1d-dependent. Antibody mediated depletion of iNKT cells revealed that tumor control during αgalcer treatment is mediated by iNKT cells. When tumors reached a certain size the iNKT cells were no longer effective at reducing the tumor growth.

Our results suggest that iNKT cells, after being activated with αgalcer, play a pivotal role in restricting metastatic growth either via direct interaction with tumor cells (perhaps by granzymes) or indirectly by modulating the immunosuppressive tumor environment increasing Th1 immunity.

47 – Tissue specific ribosomal biogenesis and its effects on size and development in *Drosophila*

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Translation of mRNA into polypeptides is essential for life and the growth and development of an organism. Specialized molecular machines called ribosomes, which are composed of rRNA and a set of proteins, known as ribosomal proteins (RPs), carry out the translational process. Mutations in RP encoding genes have been found in a variety of developmental diseases as well as certain cancers. In *Drosophila melanogaster,* haploinsufficiency of specific ribosomal proteins leads to delayed development, short thin bristles, and altered body size, consequences of compromised protein synthesis. Previously we have shown that no two ribosomal protein heterozygotes show identical phenotypes in terms of developmental rate and body size. This suggests that ribosomal proteins may not in fact perform redundant roles or be stoichiometrically equal across all tissues and organs. I hypothesize that RPs may have more defined roles in the translational process and confer tissue specificity along with mRNA selectivity. Using a combination of genetic techniques that implement the use of RNAi for gene inhibition and GAL4-UAS for overexpression we will knockout and rescue specific ribosomal proteins in different *Drosophila* tissues. We will also quantify mRNA synthesis in these tissues using qRT-PCR and polysome analysis in these tissues in an effort to characterize a set of ribosomal proteins and gain further insight into their roles in development and disease.

48 - Cytotoxic amounts of cisplatin induce either checkpoint adaptation or apoptosis in a concentration dependent manner in cancer cells

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Cisplatin is a widely used genotoxic anti-cancer drug and yet how cells die when they are treated with it remains poorly understood. We therefore investigated how cancer cells treated with cisplatin die, by exploring whether human cancer cells treated with cisplatin undergo checkpoint adaptation (entry into mitosis with damaged DNA). Cell viability assays showed that both the pharmacologically relevant concentration of 30 μM and the supra-pharmacological concentration of 100 μM cisplatin were cytotoxic to HT-29 human colorectal adenocarcinoma cells. However, light and time-lapse video microscopy indicated that cells treated with these different concentrations of cisplatin undergo different modes of cell death. Cells treated with 30 μM cisplatin died after undergoing checkpoint adaptation. By 72 h, 93% of 30 μM cisplatin treated cells were positive for γH2AX staining, 95% were positive for cyclin B1 staining and 7% were positive for phospho-histone H3 staining, as determined by immunofluorescence microscopy. Strikingly, by 96 h of treatment with 30 μM cisplatin, 81% of cells entered mitosis, as determined by time-lapse video microscopy. By contrast, only 7% of cells treated with 100 μM cisplatin entered mitosis. Instead these cells died by apoptosis, they were positive for annexin V staining, contained cleaved caspase 3, cleaved caspase 9 and cleaved PARP and did not contain Mcl-1. These data show that checkpoint adaptation is a key cellular response linking cell cycle arrest and cell death in cancer cells treated with cisplatin. Our results also show that treatment of cells with different concentrations of cisplatin induces different modes of cell death. We predict that by increasing our understanding of checkpoint adaptation, and its relationship to cell death, we might identify biochemical pathways that could be used to improve current cancer treatments.

49 - p75 neurotrophin receptor (p75NTR) regulates glioma progression through cell autonomous and cell non-autonomous mechanisms.

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Glioblastoma multiform (GBM) is the most common and aggressive brain tumor that is inevitably a fatal disease. GBM is a heterogeneous tumor consisting of tumor cells and a small population known as brain tumor initiating cells (BTICs) or glioblastoma stem-like cells. BTICs appear to drive tumor progression, underlie therapeutic resistance to current treatment and tumor relapse and have been highlighted as important therapeutic targets. The ability of glioma cells to invade into the surrounding brain parenchyma is a major clinical issue rendering glioblastoma incurable by conventional therapies. In a previous study, we found that the p75 neurotrophin receptor ($p75^{NTR}$) significantly enhanced invasion and migration of genetically distinct glioma by a cell autonomous mechanism. In addition, $p75^{NTR}$ was frequently observed in a highly invasive population of cells from freshly resected patient specimens. Importantly, $p75^{NTR}$ was found to mediate glioma invasion by neurotrophin-dependent regulated intramembrane proteolysis (RIP). Blocking of $p75^{NTR}$ proteolysis by the generation of cleavage-resistance mutants, or treatment of animals bearing $p75^{NTR}$-positive intracranial tumors with γ-secretase inhibitors, significantly inhibited glioma invasion and prolonged survival. Using a large panel of patient-derived-BTICs we have investigated the role of $p75^{NTR}$ glioma progression. Immunocytochemical studies and western blot analysis revealed that p75NTR is variably expressed on BTICs and treatment with γ-secretase inhibitors significantly decreased BTIC invasion in 3D cultures in vitro. Importantly, stable down-regulation of $p75^{NTR}$ significantly decreased BTICs invasion, proliferation and self-renewal ability *in vitro* and decreased proliferation and invasion, tumorigenicity of BTICs *in vivo* and significantly increased the median survival rate of animals bearing tumors generated by down regulated $p75^{NTR}$ BTICs. In addition we have found that, $p75^{NTR}$ was present on as a component of the cargo in BTIC-derived extracellular vesicles (EVs) that are implicated in tumor cell invasion through a cell non-autonomous mechanism. We determined that $p75^{NTR}$ containing EVs promote invasion of non-invasive glioma cells. Furthermore, $p75^{NTR}$ proteolytic cleavage fragments can be detected in glioma derived EVs. The composition of $p75^{NTR}$ containing EVs and their roles in glioma invasion are currently been investigated

50 - The impact and change in lifetime and post-diagnosis physical activity on quality of life in prostate cancer survivors

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BACKGROUND: Early detection and improved treatment have led to decreased prostate cancer mortality rates and increased numbers of survivors. Consequently, priorities in prostate cancer control have shifted to reducing the burdens of living beyond cancer to improving quality of life (QoL) and overall functioning. Physical activity is a modifiable lifestyle factor that has been shown to improve survival after cancer and may be of importance for maintaining QoL. This study examined the effects of post-diagnosis physical activity on QoL after diagnosis.

METHODS: Prostate cancer survivors (n=830) who participated in a case-control study in Alberta between 1997-2000 with histologically confirmed, invasive stage T2 or greater, identified through the Alberta Cancer Registry and were followed to 2014 for mortality outcomes. At baseline and at three times post-diagnosis, interviews/questionnaires were used to collect data on demographics, treatments, physical activity, QoL and other lifestyle factors Multivariable linear regresion was used to test effect modification and confounding.

