Immune Balance and Health
Welcome and congratulations on participating in the 2015 Canadian Student Health Research Forum, a unique venue offering students doing health research the opportunity to network, learn about cutting-edge research from internationally renowned experts and be recognized for their own outstanding scientific accomplishments.

The involvement of students in research creates the pipeline to the future of research in this country. We are grateful we can contribute to their exposures through poster presentations, a CIHR Career Development Workshop and numerous networking opportunities. The theme symposium on “Immune Balance and Health” is translational and we note the internationally recognized expert speakers who will be sharing their insights and experience.

We extend warm greetings and a ‘friendly Manitoba’ welcome to all participants from across Canada and internationally who are taking part in the CIHR National Research Poster Presentation and the Canadian National Medical Student Research Symposium. In this context we note the participants from the Canadian Society for Immunology as well as from Europe (ORPHEUS) and Shantou University, PRC. We trust that the valuable connections you make with your peers, judges and CIHR leadership will provide career-enhancing opportunities as well as a fun and enjoyable experience. A big thank-you to all the student groups for their energetic support in hosting our visitors.

Winnipeg is an exciting and vibrant place to live, work and conduct state-of-the-art health research. We hope you enjoy your visit with us at the University of Manitoba campus and have the opportunity to explore all that our city has to offer.
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<td>“Systems Vaccinology - Enabling Rational Vaccine Design with Systems Biological Approaches”</td>
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| 14:00  | Dr. Luanne Metz - University of Calgary    | University of Calgary, Calgary, AB  
  “Translation in MS - The Calgary Experience” |
| 14:45  | Coffee Break                               | Mezzanine          |
| 15:15  | Dr. WanJun Chen - National Institutes of   | National Institutes of Health, Bethesda, MD  
  “Understanding and Manipulating Regulatory T Cells for Immunotherapy” |
<p>| 16:00  | Round Table Discussion                     |                   |
| 16:30  | Awards Ceremony                            |                   |
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2015 Awards Committees

Major Awards Committee
Yvonne Myal, Chair
Benedict Albensi
Stephanie Booth
Sanjiv Dhingra
Kent HayGlass
Jeff Wigle

Oncology Awards Committee
Yvonne Myal, Chair
Sam Kung
Robert Tate
Jeff Wigle

Organizing Committee
Patrick Choy
Amanda Clinton
Kevin Coombs
Michael Czubryt
Serge Desnoyers, III, CIHR
Hani El-Gabalawy
Kent HayGlass
Ed Kroeger
Aaron Marshall
Yvonne Myal
Peter Nickerson
Nicole Szajcz-Keller, IMHA, CIHR
Kimberly Ormiston
Don Smyth
Terri Turner
Andrew Watson, AFMC
Xi Yang

CIHR/National Poster Judging Panel
Don Smyth, Chair
Jeff Wigle (Co-Chair)
Blake Ball
Dan Brown
Kris Cowley
Alysha Croker
Sanjiv Dhingra
Janice Dodd
Jules Dore
Brent Fedirchuk
Paul Fernyhough
Jean-Eric Ghia
Dan Gietz
Sari Hannila
Bob Harris
Sohelia Karimi
Thomas Klonisch
Trevor Pemberton
Bob Shiu
Tabrez Siddiqui
Louise Simard
Chris Siow
Katinka Stecina
Bob Tate
Andrew Watson

Manitoba Poster Judging Panel
Michael Czubryt, Chair
Hope Anderson
Blake Ball
Versha Banerji
Raj Bhullar
Adam Burgener
Kristine Cowley
Eftekhar Eftekharpour
Brenda Elias
Saeid Ghavami
James Gilchrist
Joe Gordon
Andrew Halayko
Sabine Hombach
Tammy Ivanco
Michael Jackson
Tiina Kauppinen
Hassan Marzban
Kirk McManus
Don Miller
Tooru Mizuno
Jim Nagy
Thomas Netticadan
Mojgan Rastegar
Bob Shiu
Miyoung Suh
Bob Tate
Jude Uzonna
Symposium
Speakers
2015
Dr. Wanjun Chen received his MD from Qingdao University Medical School, Qingdao, China in 1984. Following graduate training in Microbiology and Immunology at the Shandong University Medical School and Shandong Academy of Medical Sciences, Jinan, China, he received post-doctoral training at Harvard University Medical School.

He has been at the National Institute of Dental and Craniofacial Research, NIH since 1997 where he now a Senior Investigator and Chief of the Mucosal Immunology Section.

Among his many honours and awards, Dr. Chen has been an Invited Speaker at the NIH Director’s Seminar Series. In his introduction to that presentation he was credited with developing a revolution in our understanding of regulatory T cells and how they affect immunity and tolerance, based on their regulation of TGF beta. One of his papers on this subject has received more than 3000 citations. He has also recently received the Scientific Achievement Award from the NIH Asian and Pacific Islander American Organization, in 2013.

Dr. Chen’s current research focuses on elucidating mechanisms of TGF-beta regulation of T-cell immunity and tolerance, and manipulating T-cell immunity versus tolerance in animal models to understand the pathogenesis of autoimmunity and inflammation, cancer and infectious diseases. He is also working to develop potential therapies for relevant human diseases, with special attention to autoimmune diseases and cancer.
Understanding and Manipulating Regulatory T cells for Immunotherapy

CD4+CD25+Foxp3+ regulatory T cells (Tregs) are instrumental in the induction and maintenance of immune tolerance and in the prevention and therapy of chronic inflammation and autoimmunity. We previously discovered that TGFb induces Foxp3 in peripheral naïve CD4+CD25– T cells and converts them into Foxp3+ regulatory T cells (iTregs). We further demonstrated that TGFb also plays a critical role in the development of Foxp3+Treg cells in the thymus (tTregs). In our recent studies, we have elucidated that generation of thymic regulatory T cells indeed requires TGFb-induction of Foxp3 gene expression in Treg precursors rather than a specific TGFb-protection of Treg death. We revealed that thymic negative selection is linked to tTreg development. We have demonstrated that thymocyte apoptosis drives the intrathymic generation of regulatory T cells. We have proposed a novel model to explain the development of thymic Tregs4. In addition, we have developed a way to generate antigen-specific Tregs in vivo to treat autoimmunity. In our most recently published and unpublished studies, we induced long-term remission of the established autoimmune diseases including EAE, Type I diabetes and collagen-induced arthritis in mice by generating autoantigen-specific Foxp3+ Tregs in vivo. We showed mechanistically that apoptotic cells triggered professional phagocytes to produce TGFb, under which the autoantigenic peptides directed naïve CD4+ T cells to differentiate into Foxp3+ Tregs instead of into T effector cells in vivo. Importantly, these antigen-specific Tregs ameliorated autoimmunity without compromising immune responses to bacterial antigen. These findings and their implications in developing potential immunotherapy for human autoimmune diseases will be discussed (This research was supported by the Intramural Research Program of NIH, NIDCR).
After receiving his medical degree from the New York University School of Medicine and completing an internship at the Strong Memorial Hospital, New York, Dr Lipsky became a Clinical Associate at the NIH studying macrophage-lymphocyte interactions.

From 1975 to 1999, he worked at the University of Texas Medical Center, where he became Professor of Internal Medicine and Microbiology, Director of the Harold C Simmons Arthritis Research Center, Co-Director of the Immunology Graduate Program at the Southwestern Graduate School of Biomedical Sciences, Director of the Rheumatic Disease Division of the Department of Internal Medicine and Harold C Simmons Professor in Arthritis Research in 1995. In 1999, he returned to the NIH as Director of the Intramural Research Program at the National Institute of Arthritis and Muscoskeletal and Skin Diseases.

Dr Lipsky is internationally recognized for his research, particularly his work on T cell-macrophage interactions, mechanisms of immune cell activation, the role of B cells in autoimmune diseases and in the development of novel therapeutics for rheumatoid arthritis. He has been awarded numerous prizes, including the 2001 Carol Nachman Prize and the 2002 American College of Rheumatology Distinguished Investigator Award. In 2002, the Arthritis Foundation awarded Dr Lipsky the prestigious Lee Howley prize for his contributions to the understanding, treatment and prevention of arthritis and rheumatic diseases.

Dr Lipsky is the author of over 550 articles, is the past Editor-in-Chief of The Journal of Immunology (1992-1997), current editor of Arthritis Research and Therapy, and serves on the editorial boards of numerous scientific journals.
Dr. Yong-Jun Liu has been appointed head of research for MedImmune, the global biologics research and development organization for AstraZeneca. He joined MedImmune in January, 2014.

Dr. Liu came to MedImmune from the Baylor Research Institute where he served as Vice President and Chief Scientific Officer and Director of the Baylor Institute for Immunology Research.

In his new role at MedImmune, Dr. Liu is responsible for driving pre-clinical research in multiple therapeutic areas to help advance drug candidates from discovery to development. Dr. Liu has extensive translational research experience and is one of the most prolific researchers in immunology globally, with more than 230 published articles in top journals such as Nature, Science, Cell and the Journal of Experimental Medicine. His research has led to the development of several drug targets such as TSLP, OX40, ILT7 and pDC in the areas of allergy, autoimmune and cancer.

Dr. Liu is a graduate of the Norman Bethune University School of Medicine in China and the University of Birmingham in the United Kingdom. He spent 12 years at Schering-Plough in France, and DNAX Research Institute in California. Dr Liu was recruited by the University of Texas MD Anderson Cancer Center in 2002, where he was founding director of the Cancer Immunology Research Institute. He also served as the Chair of the Department of Immunology, building the nation’s leading immunology program as judged by top publications and grants.
After completing an MD at the University of Calgary (1983) Dr Metz studied Internal Medicine at the University of Toronto (1983-85). She then returned to Calgary to complete a Neurology Residency (1985-88) and a Fellowship in MS/Neuroimmunology (1989). She became Calgary MS Clinic director in 1992 and then Neurology Section Chief in 2014.

Dr Metz is best known for her development of the Calgary MS Clinic and of a bench-to-bedside translational research program (in collaboration with basic scientist Dr V Wee Yong). Specific areas of research interest include clinical trial design, development of novel therapies including minocycline, study of oral corticosteroids and vitamin D, and study of mental health issues in MS (in collaboration with Dr SB Patten). Dr Metz implemented an electronic health record in the Calgary MS Clinic that supports patient care, clinic management, and outcomes research.

She is widely published, holds several grants, and has led over 50 industry-sponsored clinical trials. She serves on international data and safety monitoring boards and has designed and completed her own investigator initiated studies. She is currently Principal Investigator of a multicentre Phase III clinical trial funded for 4.04 million dollars by the MS Society of Canada and is co-leader of a 5 million dollar Alberta Innovates Health Solutions Team Grant studying remyelination in MS. She was honored as one of the Top 40 University of Calgary Alumni in celebration of the University of Calgary 40th Anniversary Celebrations and she has been awarded the Faculty of Medicine Watanabe Distinguished Achievement Award for overall excellence and extraordinary contribution to the Faculty of Medicine.
The focus of the Calgary MS Research program is to translate observations in the clinic and the laboratory into science and into changes in care delivery. This means going from the clinic to the bench (and back) as well as going from the lab to the clinic. The most important factors are that it requires leadership, a team approach, and patience. In Calgary our team involves basic scientists, clinical scientists, imaging scientists, clinicians, students, government, industry, and community partners. Our community partners include patients, philanthropists, and funding agencies. The program has evolved over many years. Stable funding is also important. In this presentation I will provide examples of how we accomplish this.
Dr. Bali Pulendran is a Charles Howard Candler Professor of Pathology and Laboratory Medicine, and Director of the Innate Immunity Program, and the NIH U19 Center for Systems Vaccinology, at the Emory Vaccine Center, Emory University in Atlanta.

He received his undergraduate degree (BA Hons) from Cambridge University, and his Ph.D from the Walter & Eliza Hall Institute, in Melbourne Australia, under the supervision of Sir Gustav Nossal. He then did his post-doctoral work at Immunex Corporation in Seattle. Dr. Pulendran's work focuses on understanding the mechanisms by which the innate immune system regulates adaptive immunity and harnessing such mechanisms in the design of novel vaccines. More recently, his laboratory pioneered the use of systems biological approaches to predicting the efficacy of vaccines, and deciphering new correlates of protection against infectious diseases.

Dr. Pulendran’s research is published in front line journals such as Nature, Science, Cell, Nature Medicine, Nature Immunology, Immunity. Furthermore, Dr. Pulendran is the recipient of numerous grants from the National Institutes of Health, and from The Bill and Melinda Gates Foundation, and is the recipient of two concurrent MERIT awards from the National Institutes of Health.
Despite their great success, we understand little about how effective vaccines stimulate protective immune responses. Two recent developments promise to yield such understanding: the appreciation of the crucial role of the innate immune system in sensing microorganisms and tuning immune responses, and advances in systems biology. In this presentation, I will discuss how these developments are yielding insights into the mechanism of some of the most successful vaccines ever developed. Furthermore, such developments promise to address a major challenge in vaccinology: that the efficacy of a vaccine can only be ascertained retrospectively, upon infection. The identification of molecular signatures induced rapidly after vaccination, which correlate with and predict the later development of protective immune responses, would represent a strategy to prospectively determine vaccine efficacy. Such a strategy would be particularly useful when evaluating the efficacy or immunogenicity of untested vaccines, or in identifying individuals with sub-optimal responses amongst high risk populations, such as infants or the elderly. We have recently used a systems biology approach to identify early gene signatures that correlate with, and predict the later immune responses in humans vaccinated with the live attenuated yellow fever vaccine YF-17D, or with the influenza vaccines. I will review these studies, and discuss their broader implications for vaccinology.
Impact of the Recommended Level of Dairy Consumption in Canada on Cholesterol Metabolism

M. Abdullah\textsuperscript{1,2}, P. Eck\textsuperscript{1,2}, P. Couture\textsuperscript{3}, B. Lamarche\textsuperscript{3}, P. Jones\textsuperscript{1}

\textsuperscript{1}Dept. of Human Nutritional Sciences, \textsuperscript{2}Richardson Centre for Functional Foods and Nutraceuticals (RCFFN), University of Manitoba, Winnipeg, MB, \textsuperscript{3}Institute of Nutrition and Functional Foods (INAF), Laval University, Quebec, QC

Introduction: Consumption of milk and dairy products has been shown to variably affect circulating cholesterol concentrations, which are in turn modulated by intestinal cholesterol absorption, hepatic biosynthesis, and biliary excretion. Effects of dairy consumption on cholesterol absorption, synthesis, or clearance rates have, however, not been assessed thoroughly. Further, we have previously demonstrated associations between certain variants in key cholesterol pathway-related genes and responses of serum cholesterol to dairy, but whether the presence of those genetic variants modulates cholesterol absorption or synthesis rates is not known. This study examined impacts of the recommended 2-3 servings/day dairy products consumption on cholesterol homeostasis via stable isotope tracers, surrogate markers, and gene-diet interaction studies.

Methods: In a randomized multicentre crossover design, normocholesterolemic individuals (n = 40) consumed a prudent dietary protocol that included 3 servings/day of dairy (low and regular fat milk, yogurt, and cheese products) or dairy-free control products for 4 weeks each. Cholesterol absorption was determined by \textsuperscript{13}C cholesterol enrichment over 96 hours following oral administration as well as by plasma campesterol and beta-sitosterol concentrations. Cholesterol fractional synthesis rate (FSR) was determined by deuterium incorporation over 24 hours and by plasma lathosterol concentrations. Genotyping analyses were assessed by TaqMan assay technology.

Results: Compared to control, dairy consumption did not modify lipid profile (\(P > 0.2\)) but was associated with lower concentrations of the surrogate markers of cholesterol absorption; campesterol (−8.0\%, \(P = 0.017\)) and beta-sitosterol (−19.0\%, \(P < 0.0001\)). Overall, no changes in \textsuperscript{13}C-cholesterol absorption or FSR were observed between diets (\(P = 0.66\) and 0.43, respectively). However, when separated based on genetic variants, after the dairy diet, minor allele homozygotes of the bile acid synthesis gene \textit{CYP7A1} SNP rs3808607 showed lower \textsuperscript{13}C-cholesterol enrichment (−325.3‰, \(P = 0.046\)), whereas minor allele carriers of the cholesterol synthesis gene \textit{DHCR7} SNP rs760241 showed lower FSR (−1.6 \%/day, \(P = 0.034\)) in comparison to other genotypes.

Conclusion: Recommended dairy consumption may modulate cholesterol absorption and synthesis in individuals carrying specific genotypes within genes related to cholesterol metabolism.
Determining the Role of Specific Natural Mucosal Antiproteases in Controlling HIV Infection
L. Aboud, J. Kimani, A. Burgener, F. Plummer, T. Ball

University of Manitoba, Winnipeg, MB

**Background:** A women’s risk of becoming infected with HIV is dependent on numerous factors, including the composition of fluids produced by the genital mucosa. Specific HIV inhibitory factors, including various antiproteases, have been identified as being up-regulated within the cervicovaginal fluid (CVF), of highly-exposed sero-negative women (HESN), within the Pumwani cohort of commercial sex workers in Nairobi, Kenya. We hypothesize that CVF from HESN women will exhibit stronger HIV neutralizing activity, compared to HIV susceptible women due to the increased abundance of specific antiproteases. We believe that these overabundant antiproteases are capable of neutralizing HIV infection and will do so through both direct (e.g. blocking of viral entry into host cell) and indirect (interference with proper cell activation/proliferation) mechanisms.

**Methodology:** Neutralization assays consisted of TZM-bl reporter cells, PBMCs and C8166T cell line and employed an X-tropic, R-tropic and dual tropic human HIV isolate. These assays were performed with individual CVL samples from women within the Pumwani sex worker cohort, that were defined as HESN, HIV-positive or HIV-susceptible. Real time-PCR to determine the level of viral DNA as well as viral mRNA which determined specific antiprotease’s effect on early stages of HIV infection and transcription of viral mRNA, respectively. ACH2 cells, were used to determine the effect of the protein on late stages of the viral life cycle and confocal microscopy determined cellular entry and localization of the exogenously added antiprotease. Flow cytometry was employed to determine the effect of the protein on cellular proliferation.

**Results:** While there was no significant difference in the HIV neutralizing capacity between groups of women, considerable variation was observed in the neutralizing capacity of CVF between individuals within and between groups, with some exhibiting high neutralization, while others demonstrating enhancing effects. For many women the effect on HIV infection was consistent across multiple sample time points. Numerous mechanistic studies on one such antiprotease, revealed it is capable of inhibiting efficient HIV infection through interference with proper cellular proliferation as well as directly in late stages of the virus life cycle, likely post-transcriptionally, thus either during virus protein translation or viral assembly/budding at the cell membrane.
Introduction: Diet is an important contributor to quality of life and disease. However, limited knowledge exists on dietary intakes of Canadians in comparison with the recommended intakes. The objective of this study was to assess intakes of energy, macronutrients and vitamin and minerals in a nationally representative sample of Canadian adults in comparison with Dietary Reference Intakes (DRIs).

Methods: The DRI cut-point method and the probability approach (specifically for iron in women) were used to determine the adequacy of dietary intakes. Responses were obtained from 1,833 individuals (aged 18 and older) who completed a web-based Canadian Diet History Questionnaire in the spring of 2012, a validated food frequency questionnaire estimating dietary intakes over the past month.

Results: The average energy intake was $1644 \pm 3126$Kcal/day, with 49% of total energy from carbohydrates, 33% from total fat and 16% from protein. As well, the average energy intake of ages 18 to 30 (2086kcal/day) was higher in comparison with ages 31 to 50 (1659 kcal/day), 51 to 70 (1556kcal/day) and 71 and older (1519kcal/day). Energy intakes were lower in females (1386kcal/day) compared to males (1879kcal/day) ($p<0.05$). Compared to the DRIs, a large proportion of respondents had inadequate intakes of folate (87%), magnesium (59%), potassium (92%), calcium (78%), vitamin D (79%) and dietary fibre (89%). In contrast, most had adequate intakes of phosphorus, zinc, vitamin C, vitamin A, riboflavin and vitamin B12. Males generally had adequate intakes of thiamin (72%), vitamin B6 (71%) and niacin (93%). In comparison, the proportion of females with adequate intake of these nutrients was lower. As well, females aged 50 years and younger had a lower intake of iron compared with those aged older than 50 years (87% vs. 34%). Ages 18 to 30 have higher intakes of vitamin B6, vitamin B12, vitamin C, and calcium in comparison with ages 31 to 50, 51 to 70 and 70 and older.

Conclusion: Although, Canadians in general have intakes within acceptable ranges based on DRI recommendations, strategies are required to optimize the adequacy of Canadian’s dietary intakes and to communicate information on a balanced diet.
Uncoupling the *In Vivo* Tumorigenic Properties of Tumor Propagating Cells from Their *In Vitro* Properties

C. Aiken¹,³,⁶, L. Morrison²,³,⁶, M. Bridges³,⁶, M. Bigio⁴,⁵,⁶, T. Werbowetski-Ogilvie¹,²,³,⁵,⁶

¹Dept. of Physiology, ²Dept. of Biochemistry and Medical Genetics, ³Regenerative Medicine Program, ⁴Dept. of Pathology, ⁵Manitoba Institute of Child Health, ⁶University of Manitoba, Winnipeg, MB

**Introduction:** In the field of cancer stem cell research, there is a generally accepted principle that cells demonstrating a higher self-renewal capacity *in vitro* exhibit larger and faster tumor growth, increased tumor initiating capacity (TIC) and decreased survival *in vivo*. However, a handful of papers have revealed a contradictory correlation between these characteristics in genetic mouse models of the adult brain cancer glioblastoma (GBM). Despite these findings, this relationship has not been directly evaluated using xenograft models of human brain tumors. We have set out to investigate this relationship using multiple human brain tumor cell lines.

**Methods:** We have isolated two sub-clones from a medulloblastoma cell line, a childhood form of brain cancer, which demonstrate higher and lower self-renewal *in vitro* as well as two glioblastoma cell lines that also demonstrate differential self-renewal. We have evaluated the tumor initiating capacity, self-renewal, survival and tumor aggressiveness *in vivo* using an intracerebral xenograft model in non-obese diabetic severe combined immunodeficient (NOD SCID) mice.

**Results:** Our results demonstrate that cells with a lower self-renewal capacity *in vitro*, when injected into the frontal cortex of NOD SCID mice, result in a shorter survival and increased tumor grade, when compared to cells displaying a higher self-renewal capacity. In addition, no difference in the tumor initiating capacity and self-renewal *in vivo* was seen. MRI imaging and histological analysis has revealed these two cellular populations form very distinct tumor phenotypes with distinguishing clinical presentations.

**Conclusion:** We have established that cells exhibiting increased self-renewal *in vitro* do not always translate to an increased tumorigenic potential and decreased survival *in vivo*. This translation of *in vitro* findings to *in vivo* is a vital step in the process of drug discovery and the design of new targeted-treatment strategies. One must therefore question the utility of this measure of self-renewal and the translation of its findings when going from the controlled environment of a dish to the complex milieu of the mouse.
**Background:** Despite the effectiveness of Doxorubicin (Dox) as an anti-cancer drug, the dose dependent cardiotoxic side effects are serious life threatening concerns associated with its use. In addition to the oxidative stress, there is increasing evidence of involvement of nitrosative stress in Dox-induced cardiotoxicity. Previously, Vitamin C (Vit C) has been shown to reduce oxidative stress and improved survival of Dox-treated cardiomyocytes. However there is no evidence on the effect of Vit C on nitrosative stress. The objective of this study was to investigate the effect of Vit C on nitrosative stress in Dox treated cardiomyocytes.

**Methods and Results:** Cardiomyocytes isolated from adult Sprague-Dawley rats were treated with predetermined doses of Vit C (25 µM), Dox (10 µM) or Vit C (25 µM) + Dox (10 µM) for 24 hours. Dox treatment increased levels of nitrate in cardiomyocytes as well as released in media leading to increased production of peroxynitrite. Vit C significantly reduced the levels of cellular and released nitrate, cellular peroxynitrite as well as the nitrosylation of proteins caused by Dox. Dox induced increase in Nitric oxide synthase (NOS) activity in cardiomyocytes was blunted by Vit C treatment. Western blot analysis revealed an upregulation of inducible NOS (iNOS) and phosphorylation of inhibitory site of endothelial NOS (eNOS) by Dox. In contrast Vit C downregulated iNOS and phosphorylated activating site of eNOS. Dox induced activation of apoptosis was prevented by Vit C.

**Conclusion:** These results provide a hope and rationale for use of Vit C as an adjuvant therapy to mitigate cardiotoxic side effects by attenuating oxidative and nitrosative stress caused by administration of Dox.
Introduction: Semaphorin-3E (Sema-3E) is a member of a large family of secreted and membrane-bound proteins that play important roles in many biological processes. Sema-3E interacts with its receptor (plexins and neurophilins) on immune cell such as dendritic cells (DC) and neutrophils. Natural Killer (NK) cells are members of the emerging family of the innate lymphoid cells that play important roles in innate immunity and tissue remodeling. The interaction of NK and DC (crosstalk) is bi-directional, involving multiple cytokine signals and direct cell-cell contacts. Efficient recruitment of NK cells to peripheral organs or inflamed lymph nodes via direct or indirect interaction with DC is therefore essential in NK cell-mediated immune-surveillance, and in coordinating adaptive immune responses against cancers and infections.

Rationale and Hypothesis: Sema-3E/Plexin D1 regulates trafficking of DC during inflammatory conditions, and the secretion of IL-12 in DC. The role of Sema-3E in NK biology has not been reported. We therefore hypothesized that Sema-3E is a novel factor that regulates NK cell function(s) in NK-DC crosstalk.