RESULTS: Participants were 67 years, on average, at the start of follow-up, were mainly married with stage II cancer, and had at least one co-morbidity post-diagnosis. Both total and recreational physical activities were positively associated with the physical QoL scores. Further, when comparing changes in physical activity levels pre- to post-diagnosis, those men who consistently met physical activity guidelines had significantly higher physical (β=6.01; 95% CI= 4.15, 7.86; p<0.0001) and mental (β=2.32; 95% CI=0.29, 4.34; p=0.025) QoL scores in comparison to those who did not meet guidelines pre- or post-diagnosis.

CONCLUSIONS: There appeared to be an improvement in physical QoL in association with meeting recommended levels of recreational physical activity. Future intervention studies should focus on achieving and maintaining adherence to physical activity guidelines post-diagnosis in prostate cancer survivors.

51 - Dosimetric consequences of rotational patient setup error in stereotactic radiosurgery treatments

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Purpose: Stereotactic radiosurgery (SRS) is a highly effective method for treating many different types of brain metastases. This is due in part to its ability to accurately deliver large doses to the target tumor in a single fraction. Given that the doses delivered are relatively high and the dose gradients steep, maintaining this accuracy is critical to the success of the treatment. The amount of rotational patient setup errors needed to impact this accuracy is not well defined. The purpose of this study is to determine a rotational setup error threshold for brain SRS treatments.

Methods: Two groups were retrospectively analyzed in this study: Group A consisted of twenty cases where a single brain metastasis treatment was planned using a single isocenter located within the tumor; Group B consisted of five cases where two brain metastases were planned using a single isocenter located between the tumors. All twenty-five cases underwent simulations for rotations around the treatment isocenter of ±1°, ±3°, ±5°, and ±7° around all axes simultaneously. In addition, cases in Group B included simulations of ±2°. The percent of the planning target volume that received the prescription dose (V_p) was compared between the original plans and rotation simulations to determine what degree of rotations will cause V_p loss greater than 1%.

Results: In Group A, V_p loss ranged from 0.00% to −0.23% (median: −0.05%) for ±1°, 0.00% to −0.91% (median: −0.23%) for ±3°, −0.01% to −2.41% (median: −0.55%) for ±5°, and −0.08% to −4.56% (median: −1.13%) for ±7°. In Group B, V_p loss ranged from −0.02% to −14.71% (median: −0.83%) for ±1°, −0.17% to −21.86% (median: −3.66%) for ±2°, −0.67% to −57.84% (median: −8.47%) for ±3°, −2.37% to −69.91% (median: −20.83%) for ±5°, and −4.27% to −92.02% (median: −35.14%) for ±7°.

Conclusions: For the single metastasis, single isocenter cases in Group A, 0/20 showed V_p loss greater than 1% for rotations under ±3°. For the multiple metastases, single isocenter cases in Group B, 4/10 cases had V_p loss greater than 1% for rotations of ±1°. Additionally, V_p loss tended to increase with increasing distance from isocenter. Given these results, for single isocenter-single metastasis cases, rotations under ±3° are unlikely to cause dosimetric consequences. However for single isocenter-multiple metastasis cases, notable target coverage loss can occur for rotations as low as ±1°, especially for cases that are far away from the treatment isocenter.

52 - POPULATION-BASED URINARY INTERVENTION RATES FOLLOWING INTRAOPERATIVELY PLANNED I-125 LOW DOSE RATE PROSTATE BRACHYTHERAPY

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Purpose: To determine the rates of urinary intervention for patients with low and low-tier intermediate risk prostate cancer treated with intraoperatively planned low dose rate prostate brachytherapy.

Methods and Materials: From 2003-2012, 723 consecutive patients were treated with intraoperatively planned LDR prostate brachytherapy without external beam radiotherapy at our center. Dosimetric planning targets were uniform with a goal of D90 prostate>180 Gy, V150>74%, V200>37%, V140 urethra<24% and V150 urethra<3%. Patients were followed according to a protocol every 6 months for the first 3 years then annually. For each patient, all data were entered into a central database prospectively and retrospectively verified by reviewing a centralized electronic health record that comprehensively captures all interventions performed and visitations to any medical center in the province of Alberta, Canada. Urinary interventions included cystoscopy, transurethral resection of the prostate, urethral dilatation or catheterization for urinary retention or hematuria attributable to brachytherapy. These patients were then isolated and compared with the remaining control cohort using tests of proportions and multivariate analysis as appropriate.

Results: Median follow-up was 7.1 years (range: 2.5 – 12.5). Intervention for RTOG grade 3 toxicity was encountered in 51 patients (7%). Cystoscopy was done in 31 patients (4.3%) for RT related hematuria or obstruction. TURP was performed in 14 patients (1.9%) with a median time from implant of 20.4 months (range, 3-86) and dilatation for RT toxicity in 6 patients (0.8%) with a median time of 29 months (range, 20-85) . Median pre-implant volume was 37.1cc (20.2 – 53.0) in patients having intervention for grade 3 toxicity and 33.7 cc (13.2 – 66.9) in those without [$p<0.05$]. Time from biopsy to implant, pre-treatment AUA symptom score, PSA, clinical stage, use of hormones, and urethral dosimetry did not predict for urinary intervention in our analysis.
Conclusions: Urinary intervention rates following intraoperatively planned LDR prostate brachytherapy are low overall at 7%. The strength of our study is the ability to review all hospital records in our health region to completely capture any urinary intervention due to an integrated electronic health records system.

53 - RegulatING aging: does ING1 modulate telomere stability through TRF2?

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Cellular senescence is often thought of as the first line of defense against the development of cancer. Multiple proteins, and in particular tumor suppressor proteins, are involved in establishing this mechanism. INhibitor of Growth (ING) proteins are frequently inactivated in cancers and have been shown to regulate cell multiplication and senescence by modifying the structure of DNA. The shortening of telomeric DNA is a hallmark of cellular senescence and telomeres are well protected by proteins such as TRF2. TRF2 levels decrease as telomeres erode while in several cancer types, such as breast cancer, the TRF2 levels are 10-15 fold increased. It is known that post-translational modifications, like methylation and acetylation, play a major key role in stabilizing TRF2. Since INGs are able to direct the activity of acetylation and deacetylation complexes and can function as scaffold proteins, we hypothesize that there is a link between those two important cell regulators. Further supporting this, TRF2 and INGs have several interacting proteins in common including HDAC1, p300 and lamin A. However, the mechanism behind a potential interplay between TRF2 and INGs remains unclear, which brings us to my hypothesis: 'ING proteins act to target HDAC/HAT activity to modify TRF2, thereby affecting the modality of senescence vs. transformation'. To test this hypothesis, I will determine if INGs and TRF2 physically interact and if INGs are able to affect TRF2 stability, using co-immunoprecipitation, far western and acetylation assays. These assays will be augmented by the use of high resolution confocal microscopic analysis of the physical domains of the nucleus occupied by TRF2, INGs and lamin A in response to telomere loss and other forms of cellular stress that is known to affect subcellular localization of the ING proteins.