Methods: NK cells were isolated from spleens of Sema-3E +/+ or -/- animals. They were activated in IL-2 for 4 days before the experiments. Immature DC were generated from bone marrow cells under GM-CSF. Mature DC were prepared by stimulating immature DC with LPS overnight. Phenotypes of NK and DC were analyzed by FACS. NK-cell migrations were examined in the Trans-well assays.

Results: I examined RNA expressions of Plexin D1 and/or Sema-3E in primary NK and DC in RT-PCR. I observed that DC, resting or IL-2 activated NK cells expressed Plexin D1 transcripts. Sema-3E transcripts, however, were detected only in immature DC, but not LPS-stimulated mature DC and NK cells. To examine whether Sema-3E expression in immature DC regulated activated NK-cell migrations in vitro, I used IL-2 activated NK cells from inbred BALB/c and conditioned medium from immature or LPS-stimulated mature DC (derived from either Sema-3E+/+ or Sema-3E-/- animals) in the Trans-well assays. As reported previously, I observed that conditioned medium of the mature Sema-3E +/- DC promoted a stronger
chemotaxis of IL-2 activated NK cells than that of the immature DC. There was no significant difference in the chemotactic ability of the activated NK cells to the conditioned medium of the LPS-stimulated DC of either Sema-3E +/+ or Sema-3E -/- . However, activated NK cells exhibited a two-fold increase in the chemotactic migrations toward the conditioned medium of the immature Sema-3E -/- DC, when compared to that of the Sema-3E +/+ immature DC. Such increase in NK-cell migrations was suppressed when recombinant Sema-3E proteins (at 50 ng/ml, final concentration) was added to the conditioned medium from the Sema-3E -/- immature DC.

**Conclusions:** My data revealed a novel role of Sema-3E in suppressing NK-cell migrations in vitro. The tight regulation of Sema-3E expression in mature and immature DC suggested further that Sema-3E is a critical factor that regulates NK cell functions in NK-DC crosstalk.
Neuregulin-1, New Therapeutic Approach to Moderate Glial Scarring and Neuroinflammation Following Spinal Cord Injury

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Introduction: Reactive astrogliosis and neuroinflammation are key pathophysiological events after spinal cord injury (SCI). Activated astrocytes and microglia secrete a myriad of pro-inflammatory cytokines, nitric oxide (NO) and inhibitory extracellular matrix components including chondroitin sulphate proteoglycans (CSPGs) that cause neurotoxicity and impede tissue repair and regeneration. Our recent evidence suggests that drastic downregulation of Nrg-1 after SCI may influence astrocytes reactivity and neuroinflammatory response following injury. Here, using complementary in vitro and in vivo approaches, we demonstrate that Nrg-1 availability mitigates multiple detrimental consequences of reactive astrogliosis and neuroinflammation in SCI.

Methods: In an in vivo rat model of compressive SCI recombinant human, Nrg-1β1 (rhNrg-1β1) was delivered intrathecally at the time of SCI using mini-osmotic pumps. We analyzed SCI tissues using western blotting and stereology-based immunohistology at different intervals after SCI. For in vitro studies, we used a primary mixed culture of rat astrocytes and microglia activated using lipopolysaccharide (LPS) or transforming growth factor-beta (TGF-β). Nrg-1 or vehicle was added to the cultures of normal and activated astrocytes and microglia cultures. Conditioned media (CM) and cell lysate were collected and analyzed using immunocytochemistry, enzymatic assays, Western and slot blotting to assess cellular and molecular characteristics of astrocyte and microglial reactivity including NO, CSPGs and pro-inflammatory cytokines.

Results: We report that Nrg-1 treatment significantly attenuates several inhibitory and toxic aspects of reactive astrogliosis and neuroinflammation including CSPGs and proinflammatory cytokines (TNF-α and IL-1β) in CM of LPS-activated glia. Additionally, Nrg-1 activation can mitigate oxidative stress by reducing NO production by glial cells. Moreover, Nrg-1 availability attenuated cell proliferation and nestin upregulation, two cellular characteristics of reactive astrogliosis. Importantly, in vivo administration of Nrg-1 following SCI reduced CSPG production, scar formation and several aspects of neuroinflammation including TNF, IL-1β and MMP-9 release.

Conclusion: Our work provides novel insights into the mechanisms of astrogliosis and neuroinflammation following injury and suggests a positive role for Nrg-1 in ameliorating the outcomes of SCI.
Mevalonate Cascade Inhibition by Simvastatin Induces Farnesyl Pyrophosphate and Geranylgeranyl Pyrophosphate-Dependent Cell Death in Different Tumor Cells

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Background: Hydroxymethylglutaryl coenzyme A reductase (HMGCR) is the rate-limiting enzyme of mevalonate (MVA) cascade which can be inhibited by statins. HMGCR reduces the 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) to MVA and that can be utilized for the synthesis of some downstream products like cholesterol and isoprenoids intermediates. In the present study, we aimed at investigating the cell death mechanisms induced by MVA cascade inhibition through simvastatin in different tumor cell lines.

Methods: A wide range of tumor cells were treated with different concentrations of simvastatin (0-20 uM) in different time points (0-120 hrs) including human glioma cell lines (U87, U251), human breast adenocarcinoma cancer cell lines (MCF-7, MDA-MB-231), and human non-small lung cancer cell lines (A549, H460, H1650, H1975). MTT assay was used to determine the cell viability in each time point and also propidium iodide-based FACS assay was performed for the confirmation of apoptotic cell death. We also co-treated these cell lines with simvastatin and mevalonate (2.5, 5 mM), cholesterol (25, 50 uM), farnesyl pyrophosphate (FPP) (7.5, 15 uM), and geranylgeranyl pyrophosphate (GGPP) (7.5, 15 uM) to determine which downstream product of MVA induces cell death following simvastatin exposure in different cancer cells.

Results: According to our results, simvastatin (>5 uM) significantly (P<0.05) induced cell death (>10%) in all tumor cells (MCF-7, MDA-MB-231, U87, U251, A549, H460, H1975, H1650) after 48 hrs of treatment. Interestingly, MDA-MB-231 and U251 cells were more sensitive to simvastatin treatment compared to other tumor cells (P<0.01). Our experiments showed that simvastatin is able to induce significant apoptotic cell death (>10%) in all tumor cells which were used in this study (P<0.05). We observed that mevalonate, FPP, and GGPP significantly inhibited simvastatin-induced cell death in the all mentioned cells, while cholesterol did not significantly inhibit simvastatin-induced cell death in none of these cell lines (P<0.01).

Conclusion: Our results highlighted that simvastatin induces cell death in a broad range of tumor cells. Also, we found that simvastatin-induced cell death is not dependent on cholesterol depletion, but rather it is related to small Rho GTPase protein (FPP, and GGPP dependent) in all cell lines.
Dose Dependent Negative Regulation of NK Cell Migration in Granulocyte Macrophage-Colony Stimulating Factor (GM-CSF) Gradients

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Background: Natural Killer (NK) cells play a key role in innate immunity against viral, microbial infections and transformed cells. Impaired NK-cell trafficking or function attributes disease or cancer progression. Experimental results show specific chemokines and cytokines produced in physiological or pathological environments affect the NK-cell receptor expression level and their cytotoxic function. One such cytokine GM-CSF has its relevance in NK-Dendritic cells cross talk and cancer progression. Here we examine the role of GM-CSF on NK-cell migration.

Methods: “Y” shape microfluidic platform was used to generate stable GM-CSF gradient to study the role of GM-CSF on NK-cell migration. Human NK-cells were used for this migration study and immunostaining analyses were done on the gradient exposed cells to understand the involving mechanism.

Results: Our results demonstrated a surprising dose-dependent role of GM-CSF in regulating different NK-cell migration behavior under a stable GM-CSF gradient. At lower concentrations (100 pg/ml), GM-CSF induced cell elongation, immediate arrest of NK-cells, and little/or no NK-cell migrations. At higher GM-CSF concentration (20 ng/ml), NK-cells migrated away from the gradient (repulsive migration), followed by subsequent arrest in cell migration at a later time point. Addition of neutralizing antibody against GM-CSF-Rα abolished only the repulsive migration but not the cell elongation and arrest suggesting putative cell-arrest mechanism independent of GM-CSF-Rα. In addition, immunofluorescence analysis revealed up-regulation of activated lymphocyte factor associated antigen-1 (LFA-1) in the arrested cells which is correlated with cell migration arrest in other studies. Collectively, our current results revealed an unexpected dual role of GM-CSF in the regulation of NK-cell migrations. Future analyses will elucidate the mechanisms and physiological relevance underlying such regulation of these NK-cell migratory properties by GM-CSF.

Significance: My current work revealed novel regulatory functions of GM-CSF on NK-cells. Such properties may provide an evasion mechanism of cancerous cells to evade NK-cell surveillance.
An Epigenetic Study for Autism Spectrum Disorders Using Twins
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Introduction: Autism spectrum disorders (ASD) are neurodevelopmental disorders that are characterized by impairments in social communication and interactions, as well as restricted and repetitive behaviours, activities, and interests. Epigenetic factors are implicated in ASD based on the observation that ASD symptoms are present in some Mendelian disorders that are known to be related to epigenetic factors (e.g. Rett syndrome). Epigenetic mechanisms, such as DNA methylation, alter gene expression levels without changing the DNA sequence. The ideal research subjects for epigenetic studies are twins because they share similar prenatal and postnatal environments and are the same age; identical (monozygotic) twins also share the same genetic background, but can have different epigenetic profiles. Using twins, especially monozygotic twins, allows researchers to separate epigenetic effects from genetic and environmental factors.

To date, two groups have examined DNA methylation profiles in monozygotic twins discordant for ASD, revealing many chromosomal regions where differential DNA methylation levels were associated with ASD. Due to the small sample sizes that are inherent in twin studies, additional investigations are required to build upon these findings.

Methods: We recruited seven same-sex twin pairs with at least one twin diagnosed with ASD. Blood and buccal cell samples were collected from each twin. Using the Illumina HumanMethylation450 BeadChip, genome-wide DNA methylation profiles were generated for both the buccal and mononuclear blood cells. Comparisons of DNA methylation levels within and among the twin pairs, as well as between the cell types, will be presented. We expect to identify epigenetic risk factors for ASD. This knowledge will contribute to the development of efficient diagnostic and treatment methods for ASD in the future.
Expression Analysis of the Glycolysis Pathway Genes in HIV Highly Exposed yet Seronegative Commercial Sex Workers, Nairobi, Kenya

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Introduction: Cellular metabolism regulates immune cell functions and differentiation, thereby affecting immune response. Activated T cells have increased metabolic requirements. Aerobic glycolysis is required by these proliferating T cells to meet the metabolic needs, specifically for effector function, which involves optimal cytokine production. Specific effector functions are unable to proceed without the cell utilizing the appropriate metabolic state. It is important to understand how the glycolysis genes and enzymes are engaged, and how they can influence effector function.

Altered susceptibility to HIV-1 infection has been observed in multiple cohorts worldwide. There are individuals who are continuously exposed to HIV-1 yet remain uninfected. Through global whole blood gene expression analysis, energy metabolism pathways conducted in this cohort showed differential expression in HIV-1 exposed yet seronegative CSWs.

Methods: Study population was drawn from the Pumwani Sex Worker Cohort, Nairobi. Study groups included: HIV highly exposed yet seronegative (HESN) CSWs (>7 years); newly enrolled HIV-uninfected (<7 years); HIV-uninfected, lowly-exposed antenatal clinic attendees (low risk group) (n=14 each). Total RNA was extracted from PBMCs using Trizol (Invitrogen, USA), cDNA synthesized and relative mRNA expression determined using SYBR Green by quantitative real time PCR. mRNA expression of the 10 glycolysis genes was observed, with assays normalized using 18s rRNA gene.

Results: There was a significant difference between HESNs and newly enrolled HIV uninfected CSWs at the beginning of the pathway with expression lowest in HESNs. (Hexokinase-1 p<0.0323; Phosphoglucose-isomerase p<0.0477) There was also a significant difference between HESNs and the low risk group in phosphogluco-isomerase expression. (p<0.0348) Significant differences were observed at the end of the pathway with Phosphoglycerate mutase, Enolase-1 and Pyruvate kinase genes.

Conclusion: Significantly lower mRNA expression of 2 genes was observed in HESNs when compared to their uninfected yet susceptible counterparts. Following studies of enzyme expression and glucose uptake studies are underway to understand the role of glycolysis in HIV resistance.
Evaluating and Characterizing KIF11 as a Chromosome Instability (CIN) Gene
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**Introduction:** Chromosome Instability (CIN) is defined as an increase in the rate at which whole chromosomes or large parts are gained or lost. CIN is not only associated with virtually all tumor types, but it is associated with aggressive tumors, the acquisition of multi-drug resistance and consequently poor patient prognosis. Despite these associations, the genes and molecular defects that contribute to CIN are only poorly understood. Recently, we performed a high content screen that identified KIF11, a microtubule associated motor protein, as a candidate CIN gene.

**Methods:** Here, we couple RNAi-based gene silencing with biochemistry and cell biology to show that diminished KIF11 expression is associated with CIN. KIF11 was silenced with either individual or pooled siRNA duplexes and expression levels were evaluated by Western blots. Aberrant microtubule formation, particularly during mitosis, was examined by indirect immunofluorescence (IIF). Standard fluorescence microscopy was employed to evaluate changes in nuclear sizes and micronucleus formation following silencing, which are phenotypes frequently associated with CIN. To further evaluate CIN, KIF11 or controls were silenced, and chromosomes were manually enumerated from a minimum of 100 spreads per condition.

**Results:** KIF11 was successfully silenced and the two most efficient silencing duplexes were identified by Western blot and used in all subsequent experiments. Digital imaging microscopy revealed an increase in the number of mitotic cells 24 hours post transfection that were confirmed to have aberrant microtubule structures (spindles). Additionally, fluorescence microscopy revealed increases in nuclei sizes and micronucleus formation, that corresponded with an increase in CIN as measured by increases in chromosome complements within mitotic chromosome spreads derived from KIF11 silenced cells.

**Conclusion:** Our preliminary data indicate that diminished KIF11 expression is associated with increases in phenotypes frequently associated with CIN. They further suggest that KIF11 may be a CIN gene that normally functions to maintain chromosome stability specifically during mitosis.
Role of Sema3E in the Pathogenesis of Lipopolysaccharide induced Experimental Shock
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Introduction: Sepsis is one of the major clinical problems in Canada, which claims around 9300 deaths annually. Sepsis is a systemic inflammatory response to bacterial infection (mostly gram negative) associated with alteration of microvasculature wall integrity, eventually leading to hyperperfusion, cellular infiltration, tissue injury, ischemia and organ dysfunction. Sema3E is an axon guidance protein secreted from neurons and it is known to have antimigratory, antiproliferative and antiangiogenic effects. On the other hand, recently it has been reported that it also has a function as proinflammatory protein. However, the role of semaphorin3E (sema3E) in lipopolysaccharide (LPS) induced endotoxic shock is yet to be evaluated.

Objective: The objective of this study is to investigate the role of sema3E in LPS-induced septic shock in mouse model using sema3E gene deletion and recombinant sema3E treatment approaches.

Methods: Sema3E knockout (KO) and wild type (WT) mice were injected with sub-lethal dose of LPS (5mg/kg, i.p.) followed by eight hours observation for clinical signs and rectal temperature. Animals were sacrificed after eight hours to collect organs/tissues and lavages. Immune cells phenotyping was done in organ/tissues and lavages using flow cytometry. Cytokines and chemokines levels in serum and lavages were estimated by ELISA.

Results: LPS administration caused an elevation of proinflammatory cytokine levels in serum and peritoneal lavage in WT mice, which were significantly suppressed in sema3E KO mice. In addition, Sema3E gene deletion significantly inhibited LPS induced inducible nitric oxide synthase (iNOS) expression in peritoneal macrophages and also effectively reduced monocyte expansion in blood. Sema3E KO mice exhibited low levels of macrophage chemoattractant protein -1 (MCP-1) in peritoneal lavage and serum, as compared to wild type littermates. LPS produced significantly lesser hypothermia in sema3E KO mice when compared to WT mice.

Conclusion: These results suggest that sema3E gene deletion has protective effect against LPS-induced systemic inflammation and sema3E may be an emergent therapeutic target against septic shock. Further studies are in process to confirm the observed effects.
Role of Reelin Signaling Pathway in the Corticogenesis of the Cerebellar Cortex in Lysosomal Acid Phosphatase (Acp2) Mutant Mice

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Introduction: A mouse mutant called nax (naked-ataxia); resulting from a spontaneous mutation in Lysosomal Acid Phosphatase (Acp2) gene shows severe cerebellar defects and neuronal degeneration in its cerebellum. Cerebellum is a brain region important for motor control, cognition, and language processing. In the Acp2 mutant mouse, three layer cortex (Granule cells (gcs), Purkinje cells (Pcs) and Molecular layer) were found to decrease significantly and monolayer Pcs turn to multi-layered Pcs that ectopically invade the molecular layer. Reelin is an important large extra cellular signaling protein important in Pcs monolayer formation in cerebellar cortex. It is expressed by gcs and is required for Pcs distribution from the clusteric stage to establish a monolayer of Pcs between the molecular and granular layers of the cerebellar cortex. We hypothesize that the establishment of mono layered Pcs is independent to the Reelin pathway, however it has a migratory role in corticogenesis.

Materials and methods: Acp2 mutant mice were used for this study and molecular expression and distribution were assessed by immunohistochemistry and Western blotting.

Results: The cerebellar cortex of the Acp2 mutant mice which was characterized by the absence of the vermis, reveals the presence of Pcs in a randomized, dispersed manner spanning the entire molecular layer rather than a monolayer in the cerebellar cortex. The pattern of Reelin expression shows a down-regulation in both wild type and nax mouse, while lower amount of protein is detected in nax mutant at Postnatal day 4 (around Pc layer formation) compared to wild type.

Conclusion: Pcs differentiation is severely delayed in the Acp2 mutant cerebellar cortex while the presence of Reelin is comparable with wild type during early postnatal development. It is indicative of Reelin effect during clustric stages however failed to form mono layer Pcs. It is concluded that multilayer Pcs may be due to the failure of appropriate cross-talk between Acp2 and the Reelin signalling pathway during early postnatal cerebellar development.
Role of Hypoxia-Inducible Factor-1 in Poly (ADP-Ribose) Polymerase-1-Induced Bnip3 Expression and Mitochondrial Dysfunction

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Introduction: Excessive pathophysiological activity of the nuclear poly(ADP-ribose) polymerase-1 (PARP-1) causes neuron death in brain ischemia/hypoxia by inducing mitochondrial permeability transition and nuclear translocation of apoptosis-inducing factor (AIF) leading to caspase-independent neuron death. Bcl2-/adenovirus E1B 19KDa-interacting protein (Bnip3) is a mitochondrial pro-apoptotic Bcl-2 protein family member that is induced in hypoxia and has effects on mitochondrial permeability and neuronal death. Hypoxia-inducible factor-1 α (HIF-1α) is a key regulator for hypoxic transcription of Bnip3 by binding to hypoxia response element (HRE) in Bnip3 promoter and could be inactivated by SIRT-1 by deacetylation. Previously, we have confirmed that hypoxia dependent PARP-1 overactivation causes depletion of nicotinamide adenine dinucleotide (NAD⁺) and inhibition of NAD⁺-dependent SIRT1. The objective of the present study was to define the role of HIF-1α in PARP-1 mediated Bnip3 expression in hypoxia.

Methods: Cortical neurons cultures undergo RT-PCR and Western blotting analysis to determine levels of HIF1-α mRNA and proteins respectively in presence and absence of PARP-1. Co-immunoprecipitation and anti-acetyl-lysine are performed to measure the acetylated-lysine HIF1-α protein level in non-treated and hypoxia-treated cells (0% oxygen) in presence and absence of PARP-1. Silencing of HIF-1α using a lentiviral short hairpin RNA is used to determine whether Bnip3 expression is HIF-1 α dependent. Chromatin immunoprecipitation is performed to determine whether HIF-1 α binds hypoxia response element (HRE) upstream Bnip3 promoter and enhances its expression.

Results: HIF-1α mRNA transcripts and protein levels are PARP-1-independent. However, we see relatively higher HIF-1α acetylation in hypoxia treated PARP⁺/+ mouse neuron cultures compared to untreated PARP⁺/+ as well as to PARP⁻/-. Silencing of HIF-1α using a lentiviral short hairpin RNA reduced hypoxic increases in Bnip3 transcription. Further work is required to show whether HIF-1α driving Bnip3 promoter activity is PARP-1 dependent.

Conclusion: Together, these data illustrate a direct PARP-1 mediated hypoxic signaling pathway where PARP-1 mediated NAD⁺ depletion leads to HIF-1 α hyperacetylation and increases HIF-1-mediated Bnip3 transcription.
Hip Position Alters Pelvic Floor Muscle Activation in Women With and Without Stress Urinary Incontinence

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**Introduction:** Because of the anatomical associations between the hip and pelvic floor musculature, hip position may influence pelvic floor muscle (PFM) relaxation or activation. The purpose of this study was to determine if tonic or phasic PFM activation was altered in continent and stress incontinent women when they assumed different hip positions.

**Methods:** Women with mild and moderate stress urinary incontinence (SUI) and healthy controls were recruited using posters. Electromyography (EMG) was recorded using intravaginal differential suction electrodes located over the PFMs while participants remained at rest (tonic activation) and when they performed maximal voluntary contractions (MVCs) in lying, with the hips in different flexion (0, 60, 90 degrees flexion) and rotation (neutral, medial and lateral) positions. Separate two-way repeated measures analyses of variance were used to determine differences in peak smoothed EMG amplitudes recorded between the groups and across the different positions for each task (alpha=0.05).

**Results:** Sixteen continent women, 14 women with mild SUI and 11 women with moderate SUI participated. In all groups, tonic activation was highest when the hips were extended compared to when they were flexed to 60 or 90 degrees (p<0.05). With the hips in full extension medial and lateral rotation enhanced tonic activation in all groups (p<0.001). For the MVC data, there were significant posture by group interactions (p<0.001), therefore the data were analyzed separately by group. Compared to continent women and those with mild SUI, the women with moderate SUI generated lower activation amplitudes in all postures (p<0.05). PFM MVCs were enhanced with hip position in the continent group, where women demonstrated higher PFM EMG activation with the hips flexed to 60 degrees (p<0.001) and extended and medially rotated (p=0.02).

**Conclusion:** Changes in hip position produced significant differences in tonic and phasic PFM EMG amplitudes in women however women with and without SUI did not demonstrate consistent behaviours. Differences may be associated with alterations in loading responses of the tissues and/or motor control strategies used to compensate for altered muscle function. Treatment programs may explore differences in PFM tonic or phasic activation associated with changes in hip position.
Investigating the Mechanism of Action of the ZMAB Antibody Cocktail

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Until recently, there were very limited options for the treatment of Ebola virus infections. The treatments consisted mostly of supportive care, such as fluid administration, or experimental approaches aimed at remedying the various abnormalities caused by the infection. In 2012, three different groups showed the feasibility of using oligo- or polyclonal antibodies as a post-exposure treatment regimen. One of the three approaches used the ZMAb antibody cocktail, composed of three monoclonal antibodies (1H3, 2G4, and 4G7). Here we investigate the effector mechanisms which contribute to mediating the protection provided by ZMAb in mice. We also compare the potential of the mouse and chimeric antibodies to trigger antibody-dependent cell-mediated cytotoxicity (ADCC) in vitro using human effector cells. Overall, our results suggest that, in mice, NK cells and the FcγRI and III as well as the complement component C3 are not required for protection using the ZMAb treatment.
The Development and Refinement of a Brief Online Intervention for Parents to Prevent Childhood Obesity in Primary Care

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Introduction: Up to one-third of Canadian children meet the clinical definitions of overweight and obesity. These data have informed a growing interest in applying eHealth approaches to prevent unhealthy weight gain in children, especially in settings that families access regularly. Our objective was to systematically develop and refine a screening, brief intervention and referral to treatment (SBIRT) eHealth tool for parents to prevent childhood obesity in primary care.

Methods: Our SBIRT, the Resource Information Program for Parents on Lifestyle and Education (RIPPLE), was developed through a partnership between our research team and Evolution Health© (www.evolutionhs.com). RIPPLE was based on existing SBIRT models and literature regarding children's lifestyle habits. Subsequently, RIPPLE was refined based on feedback received through focus groups that described participants’ perceptions of the intervention. Five focus groups (6–10 participants/group) were conducted with health care professionals (n=17), parents (n=10), researchers (n=9), and administrators (n=2); focus groups were recorded using a court reporter, and data were analyzed using thematic analysis.

Results: Two central themes emerged from analysis: intervention strengths and weaknesses. Overall, participants viewed RIPPLE as a novel opportunity to enhance parents’ awareness of their children’s obesity-related behaviors. Participants thought RIPPLE was a practical, well-designed tool that may facilitate the prevention of obesity in primary care. They also believed the intervention may elicit negative reactions from parents, and specific elements (e.g., weight-related terminology) need to be improved.

Conclusion: RIPPLE may enhance parents’ awareness of their children’s healthy lifestyle behaviors, but improvements were recommended prior to implementation. Findings from this research will directly inform revisions to the intervention, which will undergo testing in a randomized controlled trial to commence later in 2015.
**First Nations Youth Perspectives on the Physical Activity-Environment Link: A Photovoice Project**

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**Introduction:** Several studies have demonstrated the benefits of physical activity (PA) for First Nations youth (Critchley et al., 2006; Tighe & McKay, 2012). It is also well-known that environmental factors like land-use mix play a critical role in the quality of physical activity (PA) opportunities available to youth (Ding et al., 2011). While the PA-environment link in urban centres is well understood, there has never been an exploration of the unique relationship First Nations youth have with their environment and how it might influence PA. The purpose of this study was to explore the PA-environment link with First Nations youth to inform the development of a culturally relevant conceptual model of this relationship that will be used to create a PA environment assessment tool for First Nations communities.