54 - The Use of Olaparib in the Treatment of Pancreatic Cancer

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Poly-ADP ribose polymerase (PARP) inhibitors attenuate the activity of PARP proteins in DNA repair and are particularly potent in cells that are defective in repair of DNA double strand breaks (DSBs) by the Homologous Recombination repair pathway. Our lab previously showed that mantle cell lymphoma and gastric cancer cell lines with depletion, loss, or inactivation of the phosphatidyl inositol kinase like protein kinase (PIKK), ataxia telangiectasia mutated (ATM) were sensitive to the PARP inhibitor olaparib and that this effect was most apparent in ATM-deficient cells lacking the tumour suppressor protein, p53. Recently, ATM was reported to be mutated in both sporadic and familial forms of pancreatic cancer. I will show that inactivation of ATM by small molecular inhibitor, sensitizes pancreatic cancer cells to Olaparib. This research could inform future clinical trials and/or provide information to test the potential for using ATM and PARP inhibitors as a novel therapy in pancreatic cancer.

55 - Gender Disparities in Non-Small Cell Lung Cancer

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Background: Although lung cancer is the second most-often diagnosed malignancy in both men and women, and the biggest cancer killer of both genders, evidence suggests that the lung cancer experience differs in women compared to men. Lung cancer incidence in men has steadily decreased since the mid-1980s, while in women it has increased. Partly, these patterns reflect sex differences in smoking behavior over the previous two decades. Additional epidemiological evidence suggests that gender impacts most facets of the lung cancer experience including the incidence, susceptibility, severity and its molecular basis. However, there is a lack of consensus on both the magnitude and etiology of these gender-based differences. The aim of this rapid review is to more precisely define this gender disparity among NSCLC patients in North America; Europe and South Asia and summarize current opinions about the molecular basis for these observations.

Methods: A systematic literature search was performed regarding gender disparities in NSCLC in four databases including MEDLINE, EMBASE, Cochrane Database of Systematic Reviews, and Cochrane Central Register of Controlled Trials. Next, we will determine gender differences in its frequency and severity utilizing data from independent studies based on rapid analysis of randomized control trials or from systematic reviews published in the past 20 years in North America; Europe and South Asia, once again using three data bases: MEDLINE; EMBASE; Cochrane.

Results: An analysis will be conducted on patients diagnosed with NSCLC, published between 1996 and 2015, from a total of 36 studies including systematic reviews, meta analysis, and randomized trials identified using MEDLINE, EMBASE, and Cochrane data bases. Main outcome measures will be frequency and morbidity of NSCLC and evidence of gender disparities.

Conclusion: Our analyses will provide a detailed description of gender disparities in NSCLC epidemiology, incidence and outcome. Additional studies characterizing risk factors and other covariates associated with female NSCLC patients will be described. Ultimately, this will lead to a change in the design of clinic trials to account for these disparites.

56 – Emerging roles of DNA-PKcs in mitosis

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DNA-PKcs has a well-established role in repair of DNA DSBs via NHEJ. We and others recently found that DNA-PKcs also plays an important role in mitosis. We recently reported that DNA-PKcs is autophosphorylated at multiple sites in mitosis and localizes to centrosomes in prophase and metaphase and the midbody in cytokinesis. We also showed that DNA-PKcs interacts with PLK1 and is phosphorylated by PLK on S3205 in mitosis. To better understand the role of DNA-PKcs in mitosis we have generated HeLa cells with CRISPR deletion of DNA-PKcs and are currently characterizing the mitotic defects in these cells.

57 - CHD6: A Potential Regulatory Protein in the Oxidative Stress Response

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CHD6 belongs to the Chromatin Helicase DNA binding (CHD) family of chromatin remodeling enzymes. An analysis by the Cancer Genome Atlas (TCGA) indicates that CHD6 gene copy number is expanded in many tumors including: colorectal, breast and lung, all of which arise from oxidatively stressed tissues. We hypothesize that CHD6 is a regulatory protein in the oxidative stress response pathway, and our goal is to investigate the mechanism of regulation and with a view towards reducing the ability of cancer cells to survive oxidative stress. We find that CHD6 protein expression increases rapidly in response to oxidative stress. CHD6 expression also increases following proteasome inhibitor (bortezomib) treatment and is, itself, ubiquitylated, indicative of a proteasome-mediated pathway regulating CHD6 function. CHD6 is predicted to operate within the oxidative stress response pathway involving KEAP1, an E3 ligase that interacts and regulates NRF2, a master transcriptional regulator of antioxidant response. In this pathway, KEAP1 binds, ubiquitylates and induces NRF2 degradation in the absence of oxidative stress and is inactivated by oxidation to enable rapid NRF2 protein stabilization and activity. We have found that CHD6 and KEAP1 interact directly and in a manner requiring the chromodomains of CHD6. Perhaps counter-intuitively, over-expression of KEAP1 stabilizes both endogenous and ectopically expressed CHD6 in multiple cell lines. We will present our ongoing work characterizing CHD6 response and its regulation following oxidative stress.

58 - Copy Number Variation in Donor KIR Genes and Motifs Titrates Natural Killer (NK) Cells' Functional Response to EBV Infections and Influences the Risk of Developing Post Transplant Lymphoproliferative Disease (PTLD) after Allogeneic HCT

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BACKGROUND: Uncontrolled reactivation of Epstein-Barr virus (EBV) leading to post-transplant lymphoproliferative disorder (PTLD) is one of the major complications after T-cell depleted HCT. Recovering within weeks after HCT, natural killer (NK) cells are deemed important in the immunopathogenesis of EBV complications. Their role however remains elusive. NK cell responses are regulated by a series of activating and inhibitory cell surface receptors, central to which are the Killer Ig-like Receptors (KIR). Here we hypothesized and tested whether diverse NK cell receptor repertoires can titrate NK cell functional responses to EBV and can potentially modify the risk of developing PTLD.

METHODS: KIR genotypes, centromeric and telomeric motifs and their variants were determined for 356 allo-HCT donors through next generation sequencing of KIR locus. PBMNCs from KIR typed healthy volunteers were co-cultured with EBV-transformed cells and degranulation and IFNγ producing NK cells were enumerated using multi-parameter flow cytometry. Effect of donor KIR profile on PTLD was tested using competing risks regression statistics. Segregation of NK cell response to EBV across various KIR repertoires was tested by Mann-Whitney U statistics

RESULTS: At least one copy of Donor tA01 motifs was required for a strong protection against PTLD ($p=0.0001$, SHR=0.17). The number of EBV induced NK cells increased with increasing tA01 motifs. There was no influence of recipients' KIR repertoire on the risk of developing PTLD.