**Methods:** Using the principles of community based participatory research (CBPR) and Two-Eyed Seeing, First Nations youth (n=14) were trained in the art of Photovoice to take photos of the PA environment. Photovoice is a method that utilizes photos to capture community expertise and experience on a particular issue. Next, the youth were asked to join a talking circle to share their experience of PA opportunities in their community. Talking circles were analyzed for themes using NVivo.

**Results:** Youth co-researchers indicated that the First Nations community environment influences their PA through 1) Policy, 2) Community and Cultural Events, 3) Infrastructure, 4) Natural Environment, 5) Social Environment, 6) Transportation, 7) PA Programs, and 8) Relations. A separate theme, labelled 9) Personal Attributes, encompasses individual characteristics that youth co-researchers noted during talking circles. Informed by the socioecological model, a conceptual model demonstrating how youth PA is influenced by the environment was created. This model will be used to develop a PA environment assessment tool.

**Conclusion:** This study was the first to explore the PA-environment link in First Nations communities from a First Nations youth perspective in order to develop a culturally relevant PA environment assessment tool. This new tool will be used to strengthen PA efforts and create culturally relevant opportunities once validated. Future research is needed to refine, test, and validate tool items.
Slug Contributes to Endocrine Therapy Resistance in Breast Cancer Cell Lines


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Background: Breast cancer is one of the most common female cancers, accounting for about 10.4% of all malignancies. During the development and progression of breast cancer, there are two important signaling pathways, estrogen receptor-α (ERα)-mediated transcriptional activation or inhibition and snail family zinc finger protein 2-E-cadherin-epithelial mesenchymal transformation (Slug-E-cadherin-EMT), establishing cross-signaling pathway through ERα and Slug. ERα was previously demonstrated to down-regulate Slug and thus suppress EMT. Recently, Slug has been shown to directly regulate ERα signaling pathway, contributing to cancer progression.

Methods and Results: By RT-PCR and Western blotting, we observed a negative correlation between Slug and ERα expression at both mRNA and protein levels in breast cancer cell lines. Knockdown of Slug by siRNA in ERα (-) MDA-MB231 cells up-regulated ERα expression; while overexpression of Slug in ERα (+) MCF-7 cells down-regulated ERα mRNA and protein expression. In chromatin immune-precipitation (ChIP) assay, we further demonstrated that Slug bound to the E-boxes of the ERα promoter, not only in MDA-MB231 but also in over-expressed Slug cell lines MCF-7/Slug. Slug bound strongly to the E-box located at about 2400 bp upstream of the ERα promoter. The Immunofluorescence staining revealed an inverse correlation between Slug and ERα nuclear staining. As ERα functions as the marker for endocrine therapy of breast cancer and endocrine therapy resistance is associated with poor prognosis, we explored the role of Slug in endocrine therapy of breast cancer. Knockdown of Slug by siRNA in MDA-MB231 cells increased the sensibility to tamoxifen treatment via restoring ERα expression, while overexpression of Slug in MCF-7 cells decreased the sensibility to endocrine therapy such as tamoxifen.

Conclusion: Slug inhibits ERα expression and affects the sensibility to endocrine therapy. This study may provide a novel insight into endocrine therapy resistance in breast cancer and eventually contribute to breast cancer survival.
Intraprofessional Nurse Collaboration: An Exploration of Professionalism and Collegiality among Registered Nurses and Practical Nurses

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**Background:** Patient safety is fundamental to nursing care and the healthcare system. Poor teamwork and communication failures among healthcare personnel, such as registered nurses and practical nurses, are the primary causes of medical errors in Canada. When patients suffer preventable harm or adverse events, it results in significant human costs and increased financial demands on the Canadian healthcare system, including unnecessary treatments and longer hospital stays. Although registered nurses and practical nurses work closely together in most healthcare settings, little is known about how they work together or how they can work better together.

**Objectives:** This research seeks to examine what knowledge registered nurses and practical nurses currently have about each others’ roles, responsibilities, and scopes of practice; and how they feel about working together. It will also identify what are the contributing and impeding factors that influence the development of effective registered nurse and practical nurse collaborative relationships in providing safe, patient-centred care.

**Methods:** In the first phase of this research, a systematic review of the current literature on registered nurse and practical nurse collaboration will be completed. In the second phase, an institutional ethnographic approach will be used to further explore the professional relationships between registered nurses and practical nurses. The complexity of the issues involved necessitates that an interdisciplinary approach be used that considers this topic from the perspective of several different disciplines, including business, education, nursing, and sociology.

**Results:** Pending.

**Significance:** This research will help nurse educators identify the barriers and enablers to collaboration in order to teach baccalaureate and practical nursing students about how to work collaboratively in their future clinical practice. It will also enable both registered nurses and practical nurses to better coordinate the care they provide and understand their respective roles and contributions to patient care.
Defining Molecular Mechanisms Linking Endophilin A2 to Metastasis in Human Breast Cancer Models

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Introduction: Breast cancers in human epidermal growth factor receptor 2 (HER2) and triple-negative breast cancer (TNBC) subtypes have high rates of tumour metastasis. This is driven by cells undergoing Epithelial-Mesenchymal Transition (EMT), and subsequent formation of invadopodia that degrade extracellular matrix (ECM) in basement membranes to spread locally and colonize distant sites. Endophilin A2 (Endo II) is an adaptor protein that coordinates internalization and trafficking of extracellular proteins. We have recently identified Endo II as a positive regulator of human TNBC metastasis, invadopodia formation, Membrane-Type 1 Matrix Metalloproteinase (MT1-MMP) endocytosis, and Epidermal Growth Factor Receptor (EGFR) internalization and signaling. Here, we extend these studies to HER2 breast cancers, and define molecular mechanisms that link Endo II to these processes.

Methods: Endo II expression was profiled by immunohistochemistry in human breast tumour tissue microarrays. Stable silencing of Endo II was achieved using lentiviral shRNAs to knockdown (KD) Endo II in HER2 and TNBC cell lines. Confocal and TIRF microscopy were used to visualize endocytosis effects in cancer cells. Mammary orthotopic xenograft assays tested effects of Endo II KD on EMT and tumour metastasis.

Results: Analysis of Endo II expression in human invasive ductal carcinomas revealed significantly higher expression of Endo II in HER2 lymph node metastases. High levels of Endo II mRNA were associated with reduced rates of relapse free survival in patients with invasive tumours. Mechanistically, this may be explained by inefficient internalization and trafficking in Endo II KD cells. These results are extended to mammary orthotopic tumour xenograft assays to further dissect the contributions of Endo II to metastatic tumour progression.

Conclusion: These findings identify Endo II as a potential poor prognosis biomarker in HER2 and TNBC. This research could open new avenues for targeted therapy focusing on endocytosis of receptors that drive metastasis.
Introduction: Pentraxin-3 (PTX3) is a member of the long pentraxins family. It activates the innate and adaptive immune systems, playing an important role in providing immunity against various pulmonary infections. Considering its role in fostering lung immunity, we examined the role of PTX3 in asthma. We recently demonstrated enhanced expression of PTX3 in bronchial biopsies of allergic asthmatics that correlated with disease severity. In this report, we assessed the effect of PTX3 deficiency in a murine model of OVA-induced asthma.

Methods: Allergic inflammation was induced by OVA administration in WT and PTX3 Knockout (KO) mice on C57Bl/6/129Sv/Ev. Flexivent was used to determine airway resistance. Airway and lung inflammation was determined in BALF and tissue by cell counting, FACS, ELISA and H&E staining. mRNA levels of mucus, collagen, fibronectin, actin genes were assessed by real time PCR. Serum immunoglobulins were measured by ELISA.

Result: Sensitized PTX3 KO mice exhibited an enhanced airway resistance in response to methacholine (MCh) in contrast to their WT counterparts. We observed an increased inflammatory cell infiltration in BALF obtained from PTX3 KO mice upon OVA sensitization/challenge as compared to their WT littermates. Airway remodelling and goblet cells hyperplasia was also found to be enhanced in allergic PTX3 KO mice as compared to their WT counterparts. Further we found an enhanced induction of Th2 cytokine production in the lungs of PTX3 KO mice as compared to WT mice upon OVA challenge. Allergic PTX3 KO mice showed enhanced OVA-specific IgE production.

Conclusion: Taken together, we conclude that lack of PTX3 predisposes mice to airway hyperresponsiveness and an enhanced inflammation.
Access Barriers to Primary Health Care: Indigenous People and the Role of the Physician Assistant in Northern Manitoba

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Introduction: This report discusses health inequities in Manitoba’s Northern Indigenous people and the relationship between access barriers to Primary Health Care and poor health outcomes. Accessible and quality primary care has a direct impact on health outcomes. Important stakeholders have yet to devise a formal framework that addresses access barriers to primary care for northern communities. This report offers a framework to assist in the development of a Northern Care Network with the overall goal of decreasing barriers and fostering independent, strong, and healthy communities in Northern Manitoba.

Methods: Qualitative data was collected utilizing: Internet search engine, Statistics Canada, Manitoba Bureau of Statistics, Health Canada, Manitoba Health, Northern Health Region of Manitoba, University of Manitoba-Faculty of Medicine-Office of Physician Assistant Studies-Aboriginal Affairs, and the Canadian Association of Physician Assistants.

Results: Northern Indigenous people suffer from some of the worst health inequities in Manitoba. The primary health care delivery model throughout Manitoba and the Northern Health Region is inefficient, fragmented and difficult to comprehend. Access barriers to Primary Health Care such as inadequate supply of health care providers exist, as well as many others, and result in negative health outcomes. While recent health indicators in Manitoba are encouraging, and progression towards a more interdisciplinary and inclusive primary health care delivery model is evident, a framework has yet to be formulated. This report discusses the NUKA System of Care and the role of the Physician Assistants as key elements in the development of a framework called the Northern Health Care Network. The NUKA system of care was implemented in Alaska and has resulted in statistically significant decreases in acute and chronic disease as well as improved access to rural and remote communities. Physician Assistants have been available to urban centers in the US and Canada since the 1960’s and the early 2000’s, respectively, but are not currently accessible to Northern and Remote communities in Manitoba. The Northern Health Care Network proposed in this report is an interdisciplinary, collaborative, and innovative approach that will decrease access barriers to primary care in northern and remote Manitoba and help foster independent, strong, and healthy communities.
Mediation of Inflammation Induced Early Protease Activation in Knee Joint Explants through Depo-Medrol Intervention


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**Purpose:** Severe injuries to the knee joint, such as anterior cruciate ligament (ACL) tears and/or meniscal damage, often results in accelerated development of osteoarthritis (OA). Clinical evidence suggests that inflamed synovium and fat pad can add to adjacent articular cartilage damage. Following ACL rupture and/or surgery, the synovium and fat pad exhibit increased mRNA levels for inflammatory and degradative markers. We tested the hypothesis that early inhibition of inflammation was essential for suppressing the resulting upregulation of degradative proteinases. Methylprednisolone acetate (MPA; Depo-Medrol®, Pfizer) is a corticosteroid that is commonly clinically used for mitigating inflammation in many chronic inflammatory diseases. The present study evaluated the efficacy of using MPA for suppression of inflammation and consequently the degradative proteases in the synovium and fat pad tissue.

**Methods:** Hind limbs from immature female sheep were obtained following sacrifice and explants were harvested from the knee joint. The explants were equilibrated in an incubator overnight in serum free medium. The explants were equilibrated and allocated into four groups a) media control, b) MPA treatment ($10^{-7}$uM), c) inflammation (IL-1β), and d) inflammation followed by inhibition with MPA (IL-1β hours + MPA). All explants were harvested at 48 hours and snap frozen in liquid nitrogen and later used for matrix metalloproteinase (MMP)-1, MMP-3, and MMP13 mRNA analysis using real time qPCR. Also, explants from each limb were tested for cellular viability. ANOVA with Holm-Sidak’s post-hoc analysis was used to determine differences in mRNA expression between groups, using Prism 6 Graph Pad software.

**Results:** The mRNA expression levels for MMP-3 and MMP-13 were suppressed in fat pad tissue when treated with MPA following induction of inflammation ($p<0.05$). There were similar trends in synovium tissue for MMP-3 and MMP-13, however not significant potentially due to considerable animal variation. Interestingly, MMP-1 exhibited a trend in this pattern of mRNA suppression with MPA in both the fat pad and synovium tissue, although significance was not noted.

**Conclusion:** Early intervention with $10^{-7}$uM MPA was successful in blocking inflammation and suppressing some degradative proteinases such as MMP-3 and MMP-13 after induction of inflammation in select tissues of the knee joint.
Respiratory Plasticity Following Exposure to Acute Intermittent Hypoxia is Not Caused by Inflammation in Healthy Humans

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Introduction: Ventilatory instability is a fundamental component of obstructive sleep apnea (OSA) pathogenesis. A key component of ventilatory stability is enhanced ventilatory chemosensitivity, with OSA patients having augmented acute hypoxic (AHVR) and hypercapnic (AHCVR) ventilatory responses. The enhanced chemosensitivity in OSA is thought to result from exposure to intermittent hypoxia (IH), but the molecular pathway is poorly understood. IH-induced oxidative stress leading to inflammation is postulated to contribute to AHVR enhancement although the role of inflammation in IH-induced respiratory changes in humans has not been examined. Employing an experimental human model of IH previously shown to increase oxidative stress and the AHVR, this study assessed the role of inflammation in IH-induced respiratory plasticity.

Methods: In a double-blind, placebo-controlled, randomized, crossover study 12 healthy males underwent 6 hours of IH. For 4 days before each IH exposure, participants ingested either 100mg lactose placebo po tid, or the non-steroidal anti-inflammatory drugs indomethacin (non-selective cyclooxygenase (COX) inhibitor; 50mg po tid) or celecoxib (selective cyclooxygenase-2 inhibitor; 200mg po bid). Pre- and post-IH AHVR and AHCVR were assessed.

Results: Pre-IH, the AHVR and AHCVR were similar across all conditions (placebo, and indomethacin and celecoxib; p≥0.093). IH increased the AHVR within all drug conditions (Placebo: 2.0±0.3 vs 1.5±0.2 L/min/% desaturation, p=0.011; Indomethacin: 1.8±0.3 vs 1.4±0.2 L/min/% desaturation, p=0.026; and Celecoxib: 1.9±0.3 vs 1.4±0.2, p=0.018). The increase in AHVR was similar across all conditions (p=0.827). Post-IH, the AHCVR was increased (p=0.003) within only the celecoxib condition.

Conclusion: Inflammation does not appear to contribute to the IH-induced AHVR enhancement following an acute (6 hour) exposure. In contrast, selective COX-2 inhibition augmented the AHCVR following acute IH exposure. With respect to OSA, these findings suggest selective COX-2 inhibition could potentially exacerbate OSA severity by increasing ventilatory instability.
Pharmacological Chaperones of the Dopamine Transporter for the Treatment of Dopamine Transporter Deficiency Syndrome

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**Background:** Hereditary dopamine transporter deficiency syndrome (DDS) is a recently described rare pediatric condition caused by loss-of-function mutations in the dopamine transporter (DAT). The disorder is characterized by parkinsonism-dystonia and raised CSF levels of dopamine metabolites. When expressed *in vitro*, the DAT missense mutations result in reduction or elimination of dopamine uptake as well as preventing DAT protein maturation. Pharmacological chaperoning, the use of small molecules to selectively bind and improve folding/maturation of a protein of interest, is an approach that has been used previously to rescue misfolding mutations leading to cystic fibrosis and diabetes. We propose that the DDS causing mutations result in ER retention of an otherwise functional DAT, which could be rescued by using pharmacological chaperones.

**Methods:** Compounds that increased surface expression of WT DAT in HEK-293 cells were identified using a β-lactamase-reporter assay, after which effects on DAT protein and function were assessed using western blotting and a dopamine uptake assay respectively. Subsequently compounds that could increase WT DAT protein and function were tested on a well-characterized ER-retained DAT mutant and several clinically observed DDS mutants. *In vivo* studies were then conducted on heterozygous DAT-knockout (DAT-HET, basal DAT levels 50% of DAT in WT mice) mice, which were treated daily with a putative pharmacological chaperone for a period of two weeks. Locomotor response to an amphetamine challenge was measured after which DAT protein levels were assessed by performing western blotting on striatal tissue lysates.

**Results:** We tested a number of known DAT ligands and have identified bupropion and ibogaine as compounds that can promote maturation of both WT and mutant DAT *in vitro*, as well as being able to rescue select DDS mutants. Furthermore, we examined the effect of bupropion *in vivo* and our data show that sub-chronic (2-week) treatment can increase amphetamine response and striatal DAT protein in DAT-HET mice.

**Discussion:** Our data suggest that bupropion and ibogaine are pharmacological chaperones of DAT and can rescue DDS mutants. These compounds could be used as a potential treatment to rescue DAT function in patients with DDS, a condition for which there is currently no treatment.
Structural Analysis of the Hybrid Sensor Kinase PA1611 Involved in Lifestyle Selection in Pseudomonas aeruginosa

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Background: Over 95% of children with CF demonstrate either serologic or culture based evidence of Pseudomonas aeruginosa infection by the age of 3 years. Successful adaptation of pseudomonas to host environment relies on tight regulation of its gene expression mediated by repertoire of two-component [TC] regulatory systems. The regulatory pathway however, is still unclear. Previously, we showed PA1611, a novel hybrid sensor kinase, regulates the choice between acute and chronic states through interaction with RetS, another hybrid sensor kinase-response regulatory protein.

Objectives: In this study, we investigated the structural basis of the interaction between PA1611 and RetS and identified PA1611 residues critical in such interactions by both molecular modeling and site-directed mutagenesis.

Methods and Results: Amino acid replacements guided by molecular models were carried out at 7 key positions in PA1611. Functional analysis of the mutants was then carried out in P. aeruginosa PAO1, and the mutants were characterized for T3SS expression (exoS promoter activity), T3SS effector protein secretion, motility, biofilm formation and exopolysaccharide production. The residues Phe269, Glu276 and Thr279 located within the Histidine kinase A and Histidine kinase-like ATPase domains of PA1611 were found to play crucial roles in the interaction between PA1611 and RetS. Alanine replacements of these mutants showed significantly decreased interaction between PA1611 and RetS. Increased exoS expression, higher swarming and swimming motility, decreased exopolysaccharide production and lower biofilm formation was observed for F269A, E276A, and T279A mutants. Conservative replacements at these positions, F269Y, E276D and T279S showed wild type PA1611 characteristics. Interestingly, functional characterization of the Y453A mutant showed increased interaction between PA1611 and RetS, while the conservative substitution Y453F showed wild type PA1611 phenotype. We further demonstrate that these mutations in PA1611 function through GacS/GacA-RsmY/Z signaling pathway.

Conclusions: It has been demonstrated that adaptation of P. aeruginosa to the CF airway is a result of adaptive mutations. There is increasing evidence now to support the role of key regulatory systems in
adaptation and colonization, however, how much they contribute or the exact regulatory pathways are still unclear. PA1611 has emerged as an important lifestyle regulator in *Pseudomonas*. Our finding that mutations within PA1611 affect the regulatory pathway significantly defines a naturally occurring mechanism for inverse regulation of acute and chronic virulence markers during infection.
Layer-By-Layer Nanocoating of Mesenchymal Stromal Cells is Feasible
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**Introduction:** Mesenchymal stromal cells (MSC) are a fibroblast-like cell population with multilineage differentiation capacity and immunosuppressive potential in response to inflammatory signals. Therapeutic MSC strategies have been limited by rapid clearance and poor homing when infused systemically with only a minority of MSC migrating to the site of injury/inflammation. This research project investigates the ability to coat MSC using layer by layer (LBL) technology with natural extracellular matrix components and to prime MSC with entrapped bioactive molecules with the goal of improving homing and function of therapeutic MSC.

**Methods:** The LBL methodology was optimized for concentration of biomaterials used (gelatin and hyaluronic acid), incubation of coating time, temperature and washing steps. To confirm the presence of nanocoating over the cells, immunofluorescence and flow cytometry was performed. After achieving the optimal LBL cell coating method, the cell morphology, cell yield, viability by 7AAD and Annexin V, was analyzed with and without the nanocoating. The cell surface molecules of the nanolayered and conventional MSC were also analyzed by flow cytometry.

**Results:** The presence of biomaterials over the cells demonstrated that the nanocoating successfully coated the MSC. Nanocoating did not change cell morphology or loss of cell number or viability. The phenotype of MSC was not changed after nanocoating, with over 95% expression of CD73, CD90 and CD105, and less than 5% expression of CD34, CD14 and CD45.

**Conclusion:** In this proof of principle study we demonstrate that nanocoating of MSC is feasible and is potentially a tool to target licensing/homing of MSC for therapeutic use. Next steps are to test whether nanocoating will increase the survival and function of MSC *in vivo* thereby improving homing potential and augmenting immunosuppressive function.
Evaluation of Antioxidant Capacity, Total Phenolics and Anthocyanins in Lingonberries (Vaccinium vitis-idaea) from Northern Manitoba and Newfoundland
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**Introduction:** Lingonberries (Vaccinium vitis-idaea) are edible fruit that belong to the Ericaceae plant family. A growing body of evidence suggests that consumption of berries provide considerable health benefits due to their high polyphenols, antioxidants, vitamins and mineral content. Therefore, it is beneficial to evaluate the antioxidant capacity of berries in order to select lines with greater contents. The aim of this study was to determine the antioxidant capacity, total phenolics and total anthocyanins of lingonberries from Northern Manitoba and Newfoundland.

**Methods:** A total of 159 lingonberry samples were collected from Northern Manitoba (Lynn Lake and Flin Flon) and Newfoundland. Samples were freeze dried, lyophilized, ground into powder and extracted with solvent methanol. In our study, total anthocyanins was determined by the pH differential method. Total phenolics was evaluated by the Folin-Ciocalteau’s assay and the oxygen radical absorbance capacity (ORAC) assay was utilized to evaluate the antioxidant capacity.

**Results:** Our study shows that Northern Manitoba grown lingonberries contain a higher level of anthocyanin compared to Newfoundland grown lingonberries. Total phenolic content for Northern Manitoba grown lingonberries was higher compared to Newfoundland grown berries. Likewise, antioxidant capacity was also higher for Manitoba grown lingonberries when compared to those from Newfoundland.

**Conclusion:** Results show that Northern Manitoba grown lingonberries have higher antioxidant capacity compared to ones grown in Newfoundland. This suggests that berries grown in different climatic conditions have different antioxidant capacity, total phenolics and anthocyanins; that is, growth in more extreme climates result in berries with higher antioxidant capacity, total phenolics and anthocyanins. Thus, the stronger antioxidant capacity of Northern Manitoba lingonberries may translate to greater health benefits.
Introduction: Using the Concept of Positive Deviance to Identify Harm Reduction Strategies Associated with Group Sex Events among Gay and Bisexual Men in Vancouver’s Momentum Health Study is a CIHR funded project involving community members, health care providers and researchers exploring the emic care practices of gay/bisexual men within the high-risk sub-culture of sexual private sex parties (PSE). By viewing gay/bisexual men as harm reduction experts this study seeks to understand the risks and rewards of group sex, poly-substance use, and the bio-cultural implications of PSE.

Methods: The Momentum Health study used Respondent Driven Sampling to obtain a sample of gay/bisexual men in Greater Vancouver, BC. Eligible participants completed a computer-assisted questionnaire to delineate the psychosocial profiles, substance use and sexual behaviour patterns of group sex attendees in comparison to participants who did not attend group sex events in the past 6 months. Positive Deviance is a concept applied to People Who Use Injection Drugs to understand how long-term users protect themselves from HIV/HEPC infection. Next, we will conduct 20 in-depth narrative interviews detailing grassroots prevention tactics for both HIV infected and uninfected Momentum participants to identify possible additional indigenous prevention tactics, norms and beliefs used by gay men within the sub-culture of PSE.

Results: Based on initial quantitative analysis of 719 Momentum Health Study respondents 25% of gay/bisexual men attend group sex events representing a distinctive gay sub-culture. The study identified a significant statistical association between the number of recent male sexual partners and group sex attendance, high-risk sexual behaviour including rimming and fisting, and illicit substance use.

Conclusions: This project positions gay and bisexual men as pleasure seeking self care experts willing to partner with researchers and other community leaders with the potential to guide future directions for gay/bisexual men’s health and community wellbeing.
Molecular Signatures of Immune Activation and Epithelial Barrier Remodelling are Enhanced During the Luteal Phase of the Menstrual Cycle: Implications for HIV Susceptibility

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Introduction: Variable infectivity and transmissibility of HIV/SHIV has been linked to the menstrual cycle, with particular susceptibility linked to the luteal phase, but the mechanism is poorly understood. Here, we performed an unbiased, mass spectrometry-based proteomic analysis to better understand the mucosal immunological processes underpinning this observed susceptibility to HIV infection.

Methods: Cervicovaginal lavage samples (n=19) were collected, characterized as follicular or luteal phase using days since last menstrual period, and analyzed by tandem-mass spectrometry. Biological insights from these data were gained using a spectrum of computational methods, including hierarchical clustering, pathway analysis, gene set enrichment analysis, and partial least-squares discriminant analysis with LASSO feature selection.

Results: Of the 384 proteins identified, 39 were differentially abundant between phases (p<0.05, ≥ 2 fold change). Cell-cell adhesion proteins and antiproteases were reduced, and leukocyte recruitment (IL-8 pathway, p=1.49E-5) and extravasation proteins (p=5.93E-4) were elevated during the luteal phase. LASSO/PLSDA identified a minimal profile of 18 proteins that distinguished the luteal phase. This profile included cytoskeletal elements and proteases, known to be involved in cellular movement. Gene set enrichment analysis associated CD4+ T cell and neutrophil gene set signatures with the luteal phase (p<0.05).