CONCLUSIONS: KIR-regulated NK cells have a profound effect on the risk of PTLD. KIR gene profile based identification of HCT recipients at high risk of PTLD will enable closer monitoring of EBV DNAemia and facilitate prompt therapy.

59 - Antithymocyte Globulin at Clinically Relevant Concentration Kills Leukemic Blasts

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Background and Rationale: Allogenic hematopoietic cell transplantation (HCT) has become a critical part of treatment for patients with acute leukemia. Despite tremendous increase in its use, the success of HCT is limited by the development of graft-versus-host disease (GvHD) and by relapse. In Alberta, of the patients undergoing BMT approximately 35% die or suffer long-term from GvHD, 20% die due to relapse, and 10% die due to other reasons, leaving only 35% of patients that survive without any complications. Contrary to cyclosporine or methotrexate, rabbit anti-thymocyte globulin (ATG) used for graft-versus-host disease (GvHD) prophylaxis with myeloablative conditioning does not increase relapse after hematopoietic cell transplantation. The reason for not increasing relapse is unknown. We hypothesized that ATG at concentrations achieved with our standard ATG dose of 4.5 mg/kg has an anti-leukemic activity. We measured ATG induced killing of leukemic blasts at clinically relevant concentrations via complement-dependent cytotoxicity (CDC) or complement-independent cytotoxicity (CIC) in marrow or blood from 36 patients with newly diagnosed acute leukemia.

Methods: Blood or bone marrow from patients newly diagnosed with acute myeloid leukemia (AML, n=31) or acute lymphoid leukemia (ALL, n=5), obtained before induction chemotherapy, was used as the source of leukemic cells. We evaluated CDC by treating leukemic cells with various concentrations of ATG (1, 10, 25 and 50 mg/L) in presence of active human serum (source of complement) for 15 minutes. For CIC, leukemic cells were treated with various concentrations of ATG in presence of heat-inactivated serum (no complement) for 4 hours. For CDC dead cells were identified as 7-amino-actinomycin D positive (7AAD+) using flow cytometry. For CIC dead/dying cells were identified as Annexin V+ using flow cytometry.

Results: ATG induced death of leukemic blasts both via CDC and CIC. Median 0.3%, 2.8%, 12.6% and 42.2% blasts were killed with 1, 10, 25 and 50 mg/L ATG, respectively, via CDC. Median 1.9%, 7.15%, 12.1% and 13.9% blasts were killed after 4 hour incubation with 1, 10, 25 and 50 mg/L ATG via CIC. CIC appeared to represent a direct induction of apoptosis by ATG. There was a high variability in the sensitivity of the blasts to ATG – at 50 mg/L, percent blasts killed ranged from 2.6% to 97.2% via CDC and from 1.4% to 69.9% via CIC.

Conclusion: ATG at clinically relevant concentration kills primary leukemic cells in vitro. Some acute leukemias are highly sensitive and others relatively resistant to ATG. Whether this applies to in vivo needs to be determined. If yes, and given that the killing of leukemic cells is ATG concentration-dependent, conditioning regimens with high dose ATG could be developed for patients with leukemia sensitive to ATG to maximize the concurrent anti-GvHD and anti-relapse effects.

60 - Treatment variations at disease progression in patients with non-squamous, EGFR-mutant NSCLC (2010-2014) - *A retrospective analysis performed at a single Canadian institution*

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Background: Treating EGFR*mut*$^+$ NSCLC patients with EGFR-TKIs extends progression-free survival (PFS) with reduced toxicity compared to cytotoxic chemotherapy. However, all patients inevitably experience disease progression (PD), and ultimately succumb to their disease. In the clinical setting, treatment at PD is less well-defined. The purpose of this retrospective study analysis is to identify how exactly EGFR*mut*$^+$ NSCLC patients are treated at PD, and investigate the association of patient and disease characteristics (with continuation and/-or discontinuation of EGFR-TKIs) at PD.

Methods: All EGFR*mut*$^+$ NSCLC patients treated with EGFR-TKI as their initial and/-or subsequent systemic therapy at Tom Baker Cancer Centre, between March 2010 and December 2014, were retrospectively analyzed. Data collected: demographic and clinical data, EGFR mutation type, treatment at PD (chemotherapy, radiation, continuation with EGFR-TKI, none). SPSS Statistics software was used to generate basic survival analyses.

Results: 130 EGFRmut$^+$ NSCLC patients treated with an EGFR-TKI were identified; (125/130 metastatic at EGFR-TKI initiation), median age at EGFR-TKI initiation: 67 years (32-95); female 63.0%; EGFR mutation exon 19/21/others: 46.2%, 36.1%, 17.7%; adenocarcinoma 90.1%; never smokers/current/previous smokers: 53.8% /10.8% /30.0%. At analysis, 107 patients had experienced PD. 47 of these continued EGFR TKI beyond progression, 40 of whom received no other systemic treatment, while 10 received palliative radiation. The overall duration of EGFR-TKI treatment for the cohort was 9.83*m*. Median overall survival (MOS) with EGFR-TKI was 21.6*m* across the complete cohort. Log-rank test showed a statistical significant difference in MOS for those with exon 19 del vs. exon 21 L858R point-mutation: MOS 27.7*m* vs. 15.5*m*, respectively. Female gender, PD with solitary-lesions and a younger age are associated with EGFR-TKI continuation at time of initial PD.

Conclusions: Our monocentric study reinforces the distinct clinical profile of EGFR*mut*$^+$ NSCLC patients and confirms the different outcome of exon 19 del vs L858R patients. Our data suggests that it is common practice for oncologists to continue treatment with an EGFR-TKI despite radiographic and/-or clinical evidence of PD. Overall, this study results can help facilitate the development of clinical trials aimed at improving therapeutic strategies beyond progression of disease after EGFR-TKI therapy.

61 – Role of differential protein synthesis in intestinal stem cell growth and proliferation.

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Stem cells undergo renewal and differentiation, and hence are responsible for the growth and maintenance of an adult organ. Stem cell studies in cell culture and model organisms highlight their requirement for highly regulated protein synthesis. Loss of this regulation can lead to stem cell over proliferation and disrupted differentiation. However, how stem cells regulate their protein synthesis remains unclear. The drosophila intestine is an excellent model to study stem cell proliferation since the intestinal stem cells (ISC) undergo rapid differentiation to replenish epithelial cells upon damage. Previous studies in our lab show that RNA polymerase III dependent tRNA synthesis is one way by which somatic cells control their mRNA translation. On testing whether tRNA synthesis plays a role in ISC proliferation, I found that inhibition of tRNA synthesis in ISCs block their division upon gut damage. In future experiments I will look at the signaling pathways underlying this regulation.