Conclusion: Taken all together, our findings indicate a strong association between proteases, factors involved in tissue remodelling, and leukocyte infiltration in the luteal phase which may represent potential hormone-associated mechanisms of increased susceptibility to HIV in young women.
Dual Role for NLRP3 in Enterohemorrhagic *Escherichia coli* induced Inflammation and Kidney Injury

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**Introduction:** Enterohemorrhagic *Escherichia coli* (EHEC) is a pathogen that causes hemorrhagic colitis and a severe kidney complication called hemolytic uremic syndrome (EHEC-HUS). EHEC produce shiga toxins (Stx) which activate cell stress responses causing inflammation and cell death. NLRP3 is an innate pattern recognition receptor, which senses a host of microbial products. In macrophages, activation of NLRP3 causes recruitment of proteolytic proteins to an intracellular complex termed the ‘inflammasome,’ which through caspase-1 mediates the secretion of proinflammatory cytokine IL-1β and cell death. Clinical studies have demonstrated elevated IL-1β in the serum of HUS patients. Therefore we hypothesized NLRPβ regulates Stx-induced inflammation and kidney injury.

**Methods & Results:** Human monocytic cell line THP1 cells were differentiated into macrophages and treated with Stx. Treatment with Stx resulted in caspase-1 activation, secretion of IL-1β, and cell death (measured by Western blot, ELISA, and a lactate dehydrogenase assay) that was abolished with the use of NLRP3 and caspase-1 inhibitors. Immunofluorescent labeling of NLRP3 and ASC in Stx treated macrophages also revealed inflammasome activation. NLRP3⁻/⁻ THP1 cells generated using CRISPR-Cas9 technology abolished caspase-1 activation, inflammasome formation, IL-1β secretion, and cell death following treatment with Stx. Renal tubular epithelial cells (TEC) underwent cell death after exposure to Stx as visualized by real time live cell imaging of Annexin-V and propidium iodide labeled cells. Analysis of cell death mediators by Western blot revealed Stx induced caspase-8, 7, 3, and PARP activation in renal TEC, which was attenuated in NLRP3⁻/⁻ TEC.

**Conclusion:** Our studies have established a dual role for NLRP3 that drives inflammation and tissue injury in EHEC-HUS. In macrophages, Stx activates the NLRP3 inflammasome leading to secretion of proinflammatory IL-1β and cell death. In addition, exposure to Stx induced NLRP3 mediated cytotoxicity in renal TEC resulting in apoptosis. As inflammation and tissue injury may regulate the severity and propagation of EHEC infection resulting in HUS, therapeutic strategies that target NLRP3 may be beneficial in reducing morbidity and mortality associated with this and other infectious diseases.
Objective: Physical activity is in decline in childhood, and it is thought that this is due to a decline in children’s active play. Play is a basic right of every child and research into this area is growing. However, little is known about the parental-level predictors of children’s active play. Accordingly, the objective of this project was to examine the association between parental perception of neighborhood safety and children’s frequency of outdoor active play.

Methods: Participants were 514 dyads of 8-11 year old children and one of their parents/guardians. Parents completed a web-based survey that assessed how frequently their child engaged in outdoor active play in 7 locations, their perception of the safety of their home neighbourhood, and several covariates. Factor analysis was used to condense the 18 neighbourhood safety questions into the following four factors: safe for children, unsafe roads, traffic calming, and fear of crime. General linear models were used to examine the association between the 4 safety factors and the frequency of outdoor active play while adjusting for covariates.

Results: The safe for children factor [β(SE) = 4.9(0.5), p<0.0001] and fear of crime factor [β(SE) = 2.6(0.5), p<0.0001] were associated with outdoor active play and explained 34% of the variability in this behaviour. Unsafe roads and traffic calming factors were not associated with outdoor active play (p>0.10). Of the 18 neighbourhood safety questions, the question “Lots of children play or hang out in our street” was the most strongly associated with outdoor active play [β(SE) = 3.1(0.3), p<0.0001, R²=0.22].

Conclusion: Parental perceptions of neighbourhood safety, particularly their perception of whether their neighbourhood was a safe place for children, was independently associated with children’s active outdoor play.
Ubiquitin Ligase Huwe1 Modulates Male Germ Cell Development by Regulating Meiotic Progression
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Introduction: Spermatogenesis involves crucial, highly regulated transitions between developmental programs such as mitotic proliferation, meiosis and differentiation. How these switches are regulated remains to be delineated. The ubiquitin proteasome system plays a significant role in protein turnover and cellular remodeling and may be involved in these transitions in spermatogenesis. We previously identified the ubiquitin ligase Huwe1 in the testis and showed that it shuttles from the cytoplasm to nucleus as gonocytes transition to spermatogonia. In addition inactivating Huwe1 in gonocytes results in a delay in their mitotic re-entry and leads to spermatogonial depletion. Here we examined the role of Huwe1 in spermatogonial differentiation, meiotic entry and progression.

Methods and Results: We inactivated it in differentiating spermatogonia by expressing Cre recombinase using the Stra8 promoter. Huwe1−/− males (KO) were subfertile siring 33% smaller litters compared to the Huwe1floX/Y (WT). The average testes weight of adult KO was only 30% of the WT with sperm concentration being 76% lower. Morphological analysis of adult testis revealed a heterogeneous phenotype with tubules displaying fewer spermatocytes and spermatids. TUNEL assay showed increased levels of apoptosis in a majority of the tubules. Since we observed fewer spermatocytes, we looked at meiotic progression more closely. While Q-PCR analysis of markers of early meiosis (Spo11, Mei1) did not show any significant alterations, markers of sex chromosome inactivation (Ube1x, Atp7A, Gla) failed to be inactivated in the KO. In addition, chromosome analysis using SCP3 or γH2AX (markers of meiotic progression) of surface spreads prepared from 28 day-old mice revealed severe degeneration of spermatocytes in the KO (2.4% WT vs. 53.3% KO) with the percentage of zygotene and pachytene spermatocytes falling in the KO by 90% and 95% respectively. Defects in meiosis were further confirmed using WIN 18,446/retinoic acid to synchronize the tubules.

Conclusion: Collectively, these results indicate a crucial role of Huwe1 in regulating meiotic progression.
NAMPT Inhibition Induces Mitochondrial Dysfunction Leading To Apoptosis in Chronic Lymphocytic Leukemia Cells


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Introduction: Chronic Lymphocytic Leukemia (CLL) is the most common blood cancer in the western world and affects 8/100,000 Manitobans. Despite recent advances in understanding its biology and emerging new treatments, the disease remains incurable. As such, novel approaches are required. FK866 is a small molecule inhibitor of Nicotinamide phosphorybosyltransferase (NAMPT) an enzyme overexpressed in CLL cells and thought to contribute to cell survival, rapid proliferation and resistance to chemotherapeutics.

We have previously shown that FK866 selectively targets CLL cells, causing NAD depletion and leading to cell death characterised by ATP depletion, loss of mitochondrial membrane potential, cytochrome C release and caspase activation. We aim to further characterise the effects of FK866 and NAD depletion on CLL mitochondrial respiration and the downstream pathway leading to cell death.

Hypothesis: FK866 induced NAD depletion leads to metabolic dysfunction which can be exploited to develop effective therapeutic strategies for CLL.

Methods: CLL cells or control B-lymphocytes were isolated from blood samples and cultured with 10 and 25 nM FK866 or DMSO (vehicle control) up to 48 hours. Caspase cleavage was measured by western blot analysis and Caspase3/7-glo assay (Promega). Mitochondrial metabolism was assessed by extracellular flux (XF) analysis using a XF24 analyser (Seahorse Bioscience). Blue native polyacrylamide gel electrophoresis (BN-PAGE) was used to detect mitochondrial respiratory complexes and supercomplexes. Complex activity was determined by in-gel activity assays.

Results: FK866-induced loss of cellular viability was not rescued by caspase inhibition. Cytochrome C and apoptosis-inducing factor (AIF) release from mitochondria were induced by FK866 both with and without caspase inhibition. Extracellular flux analysis revealed reduced mitochondrial respiratory capacity as early as 24 hours after FK866 treatment, leading to suppression of basal mitochondrial respiration by 48 hours. Altered respiratory supercomplex formation was demonstrated by BN-PAGE and differentially expressed structures were identified as supercomplexes of respiratory chain complex I.
Conclusion: FK866-treatment leads to mitochondrial NADH depletion and inhibition of mitochondrial respiration. The resulting ATP depletion leads to activation of the intrinsic apoptosis pathway as previously described.

Future Directions: The roles of glycolytic inhibition and mitochondrial NAD insufficiency in NADH depletion will be investigated by XF analysis.
Background: Multiple sclerosis (MS), a disease of the central nervous system, is the leading cause of neurological disability among young Canadian adults. Its presentation varies widely from person to person, with symptoms ranging from mild sensory alterations to severe disability limiting activity and restricting participation in life’s roles. In 2008, semi-qualitative interviews were performed to identify what matters to 190 people living with MS and their quality of life (QoL) as part of a larger investigation of gender differences in MS (Kuspinar, 2013). 62 domains were identified, many of which are rarely addressed by health care professionals. A need for a global approach targeting QoL through those domains was identified.

Self-management is a lifetime task where patients are coached to maintain wellness in their foreground perspective, rather than illness, through development of five core skills: problem solving, decision making, resource utilization, forming patient/health care provider partnership, taking action. To put those skills to use, a person needs to gain the ability to self-assess, identify and implement strategies to improve and monitor progress. The following presents the process behind the development of a global self-management intervention called the Getting on With Your Life With MS (GETONMS©) workbook.

Methods: Using the 62 domains identified, a literature review, and discussions with MS clinicians and people living with MS, 40 worksheet topics were identify for inclusion in GETONMS©. A template was created to allow for all self-management skills to be represented in each worksheet. Pages, in an appropriate language level, were developed by content experts and adapted to fit the template before being sent to people with MS for feedback.

Each worksheet was reviewed by two of the four women who volunteered to give feedback. The worksheets produced a variety of comments. In addition to grammar and wording issues, a small number of content problems or omissions were identified and addressed. The pages stimulated sharing of experiences that were later taken into account when designing vignettes. Overall, many statements expressed approval and excitement over the content, intention of implementing strategies, and anticipation for the final product.
Team meetings were held to review every worksheet before being submission for graphic layout to the company Earthlore Communications.

**Evaluation strategies:** Every section of the workbook will be peer reviewed by MS experts, using a web-based survey. Next, longitudinal qualitative interviews will be performed in people with MS to explore the usability of GETONMS® as well as how its use may affect quality of life. Finally, a randomized controlled trial is planned at the end of next year to evaluate the effectiveness of the workbook.
High Content Analysis Identifies Kinetin as a New Potential Modulator of Huntingtin N17 Phosphorylation

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Huntington’s disease (HD) is a fatal neurodegenerative disorder caused by a polyglutamine tract expansion in the huntingtin protein, which interferes with its normal biological functions and causes cellular toxicity. An important modulator of this toxicity is the phosphorylation state of two critical serine residues, 13 and 16, in the N17 region of the huntingtin protein. Mutant polyglutamine-expanded huntingtin is hypophosphorylated, and restoration of this phosphorylation in N17 can completely prevent, or dramatically reverse disease in mouse models of HD.

We conducted a high content analysis screen in STHdhQ7/Q7 cells on a natural compound library with the readout being immunofluorescence using an N17 phospho-antibody. Wells were imaged automatically and scored using a non-supervised, machine-based algorithm. From this screen, we identified compounds that were effective at modulating huntingtin phosphorylation state or localization. One of these natural products is kinetin, a plant-derived cytokine. Recent pharmacodynamic studies by others have defined that this compound is metabolized to an ATP analog that can act as a “neo-substrate” for phosphate donation to a kinase. Early data from our own lab indicates that kinetin can increase phosphorylation of N17 in both STHdhQ7/Q7 and STHdhQ111/Q111 but has a more pronounced effect on the mutant cells. We have also found kinetin to be cytoprotective in striatal cells under some stress conditions.

The discovery of the efficacy of this compound on N17 phosphorylation is promising and presents a proof-of-principle that restoring phosphorylation of huntingtin in N17 is valid therapeutic target, as well as the power of high content analysis of specific targets in the huntingtin protein coupled to chemical biology studies.
An Optogenetic Approach reveals Presynaptic Mechanism of Seizure Termination
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**Introduction:** Seizures are characterized by pathological rhythms which terminate spontaneously after a period of low complexity repetitive bursting. Identifying the mechanism through which this pathological network excitation ends is of crucial importance to improve current drug targets and for the development of unique therapies for seizure prevention. An analysis of brief inter-ictal bursts in the hippocampus showed that reduced synaptic strength due to depleted glutamate release from recurrent excitatory synapses is the mechanism which terminates each burst. The present study investigates whether the termination of neocortical seizures also entails depleted presynaptic excitatory neurotransmitter release.

**Methods:** An optogenetic approach allows for targeted activation of a subpopulation of neurons. Layer V pyramidal neurons expressing high levels of the functional ChR2 cation channel were activated by brief depolarizing light pulses while spontaneous ictal events, using a magnesium free seizure model, were recorded in vitro from layers II/III of the mouse somatosensory cortex. Local field potentials and whole cell recordings from pyramidal neurons of layers II/III were made. The cells were voltage clamped at -70mV to isolate excitatory postsynaptic currents. Short duration pressure puffs of hypertonic sucrose solution was applied using the local field electrode at a distance of 20-30μm from the patched cell to discern possible correlation between presynaptic readily releasable vesicle pool size and the optogenetically evoked excitatory post-synaptic currents (EPSCs).

**Results:** During the final stages of the repetitive bursting prior to seizure termination, a ~60% decrease in the light evoked response was observed. This lasted well into the post-ictal period. At seizure termination, the EPSCs induced by hypertonic sucrose application showed a comparable drop in activity which lasted the same post-ictal duration as the optical evoked EPSCs.

**Conclusion:** Targeting a specific neuronal population using light has identified a circuit affected at seizure termination, namely the layer V to layers II/III pyramidal cell network. These results in conjunction with a focal test of vesicular glutamate release are in line with the hypothesis that a presynaptic mechanism is involved in seizure termination.
Increased Prevalence of Gastrochisis in Northern Manitoba
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Gastrochisis is a congenital defect of the abdominal wall that results in prolapsed bowel or other intra-abdominal organs with no covering and a normal umbilicus. Previous research has reported an increase in prevalence of gastrochisis worldwide in addition to areas of significantly increased prevalence or hot spots. The authors proposed that the prevalence of gastrochisis is significantly higher in the Northern Manitoba region as compared to the rest of the province and country. Cases of gastrochisis were identified from the national birth defect registries unique to the Northern Manitoba region. Prevalence of gastrochisis was calculated and compared to national prevalence rates. It was found that there is in fact an increased prevalence of gastrochisis in the Northern Manitoba region. As a variety of risk factors have been identified as being plausible mechanisms causing gastrochisis, the authors of this study hope to further investigate this population and analyze specific maternal characteristics that may explain these results.
Cognitive Compensation in the Context of a Challenging Postural Task

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Introduction: Epidemiological research indicates a link between age-related hearing loss and mobility decline among older adults (Viljanen, et al., 2009). Our proposed explanation is that cognitive compensation occurs in both speech perception and postural tasks (Li & Lindenberger, 2002). Therefore, simultaneous listening and balancing might be more challenging for older adults with hearing loss. The aim of the current study was to investigate this hypothesis experimentally by presenting auditory stimuli in a dual-task balancing paradigm.

Methods: Healthy young adults (n = 29), older adults (n = 25) and older adults with hearing loss (n = 10) were administered baseline measures of neuropsychological, psychosocial and sensory/sensorimotor functioning. Participants then completed cognitive (modified n-back) and balance (balancing on a moving platform) tasks singly (A) and concurrently (B) in an ABA order. This design was repeated under both noisy and quiet conditions where the addition of background noise (multi-talker babble) was used to simulate age-related hearing loss.

Results: A significant interaction of age group (younger, older), attention (single, dual) and listening difficulty (quiet, noisy) indicated that older adults and older adults with hearing loss had lower accuracy on the cognitive task in dual noisy conditions when compared with younger adults. The kinematic parameter of total displacement indicated a main effect of group, whereby younger and older adults showed greater total displacement than older adults with hearing loss in all conditions.

Conclusion: Together, the results suggest that dual-tasking has a negative effect on auditory-cognitive performance and that older adults both with and without hearing loss exhibit a postural prioritization response as a means of protecting balance. Therefore, intervention techniques focused on dual-task training might prove beneficial in improving mobility.
Dynamics of Actin-based Structures in Migrating Artery Myocytes are regulated by Phosphodiesterases

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Introduction: The second messenger, cAMP, regulates vascular myocyte migration dichotomously. Indeed, while cAMP inhibits vascular myocyte migration in vitro and reduces intimal hyperplasia in vivo, it also promotes formation of integrin-based, actin-rich leading edge projections in these cells; structures which are essential for migration. Our study was undertaken to examine if distinct cyclic nucleotide phosphodiesterases (PDEs) could modulate formation of such actin-rich projections in migrating vascular myocytes.

Methods: Visualization and quantification of leading edge, actin-rich structures was assessed in primary human internal mammary artery vascular myocytes by subjecting these cells to a modified Boyden Chamber Assay. The cells were placed on the upper level of gelatin-coated (0.25%) cell culture inserts (3-μm pores) and subsequently stimulated by chemotaxis by adding 0.5% FBS to the underside of the inserts in which the cells were allowed to extend projections for 4 hours. PDE enzymes were inhibited with the use of selective PDE family inhibitors and knockdown of these enzymes was assessed through the use of siRNA.

Results: In the absence of adenylyl cyclase activation, formation of leading edge structures was promoted when either PDE1, or PDE4, were selectively inhibited using C-33 or Ro, 20-1724, respectively. PDE3 selective inhibition with cilostamide was without effect. In marked contrast, when added with forskolin, an adenylyl cyclase activator, PDE1, PDE3 or PDE4 inhibitors each potentiated the ability of forskolin to reduce formation of the actin-based structures. Knockdown of PDE1C or PDE4D, recapitulated effects observed with the enzyme inhibitors both in the absence and presence of forskolin.

Conclusion: These data are consistent with the notion that localized cAMP “pools” regulated by PDE1 and/or PDE4 enzymes at the leading edges of migrating cells can promote formation of actin-based adhesive structures, but that these events are poorly supported in cells in which cAMP is globally increased.
The Cytoplasmic Domain of the *Pseudomonas Aeruginosa* Protein FimV Modulates Virulence Phenotypes by Regulating CAMP Synthesis

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**Introduction:** Type IV pili (T4P) are a major virulence factor of *Pseudomonas aeruginosa*, responsible for surface adhesion and movement by twitching motility. T4P and other virulence factors are positively regulated by cAMP produced by the adenylate cyclase, CyaB. A screen for activators of CyaB uncovered FimV, an inner membrane protein required for T4P assembly. FimV has a periplasmic peptidoglycan-binding domain involved in multimerization of the outer membrane T4P secretin (PilQ), and two cytoplasmic tetratricopeptide repeat (TPR) motifs potentially involved in protein-protein interactions. Here, we clarify the cAMP-dependent and independent functions of FimV, and define the corresponding functional domains.

**Results:** A mutant lacking FimV’s peptidoglycan-binding LysM motif (fimV_{LysM}) had increased cAMP compared to a fimV deletion mutant, but both were non-motile, suggesting that peptidoglycan binding is required for motility, not cAMP synthesis. Complementation of fimV or fimV cyaB* (which has constitutively high levels of cAMP) with cytoplasmic domains TPR1 and/or TPR2, did not restore twitching or surface piliation, despite restoration of PilMNOP and PilQ levels by the cyaB* mutation. fimV_{LysM} had wild-type levels of PilU, PilMNOP, and PilQ, suggesting normal cAMP synthesis. Deletion of fimV in mutants lacking negative regulators of CyaB activity, which typically have high intracellular cAMP, resulted in low levels of cAMP, suggesting that FimV functions upstream of CyaB and its regulators.

**Conclusions:** The entire cytoplasmic domain of FimV is required for regulation of CyaB. Expression of PilMNOP and PilQ is a cAMP-dependent, not FimV-specific phenotype, but twitching requires both cAMP synthesis and FimV’s peptidoglycan-binding function. Our data show that regulators of CyaB depend on FimV, positioning it as a central coordination hub for virulence in *P. aeruginosa*. 
Protective Role of Relaxin Ligand-Receptor System in Glioblastoma
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Introduction: Glioblastoma (GB) is a highly aggressive brain cancer associated with poor patient outcomes. The DNA alkylating drug temozolomide (TMZ) is the standard treatment for GB along with radiation therapy. However, TMZ chemoresistance often occurs resulting in fatal recurrence events. Here, we describe a novel ligand-receptor interaction and its role in protecting GB cells through activation of the Relaxin Family Peptide Receptor 1 (RXFP1). RXFP1 is a G-protein coupled receptor (GPCR) activated under physiological conditions by its cognate ligand relaxin-2, an entity not expressed in GB.

Methods: Immunofluorescence, Caspase 3/7 apoptosis assay, Western blot analysis, siRNA RXFP1 treatment, PCR, cell culture

Results: RXFP1 transcripts, along with transcripts for the RXFP1-activating ligand CTRP8, were detected in 17/18 patient GB cells. RXFP1 activation decreased the incidence of double strand DNA breaks and inhibited caspase 3/7 mediated apoptosis induced by TMZ treatment, thereby improving GB cell survival. To understand the mechanism of RXFP1-mediated chemoresistance in GB cells, we looked for aberrant expression status of signaling molecules implicated either in cell survival and/or DNA damage repair. RXFP1 activation by CTRP8 was found to up-regulate p-STAT3, a protein ubiquitously expressed in many cancers with corresponding functions in cell survival and chemoresistance. CTRP8-mediated RXFP1 activation also up-regulated the base excision repair (BER) molecule N-methylpurine DNA glycosylase (MPG). Attenuation of RXFP1 activity reduced expression of both p-STAT3 and MPG, indicating that this signaling is RXFP1 dependent.

Future Work: Future studies will focus on further elucidating the signaling pathway that links RXFP1 to BER-mediated chemoresistance, including determining if MPG up-regulation is dependent on p-STAT3 signaling. We will also determine STAT-3 regulated genes in the context of RXFP1 signaling.

Conclusion: These results demonstrate a critical new role for RXFP1 signaling in GB chemoresistance through modulating BER in response to TMZ.
Intervention with Naringenin Enhances Weight Loss, Potentiates Improvements in Metabolic Dysregulation and Halts Progression of Atherosclerosis Induced By A High-Fat Diet in Ldlr-/− Mice.

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Introduction: Previous studies demonstrate that the addition of naringenin, a grapefruit flavonoid, to a high-fat diet prevents the development of many disorders of the metabolic syndrome and atherosclerosis in Ldlr-/− mice. Furthermore, in intervention studies, the addition of naringenin to a high-fat, high-cholesterol (HFHC) diet reversed pre-established obesity, hyperlipidemia, hepatic steatosis, insulin resistance and improved atherosclerotic lesion pathology, but not lesion size. In the present intervention study, we tested the hypothesis that addition of naringenin to a chow diet would further improve pre-established metabolic dysregulation and attenuate lesion development, compared to chow alone.

Methods: Ldlr-/− mice were fed a HFHC diet for 12 weeks to induce metabolic dysregulation. Subsequently, mice received one of 3 diets for another 12 weeks: 1) continuation of the HFHC diet, 2) an isoflavone-free chow diet or 3) isoflavone-free chow with 3% naringenin. Experimental endpoints were assessed at the end of 12 weeks HFHC-induction and after 12 weeks intervention.

Results: At 12 weeks, the HFHC diet induced significant weight gain and increased adiposity. Intervention with chow alone reduced the weight gained during induction by 22%, whereas the addition of naringenin to chow induced weight loss of 71%. Specifically, the reduction in adiposity was 2.75-times greater in naringenin-treated mice, compared to chow alone. The HFHC diet increased VLDL cholesterol 20-fold and LDL cholesterol 5-fold, which were reduced by intervention with chow (>60%) and chow supplemented with naringenin (>80%). The HFHC diet induced insulin resistance and glucose intolerance. Naringenin improved insulin tolerance (plasma glucose AUC -38%) and glucose tolerance (plasma glucose AUC -58%), which was accompanied by normalization of plasma insulin and glucose. HFHC-induction promoted the development of intermediate atherosclerotic lesions. Continuation of the HFHC diet doubled lesion size. Intervention with chow alone attenuated lesion size progression 65%. The addition of naringenin to chow slowed atherosclerotic lesion progression 90%, resulting in smaller lesions compared to intervention with chow alone (P=0.042).
Conclusions: We conclude that intervention with naringenin-supplemented chow enhances weight loss, improves metabolic dysregulation and halts the progression of atherosclerosis. These results suggest flavonoids could be useful as a therapeutic for treatment of the metabolic syndrome and atherosclerosis.
Cardiovascular disease (CVD) is currently the leading cause of mortality worldwide. It is estimated that 17.5 million people died of CVD in 2012, representing 30% of all global deaths, and of these an estimated 7.3 million were due to coronary artery disease. Vascular imaging is widely used for assessing the location and severity of arterial narrowings. Digital subtraction angiography (DSA) is widely used for vascular imaging that removes anatomic structures by subtracting a pre-injection image (mask) and post-injection images (contrast). However, cardiac and respiratory motion, swallowing prior to injection, uncooperative patients, gas and bowel movements, etc. that occurs during the time gap of several seconds between a mask and contrast image results in improper subtraction that obscures important details of iodinated vessels making DSA unsuccessful. We are investigating energy subtraction angiography (ESA) of two contrast images acquired at low and high energies in rapid succession (~10 ms) thereby making the method insensitive to motion artifacts. This method was suggested in the 1970’s, however, it was concluded at the time that image quality in terms of the iodine signal-to-noise ratio (SNR) for ESA was a factor of 2-5 lower relative to DSA and the approach was essentially abandoned. We think that reduced iodine SNR for ESA was limited by technology at the time which is why we are investigating the potential of ESA with modern technology and evaluating technological requirements to make ESA successful today. For this study we looked at comparing the fundamental iodine SNR between ESA and DSA for similar patient exposures and calculating the detector read noise necessary for ESA to overcome so that iodine SNR can be comparable to DSA. We conclude that iodine SNR for ESA is greater than DSA for lower iodine mass loadings. We show that iodine-specific images are obtained using ESA and are similar to those of DSA for similar patient exposure. With imposing a condition that read noise increase noise by only 10%, we show that detector read noise for ESA, expressed in number of interacting quanta, needs to be very low compared to DSA to achieve similar iodine SNR.
Predicting Parkinson’s disease in Patients at High Genetic Risk: Investigating Prodromal Markers in Adults with Hemizygous 22q11.2 Deletions

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Introduction: Parkinson’s disease (PD) is a serious and common neurodegenerative disorder. The development of preventative and disease-modifying therapies for PD is hampered by the difficulty of identifying patients in early stages of the disease. We recently identified the hemizygous 22q11.2 deletion and the associated multisystem syndrome, 22q11.2 deletion syndrome, as a novel genetic risk factor for early-onset PD (Butcher et al., 2013). Patients with 22q11.2DS are clinically identifiable and provide a unique opportunity to investigate PD biomarkers and disease progression.

Methods: We investigated 14 adults with 22q11.2DS (median age 39 years, range 30-54 years) for early pre-diagnostic markers of PD including subtle movement problems, associated non-motor symptoms (e.g., cognitive and sensory deficits), and neuroimaging abnormalities relative to 10 healthy age and sex-matched controls. Imaging included transcranial sonography to investigate substantia nigra echogenicity and positron emission tomography (PET) using $^{11}$C-dihydrotetrabenazine, a vesicular monoamine transporter (VMAT2) radioligand, to assess striatal dopamine neuron density.

Results: Patients with 22q11.2DS were rated significantly higher than controls on the motor component of the Unified Parkinson’s Disease Rating Scale ($p<0.00001$). The majority exhibited at least one cardinal motor symptom of PD; bradykinesia (slowness of movement) of varying severity was observed in 13 of the 14 patients and three had rigidity. Eight showed postural/action tremor. Nine were assessed to have a movement disorder, including parkinsonism ($n=5$), drug-induced parkinsonism ($n=2$), and postural/kinetic tremor with and without parkinsonism ($n=2$). The majority ($n=10, 77\%$) of patients with 22q11.2DS had olfactory deficits, and showed colour discrimination defects relative to controls ($p=0.01$). Substantia nigra echogenicity was similar between groups, however ($p=0.32$). Surprisingly, patients with 22q11.2DS showed ~30\% higher striatal VMAT2 binding than controls ($p<0.01$). Lower levels of binding showed a correlation trend with higher bradykinesia subscores ($r=-0.48, p=0.09$).

Conclusion: These preliminary results show that adults with 22q11.2DS demonstrate motor and non-motor features possibly consistent with an early pre-diagnostic stage of PD. The presynaptic
dopaminergic abnormality identified using PET imaging suggests a novel pathway to parkinsonism and/or PD in 22q11.2DS. Longitudinal studies are needed to determine if the observed imaging, motor, and other phenotypes are prodromal markers of PD in this genetic syndrome.
Plasma Oxylipins Increase the Odds of High Central Blood Pressure and Are Beneficially Influenced by Dietary Flaxseed in Patients with Peripheral Arterial Disease

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Introduction: Peripheral arterial disease (PAD) increases the risk for cardiac and cerebral accidents. Because hypertension is the leading risk factor attributed to these events, targeting hypertension may be effective in reducing morbidities and mortalities in patients with PAD. A novel therapeutic target for hypertension is a class of molecules called oxylipins. Oxylipins are highly bioactive mediators that are endogenously produced from polyunsaturated fatty acids and regulate vascular tone and inflammation. Dietary flaxseed may be a novel strategy to target oxylipins as flaxseed contains an abundant level of polyunsaturated fatty acids which can alter the oxylipin substrate profile.

Methods: Therefore, a randomized, double-blinded, controlled clinical trial, the Flax-PAD trial, was used to assess the relationship of plasma oxylipins to blood pressure (BP) and to assess the impact of dietary flaxseed on BP and plasma oxylipins (n=81). Central BP, which is more strongly correlated to end organ damage than brachial BP, was measured by pulse wave analysis at baseline and 6 months. A plasma profile of 43 oxylipins was generated using solid phase extraction, HPLC-MS/MS, and stable isotope dilution quantitation.

Results: Significant associations were observed between 17 oxylipins, primarily produced from arachidonic acid, and central blood pressure. After logistic regression analysis, every 1 nM increase in 16-hydroxyeicosatetraenoic acid (HETE) increased the odds of having high central systolic BP by 15-fold, of having high central diastolic BP by 6-fold and of having high central mean arterial pressure by 15-fold. In addition, every 1 nM increase in 5,6-dihydroxyeicosatetraenoic acid (DiHETE) and 11,12-DiHETE increased the odds of having high central mean arterial pressure by 45- and 18-fold, respectively. Flaxseed induced a significant decrease in these as well as 4 other vasoconstrictive oxylipins. At baseline, the central BP (systolic/diastolic) in the placebo and flaxseed group were, 131/73 ± 2.5/1.4 mmHg and 128/71 ± 2.6/1.4 mmHg, respectively. At 6 months the central BP in the placebo and flaxseed groups were, 132/74 ± 2.9/1.8 mmHg and 124/70 ± 2.6/1.6 mmHg (P<0.05).

Conclusion: Oxylipins are strongly associated with central blood pressure, are beneficially affected by dietary flaxseed, and may be effective therapeutic targets for hypertension in PAD.
The Small RNA Molecule Mir-16-5p Is an Early Biomarker of Neurodegeneration and a Potential Target for Therapy in Prion Disease

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Introduction: Prion disease is a uniquely infectious, fatal neurodegenerative disease that poses serious risk to human health. This disease, commonly known as mad cow disease, can be spread to humans through consumption of contaminated beef. Due to lack of knowledge on disease progression no treatments are currently available. Interestingly, the clinical stage of infection can take years to present in humans which may offer a window for therapeutic intervention. Studies on a mouse model of prion disease in our lab have uncovered a distinct alteration in expression of microRNA (miRNA) in the hippocampus during this period before clinical disease. MiRNA are small, non-coding RNA molecules that bind to complementary regions in the 3’untranslated region of target mRNA, resulting in inhibition of protein synthesis. We hypothesize that the upregulation of a specific miRNA, miR-16-5p, at the preclinical period of disease is a protective response, functioning in inhibition of expression of proteins involved in neurodegeneration.

Methods: Primary hippocampal neurons are dissected from mouse brains at embryonic day 18, cultured until maturity and then treated with a lentiviral vector. This vector either encodes miR-16 or miR-ZIP, causing overexpression or knockdown of miR-16, respectively. Evaluation of cellular morphology is performed via confocal microscopy. In order to determine which mRNA are targets of miR-16 in hippocampal cells, Argonaute immunoprecipitation (Ago-IP) coupled to microarray analysis will be utilized. Validation of targets will be carried out by real-time PCR and western blot.

Results: MiR-16 has been successfully overexpressed and knocked down in hippocampal cell cultures. Verification of neuronal specificity in culture has been performed via immunostaining. Currently, analysis of cellular morphology is ongoing. Ago-IP is also underway, and targets were predicted using a variety of software programs. Many predicted target genes are involved in hippocampal neuron excitotoxicity such as NOS1, GRIN1, GRIN2B and AKT3.

Conclusion: MiR-16 is predicted to bind to many genes involved in excitotoxicity, a process known to result in synaptic loss, one of the first signs of neurodegeneration in prion disease. This supports the hypothesis that miR-16 is involved in preventing neurodegeneration.
Characterization of the Anti-HIV-1 Activity of a Kinase Inhibitor Kenpaullone
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Background: Advances in the antiretroviral therapy (ART) have dramatically reduced the death rate from acquired immune deficiency syndrome (AIDS) and improved the life quality of many HIV-1 infected individuals. Although lifelong suppression of HIV-1 replication with ART becomes possible, the development of drug resistant strains leads to the necessity of new drug development. It is now clear that drug resistant strains are arisen from the mutations of ART targeted viral protein caused by suboptimum treatment or incomplete adherence to therapy. Therefore, it is important to develop novel therapeutic strategies that target host factors essential for HIV-1 replication.

Method and results: Host kinase mediated phosphorylation of viral and host proteins is known to be critical for HIV-1 replication in different stages such as reverse transcription, integration and transcription. Therefore, we scanned among 196 kinase inhibitors and found a CDK and GSK-3β inhibitor named kenpaullone (Ken) can significantly reduce P24 production in HIV-1 infected C8166T cells. Based on this result, we hypothesized that Ken, as a potential anti-HIV drug, can inhibit HIV-1 replication through CDK or GSK-3β pathway. Cytotoxicity evaluation of Ken by WST-1 shows that Ken under 3uM does not significantly affect cell viability of C8166T and Jurkat T cell lines and U937 monocytic cell line. Subsequent infection analysis indicated that Ken can significantly reduce HIV-1 replication in Jurkat and U937 cells at 600nM. Treatment of Ken at 2h prior to HIV-1 infection or 20h after infection did not affect the inhibition activity of Ken indicating that Ken specifically affects the late stages of HIV-1 replication. More detailed mechanistic analysis by RT-PCR revealed that Ken inhibited HIV-1 transcription. Furthermore, our infection experiment indicated that Ken can effectively inhibit the replication of nevirapine (a kind of HIV-1 reverse transcription inhibitor) resistant strain in human PBMC cells.

Conclusion: Our results clearly indicate that Ken, at non-toxic concentrations, is able to inhibit HIV-1, including the wild type and reverse transcription resistant viruses by blocking viral gene expression. More detailed studies are underway to elucidate whether Ken inhibits HIV-1 replication by disrupting a step that has not previously been targeted in current antiretroviral chemotherapy.
L1 Modulates PKD1 Phosphorylation in Cortical Neurons in the Condition of Alzheimer’s Disease

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Introduction: Alzheimer’s disease (AD), one of the neurodegenerative disorders, is characterized by the deposition of β-amyloid protein (Aβ) and extensive neuronal cell death. The neural cell adhesion molecule L1 (L1CAM) is crucial for the development of the nervous system, with an essential role in regulating multiple cellular activities. Protein kinase D1 (PKD1) serves as a key kinase given its diverse array of functions within the cell.

Methods: Immunofluorescence staining was used to study the relationship between L1 and PKD1 phosphorylation on a human cortical tissue microarray. Changes of L1 and pPKD1 levels in the frontal lobe from the AD patient were detected using immunohistochemical staining. Western blotting was used to analyze pPKD1 and the signaling pathways in primary mouse cortical neurons treated with 1) a series of concentrations (0-10 nM) of recombinant L1 (rL1) for 48 h or 2) 0, 5, or 10 nM rL1 for 2 h prior to incubation with a sublethal dose of 10 μM oligomeric Aβ1-42 for 24 h.

Results: An apparent correlation between L1 and pPKD1 and co-localization of these 2 molecules were observed on the human cortical tissue microarray. L1 and pPKD1 levels were increased in the gray matter and white matter from AD patients. L1 significantly induced pPKD1 levels, with the maximal effect observed at 2.5 nM. A similar pattern for pErk and pAkt1 levels was also observed. In cortical neurons induced by Aβ1-42, pPKD1 level was increased while pErk and pAkt1 levels were decreased. Moreover, pretreatment of 5 nM rL1 increased the levels of pPKD1, pErk and pAkt1 in Aβ1-42-treated neurons.

Conclusion: The modulation of L1 on pPKD1 may present L1 as a novel agent to the treatment of AD.
Evaluating the Drug Release and *In Vivo* Biocompatibility of a Reservoir-Based Implantable Device for the Intravaginal Delivery of Hydroxychloroquine

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**Introduction:** Hydroxychloroquine (HCQ) has been shown to exhibit anti-viral and anti-inflammatory properties. Intravaginal ring (IVR) drug delivery systems are widely evaluated in pre-clinical studies for the controlled, sustained release of antiretroviral drugs as a novel strategy for the prevention of HIV-1 infection. In this study, we have developed a novel implantable device for the delivery of HCQ and have assessed its *in vivo* biocompatibility.

**Methods:** The reservoir-based polyurethane implantable devices were fabricated via hot-melt injection molding. Each device was embedded with a RFID micro-transponder and able to be non-invasively and real-timely tracked by a RFID reader. The device was either loaded with 4 mg HCQ (mixed with a rate-controlling excipient at the weight ratio of 1:1) for *in vitro* release study at 37°C in pH 4 release medium, or loaded with 60 mg of HCQ and non-invasively implanted at the distance of 8-10 cm in the vaginal tract of New Zealand White rabbit. Rabbit cervicovaginal lavage (CVL) was collected at pre-determined intervals. HCQ levels in the release medium and CVL was quantitated using reverse-phase high performance liquid chromatography (RP-HPLC). Pro-inflammatory cytokine production was evaluated in CVL using sandwich ELISA. The histological morphology of rabbit vaginal tissue was assessed via hematoxylin and eosin staining.

**Results:** X-ray analysis demonstrated the implant device was well retained within the rabbit vagina for over 40 days. HCQ exhibited a near zero-order release profile *in vitro* for over 20 days with an average release rate of 32.23 µg/mL/day from the medical device. In vivo, there was approximately 10.67 µg/mL of HCQ released per day in CVL for 7 days. No significant differences were observed in rabbit vaginal tissue morphology or in the CVL levels of IL-1β and IL-8 among the naive (not implanted), placebo (implanted without loading HCQ), and the HCQ implant group over 30 days.

**Conclusion:** We have described a novel non-invasive implantable device for evaluating the intravaginal delivery of HCQ in rabbits. This system was non-cytotoxic and may be a suitable platform for the *in vivo* evaluation of other drug candidates for the prevention of sexually transmitted diseases.
Identification of Novel Phosphorylation Sites in Hepatitis C Virus Non-Structural 5a Protein and the Characterization of Their Effects on Viral Lifecycle through Mutational Analysis

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Hepatitis C virus (HCV) is a hepatotropic pathogen present in approximately 3% of the world’s population with 3-4 million new individuals infected every year. Up to 70% of afflicted individuals progress to a chronic disease associated with the development of hepatic steatosis, cirrhosis and hepatocellular carcinoma. One viral protein, Non-Structural 5A (NS5A) has so far eluded a defined role in HCV replication despite being essential to the processes of genome replication, viral assembly and modulation of the host cell environment. NS5A is a phosphoprotein that appears as two forms of distinct molecular weight on SDS-PAGE. These are referred to as the basal and hyper phosphorylated forms. It has been proposed that the different phosphoforms of NS5A may direct its function and act as a molecular switch between genome replication and viral assembly. Although previous research has sought to identify the specific phosphorylated sites within NS5A many of these phosphorylated residues have escaped identification. In order to obtain a more extensive understanding of NS5A function and the role of protein phosphorylation, we sought to identify novel phosphorylated residues within NS5A. Two different methodologies were applied. First, HEK293 cells stably expressing an N-terminally tagged NS5A were subjected to tandem affinity purification under denaturing conditions to obtain extremely pure NS5A protein. Secondly, NS5A was purified from Huh7.5 cells containing the JFHI replicon using size exclusion chromatography to obtain pure NS5A from the context of viral replication. Resulting samples were subjected to trypsin digestion and ESI-tandem mass spectrometry and database searching completed phosphopeptide identification and phosphate assignment. All phosphopeptide identifications were confirmed through manual discrimination of mass spectrums. We have identified a total of 29 phosphorylation sites in the NS5A protein. Using mutagenesis we created a total of 58 HCV mutants using the JFHI virus backbone where phosphorylated serine and threonine residues were changed to both an alanine (abolish phospho site) and an aspartic acid (phospho mimic). On-going studies using the phosphomutant JFHI viruses are ongoing. We are accessing the effects of the phosphorylation sites on viral genome replication, infectious viral titre and NS5A protein stability.
The human cathelicidin LL-37 is known to selectively suppress pathogen-induced inflammation, while maintaining immune responses required for the resolution of infections. The objective of this study was to elucidate the immunomodulatory mechanisms of LL-37 in chronic inflammation. We examined the effects of LL-37 on cytokine Interleukin (IL)-32γ-induced downstream responses and related signalling mechanisms. The cytokine IL-32γ is significantly elevated and directly associated with the pathogenesis of chronic inflammatory diseases such as rheumatoid arthritis, chronic obstructive pulmonary disease and Crohn's disease. In this study, we demonstrated that LL-37 significantly suppressed the production of IL-32γ-induced pro-inflammatory cytokines e.g. TNFα, IL-1β and IL-6, induced the production of anti-inflammatory cytokine IL-1RA, without altering chemokine responses in human peripheral blood-derived mononuclear cells (PBMC). Using flow cytometry, we showed that CD14+ monocytes were the major contributors of IL-32γ-induced TNFα within the PBMC population, and that LL-37 suppressed IL-32γ-induced TNFα production in CD14+ monocytes. Mechanistic studies examining IL-32γ-mediated phosphorylation, in the presence and absence of LL-37, showed that LL-37 suppressed IL-32γ-mediated phosphorylation of Fyn (Y420), a signalling mechanism known to be involved in the inflammatory process. In contrast, phosphorylation of the dual phosphatase MKP-1 (S359) was significantly increased in the presence of the peptide alone. LL-37 also induced the activity of the upstream kinase p44/42 MAPK, which is known to phosphorylate and stabilize MKP-1 (S359). Silencing of MKP-1 using siRNA resulted in suppression of LL-37-mediated downstream responses, suggesting that MKP-1 is a key intermediate in the immunomodulatory activity of the peptide. MKP-1 is known to be a target protein of TGF-β1, we therefore examined the effect of LL-37 in TGF-β1 production. We demonstrated that LL-37 significantly induced the production of TGF-β1 in PBMC. As TGF-β1 is known to play a critical role in the generation regulatory T-cells (Tregs), we further investigated the effect of LL-37 on Tregs. We showed that the stimulation of PBMC with LL-37 significantly increased the number of CD25+FoxP3+Tregs. This study suggest that the immunomodulatory role of LL-37 in chronic inflammatory diseases is mediated by the activity of the dual phosphatase MKP-1, TGF-β1 and Treg axis.
**Introduction:** Canadian men are more active than women, more likely to be moderately active in their leisure time, and fare better than women in all categories of fitness, except flexibility. Understanding gendered barriers and facilitators of physical activity is essential for developing effective strategies to promote greater population-wide participation. Gyms are common places to engage in physical activity, but they can also reinforce these very gender differences. Weight-training and cardiovascular exercise spaces are known to be respectively typecast as masculine and feminine, tied to popular perceptions of producing particular physiques. Still, little is known about the socio-spatial processes happening within the gym that may work to include or exclude people from various workout spaces and practices. By exploring a diversity of men's and women's experiences in gym environments, this study aims to better understand the role of gender in shaping the scope and nature of gym-based exercise participation and related experiences of well-being.

**Methods:** In-depth qualitative methods were used to capture experiential data. Semi-structured interviews coupled with participant-generated drawings were conducted with 52 participants (18 men, 34 women), ages 25-64, who self-identified as regular gym users, were current members of 10 different co-ed gyms in Kingston, ON, and whose gym routines included use of weight and/or “cardio” areas. A sub-sample completed 1-week gym journals. Data are analyzed using thematic coding.

**Results:** Preliminary analyses suggest three themes. First, although gyms are sites for health promotion, they are also places where gendered inequities in health opportunities emerge. Gyms were often experienced as gender divisive, yet the degree to which this affected gym practices and experiences depended upon individual characteristics. Second, conversely, gender could be a positive source of motivation. For example, same-gender social comparisons tended to encourage participants’ gym pursuits. Third, not all gyms are created gender (un)equal. Of the 10 gyms represented in the sample, gender differences appeared to matter significantly less in two.

**Conclusion:** By taking into account the gendered barriers and facilitators of gym-based exercise participation we stand to maximize the health opportunity of gyms for all and inform gym designs that are more inclusive for a diversity of users.
The Mouse *Idd2* Locus Is Linked to the Proportion of Immunoregulatory Double Negative T Cells, a Trait Associated with Autoimmune Diabetes Resistance

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Introduction: Autoimmune diseases result from a break in immune tolerance. Various mechanisms of peripheral tolerance can protect against autoimmunity, including immunoregulatory CD4-CD8- double negative (DN) T cells. Indeed, we have previously shown that diabetes-prone mouse strains exhibit a low proportion of DN T cells relative to that of diabetes-resistant mice and that a single autologous transfer of DN T cells can impede autoimmune diabetes development, at least in the 3A9 TCR transgenic setting.

Objective: Herein, we aim to understand the genetic basis for the difference in DN T cell proportion between diabetes-resistant and diabetes-prone mice.

Methods and results: We performed an unbiased linkage analysis in 3A9 TCR F2 (NOD.H2k x B10.BR) mice and revealed that a locus on chromosome 9, which coincides with *Idd2*, is linked to the proportion of DN T cells in the lymph nodes. We generated two NOD.B10.BR-Chr9 congenic mouse strains and validated the role of this genetic interval in defining the proportion of DN T cells. Moreover, we found that the increased proportion of DN T cells in lymphoid organs is associated with a decrease in both diabetes incidence and serum IgG antibody levels.

Conclusion: Together, the data suggest that *Idd2* is linked to DN T cell proportion and that a physiological increase in DN T cell number may be sufficient to confer resistance to autoimmune diabetes. Altogether, these findings could help identify new candidate genes for the development of therapeutic avenues aimed at modulating DN T cell number for the prevention of autoimmune diseases.
Chronic Inflammation and Metabolic Osteoarthritis: Evidence of Time-Course Changes in a Rat Model
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Introduction: High fat feeding and resultant obesity are associated with inflammation, which in turn is thought to exacerbate osteoarthritis (OA). We have shown that 28-week high fat/high sucrose diet leads to OA in a rat model, and is associated with little increases in systemic but large increases in local inflammation. In order to characterize the time-history of inflammatory changes involved in the trajectory of metabolic OA, earlier evaluation time points are needed. Therefore, the purpose of this study was to determine the effects of a 12-week high fat/high sucrose diet-induced obesity on OA damage and inflammation in rats.

Methods: Twenty-five rats were randomized to either a high fat/high sucrose (DIO, n=18, 40% fat 45% sucrose) group or a control group (chow, n=7, 13.5% fat, 3.7% sucrose). After 12 weeks, DIO animals were stratified into a DIO-P (Top 33% by mass, n=6) or DIO-R sub-group (bottom 33% by mass, n=6). At sacrifice, body fat percentage (DXA), synovial fluid, serum, and knee joints were collected. Joints were evaluated histologically using a Modified Mankin Score. Twenty-seven synovial fluid (local) and blood serum (systemic) inflammatory markers were quantified by Luminex xMAP®. All statistical tests were performed at α=0.05.

Results: DIO-P animals had significantly higher Mankin scores compared to both DIO-R (p=0.05) and chow animals (p=0.001). DIO-R animals had higher Mankin scores compared to chow (p=0.049). Body fat percentage had a significant positive relationship with OA-damage (r=0.74, p<0.001). Of the 27 serum analytes measured, 12 increased across all DIO animals compared to chow, and eight demonstrated a positive significant relationship with the Mankin scores. No differences were observed in serum of DIO-P and DIO-R animals. Two synovial fluid markers were increased for all DIO animals, and additionally, DIO-P animals demonstrated two increased synovial fluid analytes compared to DIO-R.

Conclusion: OA-related damage was associated with body fat percentage after a 12-week high fat/high sucrose diet. Marked increases in systemic inflammation were coupled with few changes in synovial fluid inflammation. Taken together with our 28-week DIO data, the current results suggest that metabolic OA is a dynamic process that may originate from chronic, low-level systemic inflammation.
Visually Monitored Deep Inspiration Breath Hold Radiation Therapy to Reduce Cardiac Dose in Left-Sided Breast Cancer Patients

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Introduction: Breast cancer is commonly treated with surgery followed by radiation therapy (RT) to the breast or chest wall and involved lymph nodes. Although RT reduces the risk of local recurrence, it may also result in increased risk of heart disease, especially for left-sided breast cancer patients. Deep inspiration breath hold (DIBH) during breast radiotherapy moves the heart away from the radiation beam, decreasing the radiation dose to the heart, and reducing cardiac morbidity risk. Methods for performing DIBH include active breathing control, real-time imaging, surface imaging, and other non-commercial techniques. We used portal imaging and real-time position management (RPM) to evaluate a non-commercial, visually monitored DIBH technique (VM-DIBH).

Methods: Left-sided breast cancer patients able to maintain DIBH for 20 seconds or longer were included in this study. Cine portal images were acquired weekly during the medial tangent field of patients treated with VM-DIBH. The distance between the field border and chest wall was measured at the centre of the field and used as a surrogate for breath hold position. Setup uncertainties and intra-beam motion were quantified by comparing cine image measurements and digitally reconstructed radiograph (DRR) measurements. Real-time Position Management (RPM) was used to acquire time-resolved breath hold amplitude information during cine-imaging fractions.

Results: RPM measurements found that the average (range) breath-hold amplitude and duration was 18 mm (3 – 36 mm) and 19 s (7 – 34 s), respectively. Using cine portal images the mean setup uncertainty (M) was 1.2 mm; random (σ) and systematic (Σ) setup errors were both 2.0 mm. The chest wall position was within 5 mm of the DRR position in >90% of cine images. Intra-beam motion was within +/- 2 mm in >95% of images. The skew of the intra-beam population data suggested that many patients relax or exhale slightly during breath hold.