62 - DNA length and structure dependent role of Ku80 C-terminal region in DNA-PKcs kinase activation in non-homologous end joining.

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Non-homologous end joining (NHEJ) is the major DNA double strand (DSB) break repair pathway in mammalian cells. The first step in NHEJ is recognition of DSBs by the Ku heterodimer and subsequent recruitment of DNA-dependent protein kinase catalytic subunit (DNA-PKcs), a serine/threonine protein kinase, to form the DNA-PK complex. Knockout of DNA-PKcs or Ku in mouse leads to extreme radiation sensitivity and immunological defects. The Ku heterodimer consists of 70 and 80 kDa subunits and is conserved throughout evolution. It has been suggested that the extreme C-terminal 14 amino acids of Ku80 is required for DNA-PKcs recruitment and activation. However, another study demonstrated that deletion of the Ku80 C-terminal region (CTR) does not abolish DNA-PKcs activation. Thus, there is considerable ambiguity regarding the role of the Ku80 CTR in DNA-PKcs recruitment and activation.
The aim of this study is to understand the role of Ku80 CTR in NHEJ with focus on its ability to recruit and activate DNA-PKcs kinase activity. Using clonogenic cell survival assays, I confirmed that hamster cells expressing Ku80 CTR deletions are radiosensitive and also showed sensitivity to other DSB inducing agents such as doxorubicin and neocarzinostatin. I then generated Ku80 C-terminal deletions (Ku80 residues 1-718 and 1-569), cloned them into baculovirus vectors and expressed and purified the corresponding Ku heterodimers from insect cells. *In vitro* autophosphorylation reactions, in presence of calf-thymus DNA, using purified proteins showed that Ku heterodimer with Ku80 residues 1-718 showed only a slight defect in DNA-PKcs autophosphorylation, whereas heterodimer with Ku80 residues 1-569 had significant defects in multiple DNA-PKcs autophosphorylation sites. Surprising results were observed when defined DNA structures such as 25 base pair (bp) blunt ended double stranded (ds) DNA was used. Deletion of the entire Ku80 CTR (residues 570-732) lead to abrogation of DNA-PKcs kinase activity and inability to interact with DNA-PKcs protein. These defects may underlie the radiation and chemosensitivity of Ku80 CTR deletion mutant.

63 - A novel role for a cell surface glycoprotein in organ-selective cell recruitment

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Despite significant advances in recent years, metastasis remains the cause of more than 90% of deaths from solid tumors. Adhesive cellular and molecular interactions between disseminated tumor cells and the vascular endothelium help govern metastatic progression. Clinically it has been observed that some cancers metastasize to distant sites in an organ-selective manner. Akin to leukocytes, disseminated cancer cells have been found to use common adhesion and extravasation events. The goal of this study was to identify novel cell-surface molecules that mediate adhesion and recruitment of white blood cells and metastatic cancer cells in an organ-selective manner. Using an unbiased in vivo approach we initially isolated a peptide-displaying phage that homes to the liver and lungs of animals treated with an inflammatory stimulus (LPS). Using intravital microscopy, we found that this phage, or its corresponding displayed-peptide, termed lung and liver targeting peptide (LT-peptide), inhibited the adhesion of neutrophils in the liver sinusoids in response to LPS. Excitingly, this peptide was also shown to inhibit metastasis to the lungs of animals injected with human metastatic melanoma cells (70W). Moreover, our data supports 'membrane dipeptidase' (DPEP1) – a GPI anchored cell-surface glycoprotein as the potential binding receptor for the LT-peptide. By performing immunofluorescence studies in vitro we found that the LT-peptide binds selectively to Cos-1 cells expressing either murine or human DPEP1 and not to cells expressing other membrane dipeptidase family members i.e. DPEP2 and DPEP3. In addition, although LT-peptide bound to DPEP1 it did not affect its enzymatic activity suggesting that LT-peptide may inhibit the adhesion and recruitment of cells by binding to membrane dipeptidase as an adhesion receptor. Our data would suggest that DPEP1 is a vascular endothelial receptor for leukocytes in the liver and lungs and is usurped by metastatic cancer cells in these organ sites. We are currently assessing whether LT-peptide and/or its target DPEP1 have direct therapeutic and clinical applications.

64 - Prolactin-induced breast cancer derived factors that mediate osteoclastogenesis

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Breast cancer, at an advanced stage, often metastasizes to bone, initiating a vicious cycle where the breast cancer cells induce osteoclastogenesis (osteoclast cell differentiation) that results in degradation of the bone and release of many factors that aid in metastatic cell growth. Consequently the cycle repeats, causing increased osteolysis, leading to pain, fractures and death. Prolactin, a hormone that at high serum or high cellular receptor levels, is associated with higher breast cancer risk, poor prognosis in post-menopausal women. We hypothesis that the vicious cycle of cancer triggered by prolactin stimulation of breast cancer cells occurs through via an as of yet undefined pathway(s) with multiple proteins downstream of the prolactin receptor to facilitate osteoclastogenesis. I aim to identify these pathways and factors using pathway inhibitors and secretome analysis. I will test the role of prolactin and prolactin induced factors on bone metastasis using an *in vivo* xenograft model. This will give us a better idea about the mechanistic roles of prolactin in breast cancer and in osteoclastogenesis, which could lead to the discovery of new therapeutic targets and the development of diagnostic biomarkers to identify patients at greater risk of bone metastasis.

65 - Assessing Response in Colorectal Cancer Cells and the Development of a Novel Metabolomic Biomarker of Response

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Background: Chemotherapeutic options are rapidly expanding for colorectal cancer (CRC). However, only a fraction of patients benefit from these toxic drugs. An assay that enables early identification of individuals benefiting from a particular drug would be useful to minimize patient exposure to ineffective agents. We postulate that a response to systemic therapy is associated with characteristic changes in extracellular metabolites, and some of these are agnostic to drug mechanism of action. Our objective was to identify these metabolites.

Methods: HCT-116, HT-29, Caco-2 and HCT-8 (CRC) cells were exposed to increasing doses of brivanib, oxaliplatin and 5-fluorouracil for 72 hours. Supernatant collected at baseline and at 72 hours was analyzed by gas chromatography-mass spectrometry to identify metabolomic changes. A MTT assay was done to identify conditions where cell growth was inhibited.

Results: For each drug, 82-140 metabolites changed as a result of treatment. 47-57 metabolites changed in a dose-dependent manner for each cell type. Of these, approximately 13-54 metabolites were specifically related to cell death. Interestingly, 37 metabolites were found to be associated with cell growth inhibition in all conditions (independent of cell line and drug type).