Conclusion: The VM-DIBH technique had comparable chest wall positioning accuracy to other more resource-intensive DIBH techniques. Setup uncertainties and chest wall position measurements indicated adequate breath hold setup reproducibility for the majority of patients and intra-beam motion measurements showed excellent stability of breath hold during treatment.
Acceptance Underlies Romantic-Partner Seeking Success among Persons with Chronic Pain
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Introduction: Chronic pain (CP) affects 20% of Canadians and is strongly related to depression, anxiety, and functional/work impairments (Breivik et al., 2006; McWilliams et al., 2003). Participation in a supportive, committed romantic relationship can alleviate stress and positively impact the mental and physical health of persons with CP (Holtzman et al., 2007). Coping with CP, however, often strains relationships and consequently individuals with CP are more likely to be without a partner (Wolfe et al., 2004). Existing research has not examined how CP impacts partner-seeking and early relationship development activities. Given the recognized benefits of having a supportive long-term partner, research exploring how CP affects individuals’ approaches to partner-seeking and early relationship development is warranted.

Methods: 40 partner-seeking or newly partnered (<6 months, non-cohabitating) individuals (M age = 32.2 years [SD = 8.3]) with CP were recruited online. Participants completed a demographic questionnaire followed by a semi-structured telephone or in-person interview about how CP has affected their dating outlook and experiences. Thematic analysis (Braun & Clarke, 2006) was used to extract and hierarchically categorize themes described by participants.

Results: Participants’ level of CP acceptance significantly impacted their ability to initiate/develop romantic relationships. Participants who described the lowest levels of acceptance (i.e., low activity engagement, low pain willingness, refusal to accept chronicity) were single, and expressed reluctance towards partner-seeking due to their ongoing coping difficulties. Low acceptance participants imposed more limitations and preconditions on their dating activities, desired more casual relationships and targeted partners whose interests matched their pre-CP abilities. Consequently, they were less successful at securing/sustaining partners. Conversely, more accepting participants allocated more resources to finding/developing their romantic relationships despite pain and described better strategies for communicating about pain, resulting in greater success in finding suitable partners.

Conclusions: These results suggest a relationship between CP acceptance and successful partner-seeking. Given the known benefits of supportive partners for persons with CP, efforts to increase levels of CP acceptance should be a clinical priority among health-care professionals in order to facilitate this goal.
Assessing Psychological Well-being in Women with Polycystic Ovary Syndrome and Infertility
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Introduction: Polycystic ovary syndrome (PCOS) is characterized by oligo-anovulation, hyperandrogenism and/or “polycystic ovaries” on ultrasound. Infertility, insulin resistance, and obesity are common associations. Recent research suggests women with PCOS are more likely to have depression, emotional distress, and decreased quality of life. As an initial approach to improving wellness in our fertility practice, we compared levels of depression, anxiety, stress, and quality of life in women with PCOS and regularly ovulating women.

Methods: Participants were recruited from Grace Fertility Centre, Vancouver, B.C. This preliminary analysis included 48 women with PCOS and 21 regularly ovulatory women seeking fertility treatment. The mean values ± SEM and median for age were 31.7 ± 0.81 and 33, and BMI 28.5 ± 1.1 and 26.8; corresponding values for ovulatory women for age were 34.9 ± 1.1 and 34, and BMI 24.8 ± 1.3 and 23.6. Relevant demographic, clinical and laboratory data were recorded. Participants completed two self-report items; the “Depression Anxiety Stress Scale” and the “Fertility Quality of Life Tool”. Data were analysed using Mann-Whitney tests.

Results: Women with PCOS had significantly higher anxiety than regularly ovulating women (median scores: 5.5 vs. 1.0, p<0.0001). Women with PCOS also had higher levels of depression (3.0 vs. 2.0) and more stress (11.0 vs. 6.0), and lower quality of life scores (65.2 vs. 66.7), but differences were not statistically significant. Furthermore, women with PCOS had significantly lower “mind-body” scores than regularly ovulatory women, a sub-scale of the quality of life assessment (62.5 vs. 79.2, p=0.03).

Conclusion: Increased anxiety in women with PCOS compared to regularly ovulating women may reflect concerns related to having PCOS rather than infertility itself. In addition, women with PCOS appear to be more negatively affected by their infertility physically, cognitively, and behaviourally, which has not been previously noted. Therefore, developing techniques that can enhance mind-body connection in women with PCOS may improve anxiety, mental wellness and overall health.
Introduction: Human induced pluripotent stem cells (hiPSCs) can be derived from somatic cells through a reprogramming process driven by expression of a defined set of transcription factors. These hiPSCs share the properties of self-renewal and pluripotency with human embryonic stem cells (hESCs), and can be used to generate many types of differentiated cells from three germ layers. The derivation of hESCs from sources with different genetic backgrounds is challenging because use of human embryos is limited and ethically debatable. Thus, iPSC technology provides a unique tool to derive disease-specific stem cells for research, and possibly derive normal stem cells from autologous cells of someone affected by a disease for the treatment of degenerative disorders.

However, due to the risk of insertional mutagenesis, viral transduction has been increasingly replaced by non-viral methods to generate iPSCs. One technique that has not yet been explored enough is the use of “minicircle” DNA, a novel vector that is free of bacterial DNA and has higher transfection efficiency and longer ectopic expression than virally generated iPSCs.

Method: The objective of this study was to develop iPS-like cells by reprogramming human mesenchymal stem cells (MSC). The MC.LGNSO plasmid contains a single cassette of each of the four reprogramming factors, namely Oct4, Sox2, Lin28, and Nanog, plus a GFP reporter gene. Human MSCs were transfected twice using a minicircle DNA/Lipofectamine LTX complex. The GFP-positive cells were observed by fluorescent microscopy 24 h post-transfection.

Result: Due to the dilution of minicircle DNA vector with proliferation, there was gradual loss of GFP expression in the cells. Cell colonies with a tightly packed, domelike structure began to appear 7 to 10 days after the second transfection. The pluripotency of the derived iPS-like cells was verified by RT-PCR and immunochemical staining techniques to express pluripotent marker genes and differentiated into embryoid bodies composed of all three germ layers.

Conclusion: This fast, feeder-layer-free technique benefits from using a non-viral vector for the safe development of hiPSCs. This method can have a huge effect by enabling the use of MSCs which are easily obtained from clinical wastes, now discarded after child delivery.
Construction of an Income Insecurity Index and Its Association with Health Inequalities in Mid-life and Later Life in Canada

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The objective of this study is to construct an Income Insecurity Index for Canada and examine the association between self-rated health and income insecurity, as distinct from income, and income inequality. In the health economics literature, the association between health and income and/or income inequality is well established. However, the association between income insecurity and self-rated health has been mostly ignored. Applying principal component analysis for categorical variables and using data from the Canadian Community Health Survey (CCHS) 2010, we construct an Income Insecurity Index for Canada and then investigate its association with self-rated health. While adjusted for other socio-economic factors, our ordered logit analysis shows that income insecurity has a significant association with self-rated health and this association is stronger in mid-life than in later life in Canada. Our instrumental variable (IV) analysis reconfirms the results obtained from the logit model. This study suggests that research on health inequalities should pay greater attention to the association of health with income insecurity, rather than with income alone, especially during periods of greater economic instability and financial austerity.
Influence of Different Dietary Fats on Lipid Metabolism and Gene Expression in Zucker (Fa/Fa) Rats Testicular Function

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Introduction: The unique lipid environment of testes is enriched with seminolipid, phospholipids and fatty acids which are crucial for spermatogenesis. Previous studies have shown that Zucker obese rats often have non-identical paired testes different in size, leaving one testis underdeveloped. The underdeveloped testes showed a selective decrease in docosapentaenoic acid (DPA, C22:5n-6), a dominant fatty acid in the testes, and seminolipid, with abnormal sperm morphology. This study was conducted to examine whether dietary lipids can alter these changes, and modify fatty acid metabolism by altering gene expression and enzymes controlling these pathways.

Methods: Six week old male obese fa/fa Zucker rats (n=10/group) were fed four different diets containing C18:2n-6, C18:3n-3, C20:5n-3 and C22:6n-3 enriched oils (safflower oil, flaxseed oil, EPA, and DHA oils, respectively) for 8 weeks. Lean rats were fed safflower oil only. Total RNA was isolated from testes, and global gene expression was analyzed using Affymetrix Rat Gene 2.0 ST Array containing 28,407 transcripts. Annotated gene sets from the normal and underdeveloped testes were analyzed using Ingenuity Pathway Analysis (IPA), followed by functional classification of genes. Expression values were compared based on obesity, diet, and testicular size.

Results: No significant differences were observed in the transcriptome profiles between diet and obesity groups. Out of the 3192 genes detected, 1121 and 309 were differentially expressed in the underdeveloped and normal testes, respectively. The IPA indicated that transcripts upregulated in the normal testes relative to underdeveloped testes are involved in triacylglycerol biosynthesis, sphingomyelin metabolism- eg. sphingomyelin phosphodiesterase 2 (SMPD2), and phosphatidylglycerol biosynthesis- eg.1-acylglycerol-3-phosphate O-acyltransferase 1 (AGPAT1). Transcripts upregulated in underdeveloped testes relative to normal testes are involved in production of nitric oxide and reactive oxygen species, and nuclear factor (erythroid-derived 2)-like 2 (NRF2) mediated oxidative stress response for eg.mitogen-activated protein kinase kinase 4 (MAP2K4). Moreover, downstream effect analysis revealed an increased trend towards reproductive system diseases, endocrine system disorders and cancer in the underdeveloped testes compared to the normal testes.

Conclusion: In conclusion, these results indicate that testicular lipids and their metabolism are closely related with normal testis development and function.
On March 22nd 2014, the World Health Organization (WHO) was notified by the Ministry of Health (MoH) in Guinea of an emerging Ebola outbreak in the south-eastern region of the country, bordering Sierra Leone and Liberia, which was rapidly evolving. In late June, the Public Health Agency of Canada deployed a mobile laboratory in the Kailahun district of Sierra Leone to provide diagnostic support. Over the next 6 months, the viral loads of almost 600 patients were tested and followed every few days until death or convalescence, representing a total of over 2500 samples. Because of the extended length of the outbreak, we sought to analyze how viral loads, expressed as CT values, impacted the progression of the outbreak. Initial analysis of the mean CT values revealed that as the outbreak progressed, the viral burden of patients was decreasing, from July (CT=24.35) to November (CT=28.13). When the initial CT values of patients were plotted individually, linear regression analysis revealed a 5.93 cycles’ difference between July 11th and November 30th. Due to the statistically significant difference between the CT values of the first 48 patients in July (21.97±0.66) and the last 48 patients in November (25.96±0.88), we sought to investigate the potential causes behind this trend. One factor we investigated was the number of days between the date of disease onset and the date the initial sample was collected following admission to the clinic. Statistical analysis concluded that there was no significant difference between July and November for the same 48 patients. Receiver operating characteristic also revealed that a CT value cut-off of 24 or above predicted survival, and was the same for both time periods even though the case fatality rate was 30% lower in November. Future work will be aimed at explaining this discrepancy, whether it is due to patient characteristics, changes in the virus or to the general immunity of the population.
Phenotypic Analysis of the Over Expression of A Novel Response Regulator Protein A1S_2006 in Acinetobacter baumannii

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Introduction: Acinetobacter baumannii is a Gram negative opportunistic pathogen known for its involvement in nosocomial infections throughout the world. The emergence of multi drug resistant (MDR) phenotypes of A. baumannii has had a detrimental effect on the efficacy of the current antibiotics in use and thus presenting a greater risk to human health than ever before. Therefore it is important to identify the global mechanisms contributing to the multi drug resistance in order to tackle this issue. In this study we aim to characterize a novel response regulator protein of A. baumannii in order to study its role in regulating multidrug resistance and virulence in this organism.

Methods: The putative response regulator encoding gene A1S_2006 of A. baumannii ATCC17978 was PCR amplified and cloned into an overexpression vector. The recombinant plasmid was introduced into A. baumannii ATCC17978 in order to overexpress A1S_2006. Antibiotic susceptibility and motility of the A1S_2006 overexpressing strain was analyzed.

Results: An increased resistance to cefotaxime and gentamicin was observed for the strain overexpressing A1S_2006 as compared to the wild type. The motility assays showed a reduction in motility for the A1S_2006 overexpressing strain as compared to the wild type.

Conclusion and Future Directions: Our preliminary data shows that A1S_2006 may be involved resistance to cephalosporin and aminoglycosides as well as motility of A. baumannii. The next step of this study is creating a gene knock out strain of A1S_2006 in A. baumannii ATCC17978 to examine the phenotype of the knockout strain. This would enable us to examine and correlate the preliminary data presented here in context of the role of A1S_2006 in A. baumannii.
Effects of Oxidative Stress on Expression, Localization and Activity of the BH3 Only Bcl-2 Family Member BNIP3

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Bcl-2 nineteen kildalton interacting protein 3 (BNIP3) is a member of the BH3 only Bcl-2 family that regulates cell death. BNIP3 is found at basal levels in skeletal muscle and in the nucleus of glial cells. It can promote cell death or cell survival depending on expression level and localization in the cell. Nuclear localization prevents apoptosis by repressing transcription of both apoptosis-inducing factor and death receptor-5. Under hypoxia, BNIP3 expression is increased and is localized to the mitochondria promoting cell death by inducing autophagy, a form of self-digestion, and increasing reactive oxygen species (ROS). The role of BNIP3 in regulating oxidative stress induced cell death is unknown and is the focus of this study. We found that treating HEK 293 cells with increasing concentrations of hydrogen peroxide (10-1000µM) resulted in increased ROS and cell death as determined by flow cytometer. We then lysed HEK293 cells and western blotted for BNIP3 expression levels. We found that at 1mM hydrogen peroxide, BNIP3 protein levels were significantly increased compared to untreated cells. Furthermore, using immunofluorescence staining for BNIP3 and the mitochondria we found that BNIP3 increased expression was localized to the cytoplasm and mitochondria after hydrogen peroxide treatment. These results suggest that similar to hypoxia, BNIP3 expression is increased and localized to the mitochondria under oxidative stress. In the future, we will determined whether knockdown of BNIP3 protects cells from oxidative stress induced cytotoxicity.
Direct Renin Inhibition with Aliskiren Improves Ischemia-Induced Neovascularization: Blood Pressure-Independent Effect

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**Background:** The activation of the renin-angiotensin system is associated with impaired formation of new blood vessels (neovascularization) in response to ischemia. Aliskiren is the only direct renin inhibitor that is clinically used as an orally active antihypertensive drug. Here we tested the hypothesis that aliskiren might improve neovascularization following ischemia.

**Methods and Results:** C57BL/6 mice were treated with a high dose of aliskiren (50 mg/kg), a low dose of aliskiren (10 mg/kg), or drinking water only. After two weeks of treatment, hindlimb ischemia was surgically induced by femoral artery removal. Treatment with aliskiren led to a significantly faster rate of blood flow recovery after hindlimb ischemia (Laser Doppler). Interestingly the lower dose of aliskiren, which did not reduce blood pressure, provided similar improvement of blood flow recuperation compared to the higher dose of aliskiren. At day 21 after surgery, Doppler flow ratios were significantly improved in mice treated with aliskiren (0.69 +/- 0.07 vs. 0.52 +/- 0.03; p<0.05). This was associated with an increased expression of angiogenic factors in ischemic muscles, including VEGF and eNOS. Endothelial progenitor cells (EPCs) have been shown to have an important role in postnatal neovascularisation. We found that aliskiren significantly increased the number of bone marrow EPCs at day 7 after ischemia (172 +/- 7% increase; p<0.05). Moreover, the adhesive properties of EPCs were significantly improved in mice treated with aliskiren (175 +/- 5% increase; p<0.05). In vitro, aliskiren improves cellular migration and tubule formation in HUVECs. This is associated with an increased expression of nitric oxide (DAF staining), and a significant reduction of oxidative stress levels (DHE staining). Importantly, the antioxidant and angiogenic properties of aliskiren in HUVECs are abolished following treatment with the NOS inhibitor L-NAME.

**Conclusions:** Direct renin inhibition with aliskiren leads to improved ischemia-induced neovascularization that is not dependant on blood pressure lowering. The mechanisms involve beneficial effects of aliskiren on NO and angiogenic pathways in ischemic tissues, together with an increase in the number and the functional activity of EPCs.
Background: As more individuals with serious mental illness (SMI) live in the community and as the custodial care model is shifting to more supported housing (implying more independent living), assessing and ensuring home safety has become an important issue. Although 70% of people who present home safety issues arising from self-neglect (i.e. from the inability to identify risk factors and to protect themselves against conditions which may compromise their own safety) also present a mental illness, we actually do not have a complete picture of the risk factors that individuals with SMI encounter at home.

Objectives: To identify risk factors that affect people with SMI home safety and develop a screening tool that would contextualize home safety issues.

Method: It is a developmental research where participants were recruited by a purposive and a network sampling method. Individual interviews were conducted with eight people with a mental illness, while focus-groups were conducted with seven health and social service providers, seven community stakeholders and nine relatives. The collected data were analyzed by grounded theory analysis, and the relations between the various categories were identified in accordance with the theoretical approach proposed by Paillé. We hypothesized that the Person-Environment-Occupation (PEO) Model could serve to highlight empirical relationships in the obtained data.

Results: The research team noticed and categorized several risk factors which were associated with 1) the person's characteristics, 2) the quality and nature of the home environment and living conditions, and 3) the nature of the activities in which the individual engaged themselves and how they are realised. Results show that these dimensions are interrelated so that home incidents (i.e. unnecessary harm and damage) arise from a dynamic interaction between various risk factors, which indicates that home safety is context specific.

Conclusion: The results show that the theoretical basis of the PEO is relevant to clarify, contextualize and appreciate the severity of home safety issues encountered by people with SMI and therefore, to support the elaboration of an assessment tool which will support professionals' judgment in order to ultimately foster home safety in the context of recovery in mental health.
The Impact of Patient Education on Modality Choice: A Systematic Review and Meta-Analysis

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Background: Educational interventions are increasingly used to promote peritoneal dialysis (PD), the most common form of home therapy for end-stage renal disease (ESRD). A systematic appraisal of the evidence in support of dialysis modality education is needed to inform the design of patient-targeted interventions to increase selection of PD. We performed a systematic review and meta-analysis to characterize the relationship between patient-targeted educational interventions and the selection and use of PD in adult patients with, or at risk of ESRD.

Design, setting, participants, & measurements: We searched MEDLINE, EMBASE, CINAHL and EBMR in November 2013. We included observational studies and randomized trials of educational interventions designed to increase selection or use of PD. Pooled odds ratio were acquired using a random effects model.

Results: Of 2778 citations, 15 studies met our inclusion criteria, including one randomized trial. Patient-targeted educational interventions were associated with a 2-fold increase in the odds of choosing PD (pooled odds ratio [OR] 2.15; 95% CI, 1.07–4.32; I²=76.7%) based on results from 4 observational studies (N=7,653). In the single randomized trial (N=70), receipt of an educational intervention was associated with a more than 4-fold increase in the odds of choosing PD (OR 4.60; 95% CI, 1.19-17.74). Patient-targeted educational intervention was associated with a 3-fold increase in the odds of receiving PD as the initial treatment modality (OR 3.50; 95% CI: 2.82-4.35; I²=24.9%) based on results from 9 observational studies (N=8,229).

Conclusions: This systematic review demonstrates a moderate association between patient-targeted education interventions and the subsequent selection and use of PD. Future comparative randomized trials (with well-defined education interventions and comparison groups) are required to determine the most effective components and structure of educational strategies.
Understanding Dengue Transmission: Application of an Ecohealth Approach

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Background: Last year, WHO has reported that dengue is the fastest growing vector borne disease in the world. Since 2000, Bangladesh has remained hyperendemic for dengue due to its unplanned urbanization, overcrowding, poverty, and health inequalities. To understand dengue transmission, we hypothesized that understanding dengue transmission research must cross disciplinary boundaries to encapsulate different disciplinary knowledge as well as different non academic knowledge from community members to policy makers in the society. In recognition of the need for a transdisciplinary research on this problem, we applied an Ecohealth approach to understand dengue transmission in Dhaka, Bangladesh.

Methods: By using Delphi, all city wards were classified into high, medium, and low Socio-Economic Status (SES) zones, and a total of 1200 randomly selected households were selected for conducting research from 2011-2013. Multiple disciplinary aspects were encapsulated by examination of: i) rates of human exposure to dengue virus (DENV); ii) dengue vector abundance in the same households; iii) self-risk perception of dengue between experts and lay people; and iv) knowledge, attitudes, and practices (KAP) survey of dengue and its vector by the community members. Data included in the analysis are: a) One baseline seroprevalence survey in 1126 households and two post monsoon serosurveys for seroconversion study; b) four entomological surveys in 2996 households during three monsoon and one dry season; c) KAP survey of 300 households; d) Mental Model development with 12 experts and 15 lay people; and e) 12 focus group discussions and 18 key informant interviews.

Results: 27% of all houses were infested with immature Aedes and ornamental category containers were most important for producing maximum Aedes pupae. Seroprevalence survey indicates a high seroprevalence of DENV (i.e., 79.9% IgG positive) and only 95 individuals were seroconverted during the following seasons. Multivariate logistic regression model showed that age and possession of ornamental category containers were the most significant risk factors for DENV seroprevalence and seroincidence upon adjusting other explanatory variables. KAP survey results indicated that most community members heard about dengue (91.3%) and knew (93.7%) that mosquitoes act as dengue vector. Multivariate logistic regression modeling revealed that the respondents in age group 45-60 were 2.8 times more likely to have positive attitudes towards undertaking precautionary measures than other age groups.
Conclusions: The conceptual framework developed from this research is the first novel attempt to contribute in understanding dengue transmission by using Ecohealth approach in the developing country context.
Association of a Human Growth Hormone (GH) Receptor (GHR) Gene Microsatellite Polymorphism with Idiopathic Short Stature (ISS)

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GH plays an essential role in children through multiple growth promoting and metabolic effects by binding to its receptor on target cells. Fourteen GHR mRNA variants (V) with different 5’UTRs: all code for the same protein. Many polymorphisms have been found in the GHR coding regions, certain of which result in GH insensitivity (dwarfism) due to functional defects of the receptor. Children with ISS show growth impairment without GH or GHR defects, suggesting that decreased GHR expression may be involved.

We previously found, in an adult cohort, a highly polymorphic GT microsatellite 80bp upstream of the transcription start site of the ubiquitously expressing V9 exon. To investigate the possible association between length of this polymorphism and the ISS phenotype, we screened the allelic frequencies of (GT)ₙ repeats in 39 ISS children and 61 normal stature adults and clustered the repeats into three allele classes: S (19-23 repeats), M (24-28) and L (29-35). The L/S genotype was significantly higher in ISS children than controls. This result is being confirmed in a larger cohort of short stature vs. normal height adults recruited in Quebec (CARTaGENE). We used Luc-reporter assays in HEK293 cells to test the ability of L vs. S repeats to modulate basal transcriptional activity: results show a significant difference between S and M. We are now testing if there is an allelic effect on upstream DBP and CCAAT transcription factor binding sites. GHR mRNA levels are being measured in L/S vs. M/M CEPH cell lines.

(GT)ₙ sequences form an alternative Z-DNA structure that can also modulate transcription, likely due to the binding of specific Z-binding proteins. In silico analyses show a high propensity of the GHR repeat to form a Z-DNA structure and ChIP analyses confirmed this.

Because of its close proximity to the transcription start site of the V9 GHR mRNA, the GT repeat could be an important cis-regulatory element for the GHR gene.
Magnesium Sulfate Treatment for Juvenile Ferrets Following Induction of Hydrocephalus with Kaolin

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Introduction: Hydrocephalus is a neurological condition characterized by altered cerebrospinal fluid (CSF) flow with enlargement of ventricular cavities in the brain. Previous work using rodents has shown that the pathogenesis of axonal damage in hydrocephalus includes calcium-mediated proteolysis, which can be reduced by calcium channel antagonist agents nimodipine and magnesium sulfate (MgSO₄). Ferrets are gyrencephalic mammals that exhibit similar behavioral, structural, and histological changes following induction of hydrocephalus as rodents and humans, and thus MgSO₄ treatment was tested in juvenile ferrets. It was hypothesized that MgSO₄ therapy would improve behavioral, neurophysiological, and/or neurobiochemical outcomes.

Methods: Fourteen-day old ferrets were given an injection of kaolin (aluminum silicate) into the cisterna magna. Magnetic resonance (MR) imaging was performed two days later to examine successful induction, then two weeks later to assess ventricle size and stratify ferrets to two treatment conditions. Ferrets were treated for two weeks daily with MgSO₄ or sham saline therapy, and then imaged again before sacrifice. Behaviour was examined thrice weekly.

Results: Compared to age-matched controls, hydrocephalic ferrets were not appreciably different in terms of weight and behaviour; however, those receiving MgSO₄ weighed less, were more lethargic, and were relatively weaker displaying reduced activity and less supported rearing than those receiving saline injections. Hydrocephalic ferrets developed moderate to severe ventriculomegaly and exhibited thinning of the cerebrum surrounding regions of the lateral ventricles, with significant increases in lateral and 3rd ventricles compared to controls, but there were no differences or reductions in any ventricular region for either treatment group. Though glial fibrillary acidic protein (GFAP) content was elevated in saline treated hydrocephalic ferrets, which is indicative of reactive astroglial changes, there were no significant differences compared to the MgSO₄ treated group nor to control ferrets.