Conclusions: In CRC cells, we have identified metabolites that change in association with a response to chemotherapy drugs that are independent of drug mechanism of action. The candidate metabolites identified will be assessed in clinical samples from CRC patients treated with systemic therapy. Identification of response-related changes in the circulating metabolome may represent a novel means of detecting response to chemotherapy.

66 - The molecular mechanism of CHD6 in the preservation of genome stability and cancer prevention

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CHD6 (Chromodomain, Helicase, DNA binding 6) is a class III CHD/Mi2 family ATP-dependent chromatin remodelling enzyme that, via interactions with the NFE2-related factor 2 (NRF2), is part of a protein complex that responds to oxidative stress – a key feature of IR exposure – and regulates the expression of many detoxifying enzymes following exposure to reactive oxygen species (ROS), thus maintaining cellular redox homeostasis. CHD6 has raised interest since several human ataxias have linkage map regions that encompass the CHD6 gene locus on chromosome 20q11.1-12. Further, catalytically-inactive CHD6 mutant mice exhibit motor coordination defects most consistent with a cerebellar neuron disorder (i.e. ataxia). Since ataxia is commonly seen in human syndromes lacking normal DNA strand break responses, this raises the possibility that CHD6 contributes to some aspect(s) of the DNA break response.

Data obtained in the lab to date demonstrates that CHD6 protein expression increases substantially within one hour after exposure to *tert*-butyl hydroperoxide (TBH); this increase in expression appears to occur through stabilization of CHD6. Further, CHD6 is recruited to UV-A laser DNA damage tracks as rapidly as one minute after damage induction. Interestingly, this recruitment is entirely dependent upon the enzyme PARP (Poly-ADP Ribose Polymerase), which rapidly ribosylates DNA to enable the recruitment of many proteins following break induction. Retention of CHD6 at laser tracks is abrogated by inactivating mutations within the chromodomains of CDH6, which may indicate how the enzyme is able to bind DNA. Finally, using the alkaline comet assay to assess DNA single strand break number, the most common lesion induced by ROS, revealed that depletion of CHD6 leads to an increase in DNA damage after hydrogen peroxide treatment. We hypothesize that increased CHD6 expression equates with an enhanced response to oxidative stress-induced DNA damage that is advantageous to tumor survival following ROS exposure.

67 - The role of LSPI motif containing protein partners of protein phosphatase 2A in mitosis

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Mitosis is tightly regulated by reversible protein phosphorylation. PP2A-B56 is an important mitotic protein phosphatase which contributes to the fidelity of the process of sister chromatid segregation during mitosis by dephosphorylating certain outer kinetochore proteins and thereby stabilizing attachments between sister chromatids and the microtubules of mitotic spindle. PP2A-B56 is recruited to the mitotic spindle by BubR1, a mitotic protein kinase. The minimal amino acid sequence on BubR1 required for binding PP2A-B56 has been identified to be KL*p*SPIIE or the 'LSPI' motif. Repoman, another important mitotic regulator, also binds to PP2A-B56 through the same motif. We hypothesize that LSPI is a general consensus motif for recruitment of PP2A-B56, particularly during mitosis, and that additional proteins utilize this recruitment sequence. We speculate that recruitment of PP2A-B56 is regulated by the phosphorylation status of this motif and this in turn plays an important role in controlling the progression of the cell cycle. Our preliminary results show that seven different proteins which are important mitotic regulators contain LSPI motif and also interact with PP2A-B56 in a phosphorylation dependent and isoform dependent manner. Further understanding of how interactions between the target proteins and PP2A-B56 affect mitotic progression and/or mitotic exit will provide valuable insight about the mechanisms of specific recruitment and mitotic functions of PP2A-B56.

68 - Combination oncolytic virus treatment in cancer cell lines

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Introduction

Oncolytic viruses are promising new tools for cancer therapy that are currently in clinical trials for many cancer types. These viruses can target cancer cells without hurting normal cells and cause fewer side effects than chemotherapy or radiotherapy. Reovirus, Coxsackievirus, and Adenovirus are three oncolytic viruses, containing dsRNA, ssRNA, and dsDNA genome respectively, that are being investigated for cancer therapy. Adenovirus and reovirus are in late stage clinical trials demonstrating efficacy and good safety, while one strain of coxsackievirus is in phase II clinical trials.

Despite promising efficacy and safety, the ability of these viruses to kill different tumor cells varies. It is also unknown if different oncolytic viruses can be combined to enhance the speed of cell death, the total amount of cell death, or to overcome resistance to virus infection in cancer cells. The goal of this project is to investigate how combining these oncolytic viruses to treat various cancer cell lines will affect cancer cell death and susceptibility to virus infection.

Hypotheses: 1. Combination virus treatment will result in a faster and more abundant cancer cell death.2. Resistance of some cancer cell lines to oncolytic virus will be overcome by combination virus treatment. 3. Virus will affect each other's application during combination treatment.

Materials and methods

Reovirus Type 3 Dearing (**Reo**), Coxsackievirus Type B3 (**Cox**), Adenovirus Serotype 5 (E1B deleted) (**Adeno**) were used to infect HCT116, HeLa, and HT-29 cancer cell lines. Dose-response curves of different viruses in cancer cell lines were generated using crystal violet assay. Crystal violet assay was also used to test cell viability of cells treated with Reo, Cox, and Adeno, or different combinations. Western blots were used to assess expressions of different proteins to assess viral replication (viral protein expression) and induction of cell death (confirmed by cleavage PARP) caused by different virus treatments. For both cell viability and western blot assay, HCT116 cell line was treated with 1MOI Reo, 5MOI Cox, 1MOI Adeno, or the combination of each two. Hela was treated with 5MOI Reo, 0.001MOI Cox, 10MOI Adeno. HT-29 was treated with 1MOI Reo, 1MOI Cox, 1MOI Adeno. For western blot assay, proteins of HCT116, HeLa, and HT-29 were collected after 48hpi, 72hpi, and 48hpi, respectively. Pictures of HCT116 cells were captured right before collecting protein. Fluorescent images of HeLa cells treated with Adeno (10MOI), Reo (5MOI, and Cox (0.001MOI) for 48 hours were taken on an Olympus laser scanning confocal microscope with a 60X objective (NA1.46) and 1.5 zoom. Significance was assess by 1-way ANOVA using post-test to compare differences in individual treatments.

Conclusions

1. Combination of Reovirus and Adenovirus treatment resulted in a faster cell death in HeLa cell line. Cell morphology also showed a better killing of combination of Adenovirus and Reovirus in HCT116 cell line.
2. In HCT116 cells, Adenovirus was inhibited by Coxsackievirus when combination treatment were used.
3. Co-infected cells were found in HeLa and HCT116 cells treated with combination of Reovirus and Adenovirus. This is consist with the results from Western Blots of HCT116 and HeLa cell lines, which indicated an co-cell death signal.
4. The mechanisms of combinations virus treatment are not clear, thus more work needs to be done.