Conclusion: The hydrocephalus-induced disturbances do not appear to be ameliorated by MgSO₄ treatment. This suggests that unlike lissencephalic hydrocephalic rodents, gyrencephalic hydrocephalic ferrets may not experience behavioural improvements or white matter protection from MgSO₄ therapy, which may be the same for humans with even more complex brains.
Introduction: Since their discovery as the suicide bags of the cell, lysosomes have been explored as therapeutic targets in cancer therapy. However, the efficacy of lysosomal disruption is unknown in chronic lymphocytic leukemia (CLL): the most common adult leukemia in the western world. Therefore, the purpose of this work was to determine if CLL cells were susceptible to lysosome-mediated cell death, and if so, how.

Methods: Primary CLL cells were isolated and cultured from peripheral blood donated by CLL patients through CancerCare Manitoba. Cells were treated with various inhibitors and drugs then stained with various dyes or lysed for western blot analysis.

Results: Upon testing various lysotropic drugs, it was discovered that siramesine was the most effective. Furthermore, this drug was more effective in CLL cells, even those lacking the tumor suppressor p53, compared to healthy B cells. With the use of dyes specific for the lysosome and mitochondria it was determined that siramesine first permeabilizes the lysosome and then the mitochondria leading to cell death. As lysosomes were permeabilized, production of lipid reactive oxygen species ensued, which were required to kill the cell. Translocation of TFEB into the nucleus further confirmed that lysosomes were destabilized. Unlike other model systems, cathepsins and caspases were not required for cell death in CLL cells. To determine how lysosomes may be altered in CLL cells compared to healthy B cells, we focused on changes in lysosomal proteins and sphingolipid metabolic enzymes, as sphingolipids have been previously shown to alter lysosomes. Sphingosine 1 phosphate phosphatase 1 (SPP1) was greatly over-expressed at the protein level in CLL cells compared to healthy B cells. Adding in the product of this enzyme, sphingosine, greatly enhanced lysosome-mediated cell death. Furthermore, inhibiting an enzyme that uses sphingosine also enhanced lysosome-mediated cell death. Current efforts are focused on knocking down SPP1.

Conclusions: Together these results show that CLL cells are susceptible to lysosome-mediated cell death in part due to altered sphingolipid metabolism. This work explores a novel therapeutic target in CLL: lysosomes. Development of new therapies may lead to the optimal treatment and cure of CLL.
As the population ages and retirement is delayed, the workforce is aging. This means that ailments that typically plague older individuals (ex: joint stiffness, impaired mobility), may affect these older workers. It is therefore essential to determine whether there is an increase in stiffness with age. In order to answer this question, one must first define the parameters. Both age and stiffness can be defined in two ways. Age can be defined based on the calendar (chronological), or based on the overall health of the individual (biological). Stiffness can be defined in terms of either active or passive stiffness. Active stiffness is the ability of a person to respond to a perturbation, and return to equilibrium. Passive stiffness is the inherent stiffness or flexibility of the soft tissues. In the current study, twenty subjects (10 males and 10 females) will be recruited from each of 6 age groups: 20-25 years, 30-35 years, 40-45 years, 50-55 years, 60-65 years, and 70-75 years). Their height and BMI will be recorded. This anthropometric data will be used to match subjects across age groups. Biological age will be estimated using 11 biomarkers which have been shown to be associated with age. These biomarkers include: VO₂ max, percent body fat, waist circumference, forced expiratory volume, blood pressure, hearing threshold, and five blood-protein concentration levels. Additionally, two custom-built devices will be used to estimate active and passive trunk stiffness. It is expected that an increase in passive stiffness and a decrease in active stiffness will be seen with increased biological age, but not necessarily with increased chronological age. The health and fitness habits of those chronologically older individuals, who do not show the same changes in stiffness could help lead to the development of interventions that would reduce these changes in all aging workers, thereby decreasing their risk for injury in the workplace. It is thought that these interventions would apply to all joints, not just to the low back, thereby decreasing the risk of injury cause by trips and fall, or other events.
Murine Pulmonary Slowly-Adapting Receptors (SARs): Putative Links to Neuroepithelial Body (NEB) Hypoxia Chemoreception and the Calcium Sensing Receptor (CaSR)

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Introduction: Pulmonary SARs are mechanosensitive vagal afferents, which Adriaansen (J Appl Physiol, 2006) proposed to be functionally linked to morphologically-defined, hypoxia-sensitive NEBs (Youngson et al. 1993, Nature). Post-natal NEBs express CaSR (Lembrechts et al. 2013, J Cell Sci), and vagal NEB innervation expresses P2X2 and P2X3 ATP receptors (Brouns et al. 2009, Histochem Cell Biol).

Methods: We tested in vivo murine SAR responses to quasi-static inflation (0-20 cmH2O) in: 1) hypoxia (10% O2) 2) absence of CaSR (CaSR / parathyroid hormone double-knockout mouse, KO) and 3) P2 purinergic receptor blockade (Suramin: 50 mg/kg). SAR mechanosensitivity was defined as action potential frequency (f) at tracheal pressures of 5, 10, 15, and 20 cmH2O.

Results: Hypoxia caused a small but statistically significant increase in f (9.6±2.8% at 20cmH2O; P = 0.002, mean±SEM). Loss of CaSR significantly reduced f at all pressures (-34.9% and -39.5% at 15cmH2O in hyper- and hypoxia, respectively; P < 0.001) with no impact of hypoxia within each genotype. Suramin caused a small but significant reduction in f (-10.6±1.5% at 20cmH2O; P = 0.01).

Conclusion: These data suggest that CaSR significantly impacts SAR mechanosensitivity, while purinergic signaling and hypoxia exert modest effects with as yet to be determined physiologic roles.
Blocking Orexin Receptors in the Midline Thalamus Has No Effect on Conditioned Fear but Has Anxiolytic Effects

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The paraventricular nucleus of the thalamus (PVT) is the major part in the midline thalamus where receives and projects to multiple structures in the limbic system. Some recent evidence indicates a role for the PVT in the expression of conditioned fear. Furthermore, the PVT receives a high density of orexin projections and the blockade of orexin receptors in the PVT will have an anxiolytic effect. The present study focused on the role of orexin receptors in PVT in the expression of conditioned fear. An orexin receptor antagonist was administrated in PVT, and its effect on fear expression was observed in both cued and contextual fear conditioning paradigms. Infusion of 0.5 µl of the antagonist at a concentration of 0.1, 1.0, and 10 nmol had no effect on the freezing produced by exposing rats to an auditory cue or the context associated with foot shock. In contrast, the 1.0 and 10 nmol doses were anxiolytic in an anxiety test. The results of the present study do not support a role for orexin receptors in the PVT in the expression of learned fear. The finding that blocking of orexin receptors in the PVT region reduces anxiety is consistent with other studies indicating a role for orexins in PVT in anxiety-like behaviors.
Distinct Oxylipin Profiles are Synthesized by Human EA.hy926 Endothelial Cells in their Proliferating and Quiescent States

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Introduction: Endothelial cells have a critical role in maintaining vascular homeostasis, and their dysfunction is believed to be the initial step in atherosclerosis development. Dietary fatty acids and oxylipins, oxygenated products of polyunsaturated fatty acids generated by three enzymatic pathways – lipoxygenase, cyclooxygenase and cytochrome P450, have been shown to affect endothelial functions associated with the inflammatory response. In the current study, the fatty acid and oxylipin profiles from growing (subconfluent) and quiescent (confluent) human endothelial (EA.hy926) cells were compared.

Methods: Cells were harvested at subconfluent and confluent states. Fatty acid composition of the cells was analyzed by gas chromatography while oxylipin profiles of the cells and culture media that they were growing in were determined by HPLC-MS/MS.

Results: Fatty acid analyses revealed that the oxylipin precursors linoleic acid (LA), arachidonic acid (AA), and docosahexaenoic acid (DHA) were higher in growing compared to quiescent cells (P<0.05). In contrast, 12,13-dihydroxy-octadecenoic acid (diHOME) and 9-hydroxy-octadecadienoic acid (HODE) derived from LA, 18- and 11-hydroxy-eicosatetraenoic acid (HETE) derived from AA, 20-hydroxy-docosahexaenoic acid (HDoHE) derived from DHA, and 5-hydroxy-eicosatrienoic acid (HETrE) derived from mead acid (MA) were all lower in subconfluent cells. Oxylipins secreted into the culture media showed differences in oxylipins derived from LA (12,13-diHOME, 9,10 diHOME, 9,10,13-trihydroxy-octadecenoic acid (triHOME), 9,12,13-triHOME), AA (prostaglandin F2 alpha (PGF2 alpha), 18-HETE, 12-HETE, 15-HETE, 11-HETE and 9-HETE), dihomo-gamma-linoleic acid (DgLA, 15-HETrE and 8-HETrE), MA (5-HETrE), eicosapentaenoic acid (EPA, 18-hydroxy-eicosapentaenoic (HEPE) and 15-oxo-eicosatetraenoic acid (oxoETE)) and DHA (11-HDoHE, 14-HDoHE, 4-HDoHE, 7-HDoHE, and 8-HDoHE)(P<0.05). These results reveal that all three enzymes are active in endothelial cells. The greater number of oxylipins detected in the culture media compared to the cell suggests that endothelial cells employ oxylipins as paracrine factors.

Conclusion: This study is the first to show that fatty acid profiles do not parallel their corresponding oxylipin profiles from cell extracts of both growing and quiescent endothelial cells. These findings indicate the synthetic pathways leading to oxylipin production from fatty acids are significantly different in subconfluent and confluent endothelial cells.
Evaluating of the Relationship between Pulmonary and Cognitive Function in Aging: Results from a Systematic Review and Development of a Multi-study Research Program

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Introduction: Accumulating evidence indicates an association between poor pulmonary function and impaired cognition. However, most research is correlational or relies on single-occasion measurements of either pulmonary or cognitive functioning. To address these issues, we evaluated the current literature and developed a multi-study research program.

Methods: Using the PRIMSA statement, we conducted a systematic review of the literature on longitudinal studies that examined the association between pulmonary and cognitive functioning in adults in order to understand which (if any) measure of decline in pulmonary functioning are associated with declines in cognition.

Results: Of the 582 unique records identified in the search, 4 articles, belonging to 3 different longitudinal studies of aging, met the developed protocol criteria. The findings across these studies were highly discrepant, ranging from no or modest association to significant associations between pulmonary function and various cognitive domains. Interestingly, each study employed exceedingly different statistical approaches and controls for potentially influential covariates.

Conclusion: Mixed findings within the current literature on the longitudinal association between pulmonary function and cognition indicate that further research is needed. Coordinated application of the same statistical models across multiple studies was identified as the best approach to address the identified weaknesses in the literature. As a result, we have initiated a multi-study evaluation of pulmonary function and cognition through the Integrative Analysis of Longitudinal Studies of Aging network, which incorporates 8 different international longitudinal studies into a collaborative research program. Further, this program practically addresses identified research barriers by evaluating numerous pulmonary and cognitive measures, harmonizing variables across studies, systematically addressing important covariates, and employing reproducible research methodologies.
The Role of DCC Proteolysis in Axon Outgrowth and Guidance

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Introduction: The establishment of functional neuronal circuits requires a large number of precisely coordinated events. Before communication between neurons can take place, an axon (output) must come in close proximity to its target dendrite (input). The netrin/DCC signaling pathway was identified as one of the key signaling pathways involved in axon guidance. It has been demonstrated that DCC is highly serine/threonine phosphorylated but its functional significance remain elusive. In addition, DCC was shown to be cleaved by several proteases. Receptor proteolysis is a conserved regulatory mechanism during axon outgrowth and guidance. We propose that threonine phosphorylation protects DCC from proteolysis and explore its functional significance during axon outgrowth and guidance.

Results: We identified threonine 1210 as a phosphorylation site in the cytoplasmic tail of DCC. Surprisingly, the expression of both phospho-null mutants T1210A and T1210V in cultured HEK 293 cells led to a truncated protein lacking part of the cytoplasmic tail. We next generated a phospho-mimicking mutant DCC–T1210E and found that this mutant behaved like the wild-type DCC, suggesting that the negative charge is protective. We found that DCC–T1210V and T1210E, but not –T1210A were localized at the cell surface. To determine the identity of the protease, cells were treated with various protease inhibitors. Interestingly, we found that the endogenous calpain inhibitor, calpastatin, was able to partially block proteolysis. Indeed, calpain was able to cleave DCC in vitro. We next wanted to determine if DCC could form a complex with calpastatin to further support the hypotheses that calpain cleaves DCC and that this is inhibited by calpastatin. We did observe the presence of both DCC and calpastatin within the same complex when overexpressed in HEK 293 cells. To test the effect of netrin-1 on this interaction and to see if this interaction occurs with endogenous proteins, we stimulated E17.5 primary rat cortical neurons for various time-points and found that DCC interacted at 0 and 5 minutes and that this interaction disappears thereafter.

Conclusion: We propose a novel role for Calpain in regulating the netrin/DCC signaling pathway by DCC proteolysis.
Inhibition of LAR and PTPσ Receptors Blocks CSPGs Inhibitory Effects on Spinal Cord Neural Precursor Cells
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Introduction: Multipotent neural stem/progenitor cells (NPCs) reside in the spinal cord and are capable of replacing lost oligodendrocytes following spinal cord injury (SCI). Despite this intrinsic capacity, adult spinal cord NPCs mainly differentiate into astrocytes, with only a limited number becoming oligodendrocytes. This evidence emphasizes a key role for the post-SCI niche in modulating the regenerative response of spinal NPCs. We have reported that injury-induced upregulation of chondroitin sulfate proteoglycans (CSPGs) potently restricts the survival, integration and differentiation of transplanted and endogenous NPCs in SCI. Given the inevitable and long-lasting upregulation of CSPGs in NPCs niche after SCI, it is important to unravel the potential mechanisms by which CSPGs influence the properties of NPCs.

Methods: Using in vitro models recapitulating the extracellular matrix of SCI, we investigated the direct role of CSPGs and its signaling receptors protein tyrosine phosphate receptor sigma (RPTPσ) and leukocyte common antigen-related phosphatase (LAR) in modulating adult spinal cord NPCs. In primary cultures of adult spinal cord NPCs, using cell viability, western blotting, and immunocytochemistry assays, we assessed CSPGs direct effects on NPC growth, attachment, survival, proliferation and differentiation.

Results: We show that CSPGs negatively modulate the properties of spinal cord NPCs by decreasing their growth and attachment, survival, proliferation and oligodendrocytes differentiation. Genetic down-regulation of CSPG receptors protein tyrosine phosphate receptor sigma (PTPσ) and leukocyte common antigen-related phosphatase (LAR) in NPCs attenuated the inhibitory effects of CSPGs on NPCs. Similarly, pharmacological inhibition of LAR with inhibitory LAR peptide (ILP), and of PTPσ with inhibitory sigma peptide (ISP), significantly blocks all of CSPGs inhibitory effects on NPCs. At the intracellular level, we find that CSPGs inhibitory effects are mediated through the Rho/ROCK pathway and inhibition of Akt and Erk1/2 phosphorylation.

Conclusion: Our data suggest the impact of CSPGs and its signaling receptors in governing the response of NPCs in their post-SCI niche, and identify new therapeutic targets for enhancing NPC-based therapies following SCI.
An Essential Role of Chromogranin-A In Pathogenesis of Experimental Colitis

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Lesions accompanied by a prominent infiltrate of activated immune cells, and alteration in chromogranin A (CgA) producing enterochromaffin (EC) cells. Due to the strategic location of EC cells in gut mucosa, it is very likely that CgA plays an important role in immune activation and regulation of inflammation in various intestinal disorders including IBD. In this study, we investigated the role of CgA in the development of colitis in a model of experimental colitis mimicking ulcerative colitis in CgA deficient mice.

Methods: Colitis was induced in CgA-deficient on C57BL/6 background (CgA⁻/-) mice that lack CgA, and wild type (CgA⁺/⁺) mice by dextran sulfate sodium (DSS 5%) in drinking water for 5 days. Disease activity index was evaluated daily after induction of colitis. At sacrifice, composite macroscopic score including diarrhea, hyperaemia, thickness and adhesion were evaluated. Myeloperoxidase activity (MPO), pro- and anti-inflammatory cytokines levels were quantified using RT-qPCR.

Results: Delayed onset and decreased severity of clinical disease as assessed by loose stools, weight loss and rectal bleeding were observed in CgA⁻/- mice as compared to CgA⁺/⁺ mice after induction of colitis by DSS. No rectal bleeding and loose stools were detected in CgA⁻/- mice and weight lost was 4 % vs. 36% in CgA⁻/- and CgA⁺/⁺ mice, respectively on day 4 post-DSS. Macroscopic and histological damage scores were significantly decreased by 39 % and 43 % in CgA⁻/- mice as compared to CgA⁺/⁺ mice, on day 5 post-DSS. This was correlated with a significant down-regulation of MPO activity, and production of IL-1β, IL-6, Mcp-1 and TNF-α, conversely a significant up-regulation of IL-10 was detected in CgA⁻/- mice compared to CgA⁺/⁺ mice.

Conclusions: These observations suggest that lack of CgA in CgA-deficient mice reduced the severity of DSS-induced colitis and that CgA is critical in the pathogenesis of inflammation in that model of experimental colitis. These observations endow new insights into the mechanisms of inflammation, which may ultimately lead to improved therapeutic strategies to combat gastrointestinal disorders.
Progressive Post-Operative Contralateral Hippocampal Atrophy Following Temporal Lobe Epilepsy Surgery

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Introduction: Medically refractory temporal lobe epilepsy is an extremely disabling focal epilepsy. Surgical resection of the anterior temporal lobe (ATL) or only the amygdala and hippocampus (AH) achieves seizure control in 58-75% of patients. However, the optimal surgical approach and the neurological consequences of surgery remain controversial. Neuroimaging is a non-invasive way of comparing surgical approaches and assessing their consequences on brain structure.

Methods: Our objective was to determine the time course and extent of changes in the hippocampus—a critical memory structure—following epilepsy surgery. We hypothesized that contralateral hippocampal volume would increase following surgery because of seizure control.

We recruited 26 adult epilepsy patients referred for surgery and 9 healthy controls and obtained serial T1-weighted MRI brain scans pre- and post-operatively. Hippocampal volume was measured by a blinded observer using quantitative volumetry. Patients were imaged in two groups: 1) a longitudinal group [ATL (n=8); AH (n=2)] on post-operative days 1, 2, 3, 6, 60, 120 & >360; and 2) a single post-op group [ATL (n=6); AH (n=10)] obtained on average at >2100 days.

Results: In the single scan group the mean hippocampal volume decreased significantly (73.2 ± 8.5%; p<0.001) relative to baseline. In the longitudinal group, there was statistically significant and progressive atrophy in hippocampal volume from baseline to post-op day 4-8 (72.6 ± 6.5%; p<0.001), post-op day 60-360 (69.7 ± 12.3%; p<0.0001) and >360 days (58.5 ± 10.6%; p<0.0001). There was no significant atrophy over time in the hippocampal volume in the control group. There was no statistically significant difference in hippocampal volume at the most delayed exam day compared by surgery type (ATL or AH; p=0.13).

Conclusions: We report novel evidence of a dramatic contralateral hippocampal atrophy following epilepsy surgery as early as 24-72 hours post-operatively; furthermore, this atrophy progresses over time. Atrophy is independent of the type of surgery (ATL or AH) and may have implications for memory function after epilepsy surgery. Our next step is to assess other structural consequences of temporal lobe epilepsy surgery, and how they relate to seizure control, disease duration, and long-term neuropsychological outcome.
Centering Women and Newborns in Health Human Resources (HHR) Planning: A Needs-Based Approach to Primary Maternity Health Care in Nova Scotia

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Introduction: In the midst of a global health human resources (HHR) crisis and with increasing health needs, new ways of designing health care in all clinical settings, including primary maternity health care is required. The purpose of this sequential quantitative-qualitative mixed methods study was to answer the primary research question: What are the primary maternity health care needs of women and newborns in Nova Scotia (NS)?

Methods: Informed by established HHR frameworks, data from the Canadian Community Health Survey (CCHS) and the NS Atlee Perinatal Database were analyzed to determine the predictors of health needs of women and newborns based on various health needs indicators. Focus groups and interviews with women, health care providers and health leaders were analyzed using thematic analysis.

Results: Associations between specific determinants of health and maternal and newborn health needs were identified in the initial quantitative analysis and used to inform data collection and discussions in the qualitative phase. Using multiple regression analysis, a number of predictors (associated with a broad definition of health) were found to increase maternal and newborn health needs. Qualitative analysis identified that women, health leaders and providers recognize a lack of patient-centredness in our current system influenced by differing philosophical approaches, professionalization and health care funding models as well as a need for interprofessional and full scope practice. Together, the integrated findings provide a contextualized, comprehensive understanding of maternal and newborn health needs as well as statistically significant, generalizable findings. Improvements to how we understand and measure health and health needs to inform how we design and deliver health care were also identified.

Conclusion: The design of health human resources and the delivery of primary maternity health care require creating diverse health workforces with the competencies for culturally sensitive, community-focused care provided by a team of providers who are grounded in the principles and skilled with the practices to address the multiple factors that impact health. The integrated findings from this research will inform HHR and health system planning in Nova Scotia and will also identify gaps in services for specific populations of women to inform targeted planning.
Glucagon-Like Peptide-1 Reduces Chylomicron Secretion despite Adequate Intestinal Lipid Availability

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Introduction: Chylomicron overproduction and postprandial dyslipidemia are common features of insulin resistance and the metabolic syndrome. This results in heightened levels of atherogenic chylomicron remnants that can increase the risk of cardiovascular disease. Recently, the hormone glucagon-like peptide-1 (GLP-1) has been shown to impair chylomicron production and prevent the development of diabetic dyslipidemia. The mechanism by which this occurs is unclear, but GLP-1 is known to slow gastric emptying which may contribute to its effects on chylomicron output. Our study aimed to assess whether GLP-1 could still lower chylomicron production even during adequate intestinal lipid availability.

Methods: Hamsters were administered olive oil intraduodenally to bypass the stomach, preceded by an intraperitoneal injection of the GLP-1 receptor agonist exendin-4 or vehicle. They then received triton intravenously to prevent lipoprotein catabolism and clearance. Plasma was collected over 4 h and the triglyceride (TG)-rich lipoprotein (TRL) fraction of the plasma was isolated for TG and apolipoprotein B48 (apoB48) measurements, the latter reflecting the number of chylomicron particles present. Particle size was assessed by FPLC, and jejunum was collected for additional measurements.

Results: Exendin-4 treatment had no effect on plasma or TRL TG levels, but significantly lowered TRL-apoB48 levels at 4 h. This resulted from an increase in lipoprotein particle size (TG loading), despite the presence of fewer particles. While exendin-4 had no effect on 4 h jejunal activity of microsomal TG transfer protein, responsible for apoB48 lipidation, there was an increasing trend in jejunal tissue TG levels (p = 0.084). Furthermore, a reduction in jejunal CD36 levels was observed at 4 h with exendin-4 treatment, and CD36 is needed for proper formation of pre-chylomicron transport vesicles for intracellular chylomicron trafficking within the enterocyte.

Conclusion: Overall, we demonstrate a role for GLP-1 in reducing the number of chylomicron particles secreted by the intestine, even during adequate lipid availability and elevated particle lipidation. This was associated with lower jejunal levels of CD36, involved in intracellular chylomicron trafficking. Future studies will aim to assess the effects of GLP-1 on intracellular signaling pathways linked to chylomicron trafficking, providing mechanistic insight into the actions of this anti-diabetic hormone.
Knockout of the Putative AdeFGH Drug Efflux Pump Regulator AdeL in A. baumannii ATCC 17978
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Introduction: The gram-negative pathogen Acinetobacter baumannii is found in hospital settings causing major morbidity and sometimes death in immunocompromised patients. The organism generates drug resistance through intrinsic and acquired means and causes complications during treatment. The Resistance Nodulation Division Efflux (RND) pumps are intrinsic mechanisms in gram negative bacteria capable of expelling antibiotics, detergents, dyes and metals. RND expression is controlled by local regulators that are divergently transcribed. AdeL, is a putative regulator of adeFGH expression. AdeFGH has been shown to efflux multiple classes of antibiotics including fluoroquinolones, chloramphenicol, trimethoprim and clindamycin.

Methods: Creation of ΔadeL ATCC 17978 was carried out using SOEing PCR, incorporating gentamycin (Gm) marker for selection of adeL mutants and flippase recognition target (FRT) sites for excision of Gm marker. Growth curves performed in a 96-well format. Minimum inhibitory concentrations (MIC) for antibiotics were determined using the two-fold broth dilution method.

Results: The fitness of strains lacking the adeL regulator did not show any significant difference compared to the wild type strain ATCC 17978. The measurement of minimum inhibitory concentrations in the adeL knockout against the wild type strain ATCC 17978, showed at least 2-fold increased susceptibility to chloramphenicol and clindamycin. No change was observed for moxifloxacin and trimethoprim.

Conclusion: The AdeL regulator may not play a major intrinsic role in drug resistance seen in ATCC 17978 with respect to regulation of adeFGH expression. Clinical isolates that have shown mutation in adeL causing overexpression of AdeFGH and decreased susceptibility to antibiotics may be due to other uncharacterized mutations in the genome. Clean deletion of the adeL gene allows for study of changes in gene expression without the presence of disruption marker in a characterized strain. Subsequent studies using RNA-seq and proteomics will help to elucidate the function of AdeL.
**The Regulation of Ski and Scleraxis in Mediating Pathological Fibrosis in the Post-MI Heart**

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Introduction: Coronary heart disease is the foremost cause of congestive heart failure, which is often attended or caused by severe pathologies including myocardial infarction (MI) and cardiac fibrosis. Upon myocardial injury, resident cardiac fibroblasts phenoconvert into myofibroblasts and synthesize large amounts of fibrillar collagens to produce scar tissue. Although the majority of myofibroblasts are removed from the infarcted area following wound closure a sub-population of cells persist in the wounded area, leading to maladaptive chronic remodeling of the myocardium. The Ski protein has been identified as a key inhibitor of the canonical transforming growth factor-β1 (TGF-β1) pathway, attenuating the myofibroblast phenotype and its functional properties. Conversely Scleraxis has been implicated in canonical TGF-β1 signaling by working together with Smad3 to promote collagen1α2 expression. The purpose of this study is to investigate how Ski and Scleraxis contribute to physiological and pathological wound healing in vivo and identify whether these two proteins would serve as therapeutic targets to limit fibrosis in a living system.