69 - The role of Telomerase in the development and maintenance of Glioblastoma Multiforme

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Introduction: Glioblastoma Multiforme (GBM) is a highly aggressive and devastating brain tumour which has a median survival of only 15 months following diagnosis. The current standardised treatments for this tumour have been largely unsuccessful due to the vast degree of genetic variability between patients with these tumours. Activity of the enzyme telomerase is present in <70% GBM tumours and is thought to be involved early in their development making it a potential target for therapy. In this study we focus on the role of telomerase in GBM and its role in malignant transformation.

Methods: Human BTIC) and mouse cells derived from the sub-ventricular zone (SVZ) were used. Mouse SVZ cells were cultured differentially in EGF/FGF or PDGF following a protocol developed previously by the Cairncross and Weiss labs in which cells treated with PDGF transform to a GBM like phenotype and those in EGF/FGF do not qPCR and TRAP assays were performed to analyse the levels of TERT mRNA and Telomerase activity respectively.

Results: All human BTIC cell lines tested showed increased levels of TERT mRNA expression compared to BJ fibroblast controls. This suggests increased hTERT levels in the cells which was confirmed via TRAP assay showing increased telomerase activity in all cell lines tested. Transformed and untransformed mouse cell lines showed varying degrees of telomerase activity by TRAP. Interestingly, the 16P transformed cell line showed no telomerase activity, suggesting this particular line may be employing the ALT telomere maintenance mechanism, independent of telomerase.

Conclusion: All human BTIC cell lines with increased TERT mRNA expression showed increased telomerase activity, supporting the role of telomerase in the maintenance of these tumours. However, the transformed mouse cell lines showed varying telomerase activity suggesting the presence of an ALT mechanism in one cell line. The presence of telomerase activity in one untransformed line also suggest the possible self-immortalisation of these cells in culture, although it has been confirmed previously that these untransformed lines, even in the presence of telomerase, do not form tumours in mice.

70 - Urinary Bladder Cancer Regulation: Exploring the Role of Proteinase-Activated Receptors and Transient Receptor Potential Ion Channels

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Background and Aims
Once detected by haematuria, bladder cancer is well developed. The challenge is to develop 'biomarkers' for the early detection of this disease and for monitoring recurrence after treatment. Our hypothesis is that bladder cancer growth, invasion and metastasis is driven by tumour and parenchymal cells that can direct cell signaling via two main mechanisms: (1) the activation of proteinase-activated receptors (PARs: regulated by proteolysis and release of an N-terminal fragment) and (2) coordinate regulation of tumour cell ion channels linked to PAR activation, like transient receptor potential vanilloid-4 and -M8 (TRPV4/TRPM8). We suggest that bladder tumour development involves the up-regulation of the abundance and activation of PARs, their activating proteinases, and TRP channels. This oncogenic process in principle would be accompanied by an increased urinary secretion of PAR fragments and their activating proteinases, and by an increase in TRPV4/M8 activation. Our aims were therefore to validate in two bladder cancer-derived cell lines (T24 and HTB-9): (1) the presence of functional PARs 1 & 2 and TRPs -V4 and-M8, and (2) the relative abundance of PAR- and TRP channel mRNAs.

Methods
HTB-9 and T24 are the urinary bladder cancer cell lines used in the following experiments: 1.) The presence and relative abundance of PARs and related channels (TRPV4 and M8) was evaluated using semi-quantitative PCR, normalized to an actin signal. 2.) The functionality of PARs (1, 2, and 4) and related channels was evaluated by calcium signaling (JPET 288:358, 1999) stimulated by increasing concentrations of target-selective agonists (PAR_1:TFLLR-NH_2, PAR_2:2fLIGRLO-NH_2, PAR_4:AYPGKF-NH_2, TRPV4:GSK101, TRPM8:icilin).

Results
Bladder tumour derived HTB-9 and T24 cells posses functional PAR1and PAR2 with a differential sensitivity for PAR2 (HTB9>T24) vs PAR 1 (HTB-9=T24). These cell lines also express functional TRPV4 receptors. Neither cell line possess PAR4 nor TRPM8.

Conclusions
Bladder cancer cells possess functional PARs (1 and 2) and TRPV4 channels which may drive tumour progression. Thus, these receptors may represent novel therapeutic targets. If these receptors are important for tumour progression, regulating factors may be present within the tumour microenvironment; a theory that is currently being explored.

71 - Identifying barriers to cervical cancer screening among South Asian Muslim immigrant women.

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Objectives: We sought to identify the barriers to cervical cancer screening among South Asian Muslim immigrant women in Calgary. Understanding their ideas and needs will enable development of educational programs and services so they can benefit from screening and reduce the effect of this disease.

Approach: Qualitative, semi-structured in-depth interviews, by purposive sampling, were conducted with South Asian Muslim immigrant women of Calgary who were unscreened or infrequently screened for cervical cancer. Thematic analysis was conducted for data analysis using Microsoft Word.

Results: 18 women were interviewed and the majority (66%) never had a Pap test. Findings were categorized into five major themes: Attitude, knowledge & beliefs, healthcare seeking practices, experience with healthcare system & services, barriers and strategies to Pap testing. Major findings include: misunderstanding about Pap test reminders, strong preference for a female physician who also speaks their language, seeking symptomatic treatment not prevention, negative experiences with healthcare providers including painful Pap test experience. Major barriers involved: lack of knowledge about cervical cancer and the term cervix, fatalist beliefs, dependence on husband, transportation, language and unavailability of female physicians. Separate centers for Pap testing, awareness and encouragement by social workers and family physicians to get tested were strategies participants suggested.

Conclusion: Different healthcare strategies are needed at the system and provider level to improve healthcare experience of these women and to promote cervical cancer screening. Providing female physicians, knowledge and resources such as transportation and a separate center, and screening reminders that explain the procedure and the disease in detail could potentially increase screening practices.

72 - Association between immigration status & cervical cancer screening: Systematic review & meta-analysis

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Background: In developed countries, much invasive cervical cancer, and the highest mortality rates occur in women who never had a Pap test. Immigrants appear less likely to have been screened for cervical cancer than non-immigrants. Education, marital status, income, primary care provider characteristics, acculturation, and women's knowledge and beliefs about cervical cancer screening seem to be associated with low levels of screening among immigrants.

Objective: We aimed to determine the magnitude of association between immigration status and cervical cancer screening (ever been screened) among women in developed countries.

Approach: The search used guidelines of the Center for Reviews and Dissemination, using a combination of keywords related to cervical cancer and screening. Data was extracted using the 2009 PRISMA checklist. The Newcastle-Ottawa Quality Assessment Scale was used for confounding and quality assessment.

Results: From 7426 citations, ten articles were included in the systematic review and eight in meta-analysis. The studies were published between 2001 to 2013 from Australia, UK, USA, Canada & Spain. Immigrants are less than half as likely to have ever been screened as non-immigrants in Canada (pooled OR = 0.44; 95% CI:0.386- 0.511), Spain (OR = 0.41; 95% CI: 0.365-0.467), and Australia (OR = 0.44; 0.376-0.508). In the UK, the ratio is worse (OR = 0.23; 0.210-0.244) In the USA, the trend was similar but not significant (polled OR = 0.62; 0.190-2.083). Demographics showed immigrants are less likely to be educated, have lower income and are uninsured. Women born in Asia had lower odds of ever being screened compared to other immigrant groups.

Conclusion: A statistically significant association was found between immigration status and cervical cancer screening but there are limitations due to data reporting. Efforts to increase cervical cancer screening should focus on newly arrived immigrants, immigrants with low levels of education, with low household annual income, and particularly from Asian background. Improving access to care is important to increase cervical screening practices among immigrant populations.

73 - Modulation of cancer cell susceptibility to Reoviral infection via manipulation of microRNA expression.

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The development of effective cancer therapeutics with minimal toxicity is of critical importance. Oncolytic viruses are an attractive alternative to current therapeutics as they selectively replicate in and kill cancer cells. Reovirus is a well studied oncolytic virus has shown potential as a novel cancer therapeutic and has entered phase I, II and III human clinical trials with FDA approval granted recently as an orphan drug for certain cancers. Reovirus replicates within a broad range of cancer cell types such as breast, colon and ovarian cancers. However, not all cancers are susceptible to Reoviral infection. The selectivity of Reoviral replication has been a topic for much debate and the exact mechanisms that govern cell responsiveness to Reoviral replication have yet to be fully elucidated. Successful completion of the viral replication and lytic cycle is largely dependent on the interaction between both the host cell and viral genomes. microRNAs, which are small non-coding regulatory RNAs, help govern the expression of the host genome and are involved in the regulation of complex signalling networks. It is possible that altered microRNA expression profiles in cancer cells may result in the stimulation of pathways that are essential for viral replication and subsequent cellular apoptosis. Through microRNA microarray analysis, the global change in microRNA expression in cancer cells after Reoviral infection has been determined. Numerous intriguing candidate microRNAs have been identified as vastly up- or downregulated in response to Reoviral infection. These microRNAs may be implicated in a cell's permissiveness to Reoviral replication. The aim of this investigation will be to determine which of the microRNAs contribute to cell susceptibility to Reoviral infection. Once identified, these microRNAs will be used to arm additional viral vectors, such as the commonly used Adenoviral vector. These vectors will then be used to prime cancer cells that are non-permissive to Reoviral infection and possibly enhance their susceptibility to infection. Understanding the interaction between microRNA expression and Reoviral replication could allow for informed engineering of novel therapeutic vectors to be used in concert with Reovirus, ultimately expanding the utility of Reovirus as an oncolytic agent.

74 - A Novel Drug Discovery Approach for Refractory Pediatric Leukemias: Dual-function Antimicrobials as Anti-leukemic Agents

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Introduction: Currently, relapsed or refractory leukemia remains a leading cause of death in children. Novel therapeutic approaches are urgently needed to design new treatment protocols. In the world of anti-cancer drug discovery, finding new leads for drug development can be quite time-consuming, costly and bears many unknowns with regards to drug toxicities. One approach to circumventing these concerns is to screen compounds with previously established pre-clinical data, such as that of antibiotic medications. Here, we screen a collection of antibiotics for their anti-leukemic potential, acting as a stepping point from which to create a new anti-leukemic therapy.

Methods: Using leukemia cell lines established from refractory leukemic blasts from children (including Molt-3, C1 and Poetic 2.2), a panel of antibiotics (n=110) were screened for their ability to inhibit cell survival. Drugs were dissolved in DMSO to a 10mM concentration and further diluted with culture media to produce final drug concentrations ranging from 0.001uM to 40uM. Exponentially growing leukemia cells were treated with various concentrations of individual agents. After four days, cell viability was measured via automated microscopy, enabling the calculation of 50% cell inhibitory concentrations (IC50). The "hits" from these screening assays are being analysed further including for their structural similarities, both computationally and biochemically, to determine potential pharmacophores and/or mechanistic routes with which to gain further insight into potential new targets.

Results: Of the 110 antibiotics screened, numerous antibiotics showed anti-leukemic activity against the Poetic 2.2 cell line (30 drugs), the C1 cell line (23 drugs) and the Molt-3 cell line (22 drugs). Several of the drugs showed activity across all three cell lines with promising IC50's, including a subset of antiviral compounds with distinct molecular mechanisms.

Conclusions: In screening a panel of known drugs—antibiotics, we have found several "hits" with anti-leukemic properties. These hits demonstrate the potential for repurposing antibiotics as anti-leukemics. Ongoing studies are focussing on the identification of unique targets and mechanisms of agents with promising activities. Further development of these compounds by targeted optimization may lead to the development of safe and effective clinical trials of such agents and combinations for the treatment of relapsed childhood leukemia.

75 – Analysis of Longitudinal Data

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The handling of statistics for animal models is always difficult. This study evaluates Drug A and Drug B combination effects in treating a blood cancer in a syngeneic mouse model. The data collected are in the form of longitudinal data that evaluates whether certain serum protein levels (indicative of tumour burden) in mice harbouring the blood cancer are significantly different between 4 treatment groups and the control group over time. Analytical challenges include:
Only 5 subjects in each group and 2 missing values at time point 3. Between time points within each group a possible unknown correlation may exist. This data structure makes GEE (Generalized Estimating Equation) a good approach to fit the data.

After plotting the data, testing the linearity of response in each group, and transforming the data, the criterion QIC (Quasi Information Criterion) is used to select the best fitting GEE model. The model considering group effect, time effect and interaction between these two effects, the full model, is shown to most accurately reflect the differences.

Based on the estimates of this full model, we determined that there are statistically significant differences between the 4 treatment groups and the control group over time.

76 - PAactivPAL: An R Package for Statistical Analysis on Physical Activity Data

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Researches for the links between physical activity and cancer risk have traditionally been based on fairly crude self-report instruments such as questionnaires. This field is being revolutionized by the availability of relatively cheap accelerometers. ActivPAL™ is one of the most popular wearable devices in application. The device could produce over 10,000 observations per person per day on a second by second basis. It measures whether the person is lying down, sitting, standing or moving, and produces estimates about the amount of energy expended. However, the raw ActivPAL data can be complicated for statistical analysis. We develop an R package PAactivPAL to solve this problem. The functions embedded in this package can summarize variables recorded in physical activity. For example, it calculates the time and energy spent in sedentary, light, moderate and vigorous activities. The package also includes functions for plotting and group comparison, which facilitates users to analyze the data for different perspectives.