Methods: 64 male Sprague-Dawley rats underwent ligation of the left anterior descending (LAD) coronary artery ligation to induce a myocardial infarction. Control (sham) operated animals underwent surgery without ligation of the LAD artery. Animals were sacrificed at 2, 4, and 8 weeks post-MI and tissue collected for Western blot and qPCR studies.

Results: Scleraxis mRNA expression remains at baseline at 2 and 8 weeks post-MI, but is increased 4 weeks post-MI. Scleraxis protein expression was down regulated within the scar area of infarcted hearts as compared to control samples 2 and 4 weeks post-MI. Ski mRNA expression is upregulated within the scar area of infarcted hearts 2, 4 and 8 weeks post-MI.

Conclusions: Our results indicate that Scleraxis may undergo down regulation or degradation within the scar during the chronic stages of myocardial infarction. Though Ski mRNA is increased within the scar area, protein expression remains to be elucidated. By tracking the expression and localization of Ski and Scleraxis we hope to learn more about the mechanisms they are involved in and how they influence the progression of cardiac fibrosis.
Microbial Analysis of Intestinal Biopsies Investigating the Association of Anatomical Site and Inflammatory Status in Patients with Inflammatory Bowel Disease

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Crohn’s disease (CD) and ulcerative colitis (UC) are clinically distinct prototypes of inflammatory bowel disease. They are multifaceted intestinal disorders with uncertain etiologies thought to be influenced by a dysbiosis (microbial imbalance) of the gastrointestinal tract. We resected biopsies from different mucosal sites (ileum, cecum, colon, rectum) at colonoscopy and histologically defined them as inflamed or noninflamed. We performed 16S rRNA sequencing to analyze population structures. Quality control and OTU classification of reads were performed using mothur with statistical analyses executed in the R package, phyloseq. The structure of microbial communities varied among CD and UC patients and healthy controls. Genera including Fusobacterium (p=0.05) and Marinobacter (p=0.04) were more abundant in inflamed CD versus inflamed UC. The abundance of Clostridium (p=0.008) and Haemophilus (p=0.0007) were elevated in noninflamed CD versus noninflamed UC. Within each disease, different segments of the intestine demonstrated insignificant variation of microbial communities. However, for CD, the abundance of Pseudomonas (p=0.003) varied between anatomical sites, which were highest in the colon and rectum. Mucosal sites between disease groups presented more conclusive results. Bacteroides (p=0.03) was highest in the ileum of UC. In the colon, Pseudomonas (p=0.0002), Sporacetigenium (p=0.05) and Actinomyces (p=0.01) were more prevalent in CD. Pseudomonas (p=0.0007) and Bacteroides (p=0.0007) in the rectum were most abundant in healthy controls and UC, respectively. Distinct microbial communities were observed between disease groups dependent on mucosal site and whether inflamed or noninflamed tissue was sampled. The relative lack of intra-individual variation among mucosal sites presents similarly to previous studies.
Nanoparticles Encapsulated with Serpin A1 and LL37 Promote Wound Healing *In Vitro* and Possess Antibacterial Properties

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**Introduction:** Chronic wounds (CWs) are a serious health care concern as they fail to heal and are characterized by a “non-resolving” chronic inflammatory phase. Most current therapies target only a single aspect of the CW process making it difficult to achieve effective healing; hence, a new paradigm is urgently required. Serpin A1 (α1 anti-trypsin), is a neutrophil elastase inhibitor and an anti-inflammatory agent in CW healing. LL37, a host defense peptide possesses anti-infective, and wound healing properties. Thus, the combination of these peptides (A1:LL37) may show synergistic potential in accelerating healing as well as establish control over the bacterial bio-burden, when delivered to the wound site simultaneously over a prolonged period using nanoparticles.

**Objective:** To evaluate nanoparticle encapsulated with A1:LL37 as a new potential combination strategy for accelerated wound healing and antibacterial properties.

**Methods:** Solid lipid nanoparticle (SLNs) encapsulated with A1:LL37 were made using solvent-diffusion double emulsion technique. The *in vitro* release study was performed in artificial wound fluid (pH 7.4) as release media, incubated in an orbital shaker at 37 °C. Aliquots were withdrawn at fixed intervals and analyzed by HPLC. The *in vitro* wound healing assay and cytotoxicity studies were performed on fibroblast cells. Minimum inhibitory concentration of A1:LL37 against *E.coli* and *S.aureus* and synergy of the drug combination was evaluated using checkerboard microtiter plate assay.

**Results:** SLNs had an average particle size of 210 ± 5.6 nm and encapsulation efficiency of 85.4 ± 3.2. In *in vitro* release studies in artificial wound fluid demonstrated a daily release of ~15.2% of the initial loaded concentration of A1:LL37, over a 72 h study period. *In vitro* scratch assay showed high level of healing in fibroblasts treated with A1:LL37 loaded SLNs (500 μg each), in comparison to untreated cells. No cytotoxicity was observed when fibroblasts were treated for 24 h with drug-free SLNs as well as A1:LL37 SLNs. ΣFICI of A1:LL37 was ≤ 0.5, demonstrating synergy in comparison to individual drugs alone.

**Conclusion:** This is the first study to develop a nanoparticle formulation for the combination delivery of serpin A1 and LL37 as a novel strategy for the treatment of chronic wounds.
Effect of Transcription Factor Accessibility on Endogenous Versus Ectopic Expression of Placental Members of the Human Growth Hormone Gene Family

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The human (h) growth hormone (GH) and chorionic somatomammotropin (CS) gene family has been used as a model system to study tissue-specific expression, and specifically the role of histone modifications, DNA methylation, chromatin remodeling as well as transcription factor availability and accessibility. In spite of considerable (>90%) nucleotide sequence similarity, hGH-N is preferentially expressed in the pituitary, while the genes coding for chorionic somatomammotropin (hCS-A and hCS-B) and placental GH (hGH-V) are expressed efficiently in the placenta; the fifth gene, hCS-L, is a pseudogene. Less is known about the control of placental gene members, although they are expressed differentially (hCS-A>hCS-B or hGH-V) and transcripts are detected at extremely low levels in human choriocarcinoma BeWo, JAR and JEG-3 cells. There are, however, reports of hGH/CS transcripts detected outside of the placenta, including tissue/cells of breast, dermal, ovarian, testis and uterine origins. Here, we have begun to assess: (1) the relative expression of hGH/CS gene expression in human term placenta versus cells of placental and non-placental origin, and if detected the relative expression of hCS-A, hCS-B and hGH-V; and (2) whether the expression observed corresponds to availability of transcription factors capable of trans-activating the hCS promoter and/or (downstream) enhancer region. Assessment of relative hGH/CS RNA levels by RT-PCR using a common set of exon 3/exon 4 primers reveals that expression is ~80-fold higher in human term placenta versus non-placental tissue. Transcripts were detected in placental BeWo, JAR and to a much lesser extent JEG-3 cells, but were ~90-fold lower than in term placenta. Low levels, comparable to JEG-3 cells, were also detected in non-placental HeLa (cervical carcinoma), MCF-7, T47D (breast cancer) and Hec1A (uterine adenocarcinoma) cells. Specific assessment of hCS-A, hCS-B and hGH-V transcripts reveal >4-fold higher levels in placental BeWo and JAR versus JEG-3 cells, which in turn was comparable to non-placental HeLa and MCF-7 cells. To assess available levels of CS promoter and enhancer factors, placental and non-placental cells (~60% confluent) were transfected with hybrid luciferase genes; the activity of the hCS-A and hGH-N promoter (0.5 kilobase) region was compared, as well as the effect of the hCS-B downstream enhancer region on CS promoter activity. There was no
apparent preference for use of the hCS versus hGH promoter, but a >10-fold potential for activation by the CS enhancer region was observed in BeWo, JAR and JEG-3 versus non-placental HeLa and MCF-7 cells. These observations suggest that low hCS expression in placental and non-placental cells versus term placenta is related, at least in part, to inaccessibility of regulatory sequences, but predict different outcomes if these regions, including the enhancer, were exposed.
Determining the Function of Transcription in Targeting Activation-Induced Cytidine Deaminase (AID) To Immunoglobulin and Oncogene Loci

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Introduction: Activation-induced cytidine deaminase (AID) mutates antibody genes in B lymphocytes to enhance antibody response. AID is required for efficient immunity. However, AID also mutates off-target leading to cancer. Therefore, understanding AID targeting to different genes is important. AID mutates single-stranded DNA (ssDNA) and acts on actively transcribed genes. There are two proposed models for AID targeting: first, binding partners chaperoning it to specific genes, and second, recruitment by specific DNA sequences. We and others showed that AID does not require any other proteins for activity and that many proposed interactions are likely artificial. We suggest a new model wherein the primary sequence of genes, combined with dynamics of their transcription determine the amount of ssDNA available for AID to act on.

Methods: To test our hypothesis, we developed a cell free transcription/deamination (T/D) system where AID acts in concert with transcription on the same stretch of DNA. We optimized T/D for a variety of targets including super coiled, relaxed or linear DNA. To examine the impact of transcription on AID activity, we are able to fine-tune transcription dynamics, such as initiation and/or elongation rates of the RNA polymerase. Using a PCR-based method, we can analyze mutation frequencies and spectra on each individual DNA molecule in the reaction. If our hypothesis is correct, we expect to find that the primary sequence of each gene, and transcription dynamics, determine the pattern and frequency of AID mutations in a unique manner. A secondary prediction of our hypothesis is that AID would have co-evolved with the sequences of antibody genes. To test this, we have purified AID from numerous species that are phylogenetically distant, and we will each with its cognate antibody genes in our T/D assay.

Results and conclusion: We found that AID can indeed mutate breathing double-stranded DNA in the absence of any cofactors at similar rates to its action at the antibody genes during an immune response in vivo. We also found that transcription initiation and elongation rates regulate AID activity. These results strongly support our model that AID targeting is primarily a function of DNA sequence and topology.
Research Tool Development for Prenatal Nutrition among First Nations Women Living In Manitoba: Implications for FASD

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Introduction: First Nations communities often face health inequalities and are at greater risk for disease than the overall population. Fetal Alcohol Spectrum Disorder (FASD) is one health issue that is over-represented. Optimal prenatal nutrition plays a role in the severity of FASD and is an under-researched area among First Nations women. Therefore, studies that determine food intake patterns and nutrient status of pregnant women who may be consuming alcohol during pregnancy are needed. However, there is no nutrition research tool that has been developed for pregnant First Nations women who are at risk for alcohol use. Therefore, it is necessary to develop an effective, culturally appropriate and interactive nutrition research tool. This study, as the first phase of a series of studies, will address this gap by: (1) developing a culturally appropriate, interactive iPad research tool that combines nutrition, alcohol consumption, pregnancy, and determinants of health, and (2) pre-testing and evaluating the research tool with a sub-sample of the population.

Methods: Determined top sources of 5 key brain development nutrients, engaged with prenatal health workers from 14 First Nations communities, converted the tool to an iPad app with the help of a software development firm, Function Four, had prenatal health workers who support at-risk pregnant women review the tool, and completed a pre-test and evaluation with several at-risk pregnant women.

Results: Results show positive and valuable feedback from reviewers and pre-test participants which will be used to finalize the tool.

Conclusion: This study, and the research tool developed, will pave the path for future prenatal nutrition research focused on First Nations women which will help inform programs and policies which strive to improve food and nutrition security and ultimately reduce the severity of FASD.
Introduction: The high frequency of poor surgical outcomes and chronic pain after shoulder rotator-cuff injury prompted this study which explored the potential for promoting muscle regeneration as a way to improve function after injury. Muscle was studied to test the hypothesis that muscle stem-cells in injured supraspinatus will be more responsive to activation by a nitric oxide donor-drug, isosorbide dinitrate (ISDN) than those from control muscle, and show a correlation to changes in fiber size and distribution, acetylcholine receptor (AChR) pattern, and AChR subunit ratio.

Methods: During arthroscopic surgery biopsies from 13 participants were collected for histological analysis (fiber diameter) and α-bungarotoxin staining (AChRs). A portion of each biopsy was cultured for 40-hr with a nucleotide, bromodeoxyuridine (BrdU), and with/without ISDN, to activate satellite cells. Cell activation was quantified via immunostaining analysis of BrdU nucleotide incorporation. The γ:ε AChR subunit ratio was also examined as an indicator of denervation. Ipsilateral deltoid was used as a control and t-tests and Chi-squared analysis were used to determine significance.

Results: Mean age was 57.6-years (49-65) and 12 participants had a full-thickness tear. Histological analysis revealed signs of atrophy in the supraspinatus, including a higher frequency of small-diameter fibers than control and a smaller mean diameter (17.9±0.3µm) than control (21.8±0.3µm, p< 0.01). Alpha-bungarotoxin staining revealed the supraspinatus tended to have more linearly-arranged AChRs than clusters. The supraspinatus also tended to have a higher γ:ε AChR subunit ratio. Culture with ISDN induced a significant increase over baseline (up to 1.8-fold), in the active (BrdU+) proportion of Pax7+ satellite cells in supraspinatus, but not in control, after 40 hr in culture.

Conclusions: Supraspinatus muscle is atrophic relative to control, and results suggest that NO-donor treatment could be used to promote muscle growth in atrophied supraspinatus of rotator cuff injury and may improve surgical success. Injured muscle exhibits features that may suggest denervation at the time of reparative surgery including a trend toward more AChRs in a linear arrangement, and a trend toward a higher γ:ε AChR subunit ratio, than control. These features would increase muscle susceptibility to traumatic injury and failure to repair tendon tears surgically.
Development of a Mass Spectrometry-Based Absolute Quantitation Method of Griffithsin, a Viral-Entry Inhibitor and Candidate Microbicide against HIV

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Introduction: Since HIV transmission occurs over mucosal surfaces, including rectal and vaginal compartments. Microbicides, compounds that can be applied inside the vagina or rectum, are being investigated as a preventative strategy against HIV transmission. PREVENT (Pre-exposure prevention of viral entry) is a new pre-clinical/clinical trial with the goal of determining the efficacy of griffithsin, an extremely potent HIV-entry inhibitor, as a rectal/vaginal microbicide. In order to study the pharmacokinetic and pharmacodynamic responses to griffithsin in biological fluids and tissues of non-human primates and humans, an accurate quantitation method of griffithsin is required. The ultimate goal of this study is to design a triple-quadrupole mass spectrometry-based approach for the absolute quantitation of griffithsin in biological samples.

Methods: Griffithsin protein was digested with trypsin and prepared for mass-spectrometry analysis (method previously described by Birse K., et al. (2013) Unbiased Proteomics Analysis Demonstrates Significant Variability in Mucosal Immune Factor Expression Depending on the Site and Method of Collection. PLoS One, 8(11): e379505). Peptides were analyzed by both MALDI-TOF (Bruker Daltonics Autoflex III Smartbeam) and triple-quadrupole mass spectrometers (ABSciex QTRAP 6500). Data was analyzed using Mascot (v2.4, Matrix Science).

Results: The MALDI spectra confirmed presence of both intact and digested griffithsin. Two peaks were seen for the intact griffithsin, one corresponding to a singly charged griffithsin monomer (m/z 12730.164) and the other corresponding to a doubly charged monomer (m/z 6365.454). Three peaks corresponding to griffithsin peptides (m/z 1682.854, m/z 1818.932 and m/z 1712.820) were seen on the MALDI spectra. These peptides represent a substantial amount (42%) of the griffithsin sequence. Optimization of the detection of griffithsin by the triple-quadrupole is ongoing.

Summary & Future directions: Our goal is to develop an efficient method to detect griffithsin in biological samples using mass spectrometry. Once the detection of griffithsin is optimized on the triple quadrupole, a next step will be to use heavy-isotope labeled peptide standards to perform absolute quantitation of griffithsin in biological samples. This method will be important for the evaluation of the pharmacokinetic and pharmacodynamic responses observed in biological matrices of non-human primates and humans treated with griffithsin as a rectal/vaginal microbicide.
A Chemogenetic Approach for Profiling Bioactives by Next Generation Sequencing Reveals a Novel Antibacterial Target in *Burkholderia cenocepacia*

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**Introduction:** The prevalence of antibiotic resistance and a lack of antibiotic development is a global health crisis. A key obstacle for generating new antibiotics is identifying their mode of action (MOA). We previously developed an enhanced sensitivity assay (ESA), a chemogenetic approach to match antibiotics to their respective targets, using a library of *Burkholderia cenocepacia* conditional growth (CG) mutants. In each CG mutant the expression of a gene that is essential for growth in rich media is controlled by a transposon-inserted rhamnose-inducible promoter. Depleting an essential product to a point where growth is inhibited sensitizes CG mutants to antibiotics targeting that essential product, revealing antibiotic-target matches. The ESA is laborious and time consuming because it measures the growth of individual mutants by OD600. To expedite the development of novel antimicrobial compounds into new antibiotics by finding antibiotic-target matches, the ESA is being developed into a high throughput (HTP) screen using next generation sequencing to quantify CG mutants when grown together in a pool.

**Methods:** The ability to detect enhanced sensitivity was established using a gyrB mutant that is hypersensitive to its cognate antibiotic, novobiocin. Twenty-five strains from a library of *B. cenocepacia* CG mutants, including a gyrB mutant, were screened against antibiotics with known MOAs in a pilot HTP ESA. Multiplexed sequencing was used to track the growth of each CG mutant by their transposon-genome interface to determine if the enhanced sensitivity of a gyrB CG mutant to novobiocin can be quantified after growth in a pool.

**Results:** The enhanced sensitivity of a gyrB CG mutant to novobiocin was quantified using multiplexed sequencing and the sensitivity of detection is magnified when strains are grown in a pool versus when they are grown clonally. The pilot HTP ESA revealed a histidine sensor-kinase required for resistance to different classes of antibiotics, providing the first evidence of an essential two-component system that can be targeted to reduce antibiotic resistance in *B. cenocepacia*.

**Conclusion:** The pilot HTP ESA results highlight the promise of the HTP ESA for finding targets of novel antibiotics and characterizing essential gene function once it is scaled up.
Early Detection of Cardiac Dysfunction in Colorectal and Renal Cell Cancer: Bevacizumab and Sunitinib Mediated Cardiotoxicity

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Introduction: An increasing number of cancer survivors experience cardiovascular complications due to anti-cancer drugs, suggesting a need for research studies focused on early detection and prevention of cardiotoxicity. Two types of targeted therapy currently used for colorectal (CRC) and renal cell cancer (RCC), respectively, include the monoclonal antibody Bevacizumab (BVZ) and the tyrosine kinase inhibitor Sunitinib (SNT). Despite the benefit of improved overall survival, an unexpected side effect is development of cardiotoxicity in nearly 1 in 4 patients.

Objectives: To evaluate whether novel echocardiographic techniques, including tissue velocity imaging (TVI) and/or strain rate (SR), can detect cardiotoxicity prior to changes in left ventricular ejection fraction (LVEF).

Methods: In an acute murine model, 75 wild type C57Bl/6 mice were randomly assigned to: i) 0.9% saline, n=5; ii) BVZ [n=35]; or iii) SNT [n=35] and followed for 14 days. Echocardiography was performed to determine baseline cardiac function and serially for 14 days.

Results: BVZ- and SNT- treated mice demonstrated that TVI and SR can detect early LV systolic dysfunction at day 8 as compared to conventional LVEF parameters at day 13. In mice treated with either BVZ or SNT, the LVEF decreased from 75±2% at baseline to 48±3% and 47±2%, respectively, at day 13. While LVEF values decreased at day 13, both TVI and SR values confirmed evidence of subclinical LV systolic dysfunction 5 days earlier. In mice treated with BVZ, V_endo decreased from 3.5±0.3cm/s at baseline to 2.4±0.1cm/s at day 8 and radial SR decreased from 21±1s-1 at baseline to 14±2s-1. In SNT treated mice, V_endo decreased from 3.4±0.2cm/s at baseline to 2.5±0.2cm/s at day 8 and radial SR decreased from 21±2s-1 at baseline to 15±2s-1.

Conclusions: In a murine model of BVZ or SNT mediated cardiomyopathy, non-invasive assessment by TVI detected early LV systolic dysfunction prior to alterations in conventional echocardiographic indices. Future research studies are required to investigate the potential use of TVI/SR parameters for early detection of subclinical alterations in cardiac function among CRC and RCC patients treated with either BVZ or SNT in the clinical setting, potentially avoiding the development of advanced heart failure.
Assessment of the Effect of Artificial Sweeteners on Gut Microbiota and Glucose Metabolism

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Introduction: Non-caloric artificial sweeteners (NAS) have grown very popular since their introduction to the market. Currently, there are six NASs approved as “table-top sweeteners” by Health Canada, including acesulfame potassium, aspartame, erythritol, neotame, steviol glycosides, and sucralose. The NASs under review in this project are sucralose and aspartame, which are frequently used in Canada. Recent research has been focused on illuminating the negative health effects of NASs. For example, consumption of the NAS saccharin in mouse models reportedly induces glucose intolerance by altering the microbial composition of the gut (referred to as “gut microbiota dysbiosis”). The gut microbiota may have the ability to metabolize NASs into short-chain fatty acids (SCFA), which hold a wide range of consequences including the potential to shift the normal bacterial balance, potentially leading to alterations in glucose metabolism.

Methods: Ten eligible (young, healthy, non-pregnant, non-diabetic) participants will be selected, between the ages of 20-30 years old, with a BMI of 20-25 (i.e. normal weight), and a fasting blood glucose (FBG) < 5.7 mmol/L. They will undertake two-week treatment diets for both sweeteners, separated by washout periods and sample collections in a randomized, double-blind, crossover design. According to the Academy of Nutrition and Dietetics, the estimated daily intake (EDI) for aspartame is 4.1 mg/kg body weight/day, while that for sucralose is 2.0 mg/kg body weight/day. The amount consumed by each participant will be determined individually, in order to reach the 90th percentile of the EDI. Fecal and blood samples will be analyzed for SCFAs and microbiome, and glucose, insulin, glucagon, incretins, and leptin, respectively.

Conclusions: Although there has been a good deal of research conducted on the gut microbiome, there is a gap in that there have been no studies regarding the effects of the NASs sucralose and aspartame on its composition and function. In the current study, our aim is to quantify these effects. This study aims to determine the effect of sucralose and aspartame consumption on gut microbiota composition, diversity, and community structure, and to determine whether NAS-associated changes in glucose metabolism are mediated by gut microbiota dysbiosis induced through sucralose and aspartame consumption.
**The Cardioprotective Role of NACA in Doxorubicin and Trastuzumab Mediated Cardiac Dysfunction**

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**Objective:** To investigate whether an anti-oxidant, N-acetylcysteine amide (NACA), can attenuate the drug-induced heart failure caused by DOX+TRZ.

**Methods:** A total of 100 female mice received one of the drug regimens: i) 0.9% saline; ii) NACA; iii) DOX; iv) TRZ; v) DOX+TRZ; vi) NACA+DOX; vii) NACA+TRZ; and viii) NACA+DOX+TRZ. LV systolic function was assessed using echocardiography. At day 10, mice were euthanized and hearts were measured for oxidative stress (OS) and apoptosis.

**Results:** In mice receiving DOX, left ventricular ejection fraction (LVEF) decreased from 73±4% to 43±2% at day 10. In mice receiving DOX+TRZ, LVEF decreased from 72±3% to 32±2% at day 10. Prophylactic administration of NACA to mice receiving DOX or DOX+TRZ was cardio-protective with an LVEF of 62±3% and 55±3% at day 10, respectively. There was a 3-fold and 4-fold increase in superoxide production in hearts of mice treated with DOX or DOX+TRZ, respectively. Prophylactic administration of NACA reduced degree of OS in both groups. Furthermore, there was a 1.5-fold and 2 fold increase in the Bax/Bcl-xL ratio in hearts of mice treated with DOX or DOX+TRZ, respectively. Prophylactic administration of NACA reduced the degree of apoptosis in both groups.

**Conclusion:** NACA attenuates the cardio-toxic effects of DOX+TRZ in a murine model of chemotherapy induced cardiac dysfunction.
Role of Receptor Tyrosine Kinase AXL in of HER2 + Breast Cancer Progression
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Introduction: More than 90% of breast cancer deaths are caused by metastasis. HER2+ subtype is one of the most aggressive for growth and metastasis and is linked to poor survival. The receptor tyrosine kinase AXL is known to be implicated in many oncogenic processes and its expression is associated with lymphatic vessel invasion and metastasis. While findings highlight a role for AXL in metastasis, its exact functions remain poorly defined.

Methods/Results: We used a bitransgenic mouse model of HER2+ breast cancer (MMTV-Neu) crossed with AXL KO animals (MMTV-Neu:AXL KO) and found a strong reduction of lung metastasis in AXL mutants mice when compared to control mice. In contrast, the KO mice for AXL ligand, Gas6, had a metastatic burden identical to controls. If Gas6 is not required for HER2-driven metastasis, how is AXL activated? Biochemical studies showed that AXL forms a complex with HER2, which promotes the trans-phosphorylation of AXL activation sites. In addition, we have determined the transcriptomes of control and AXL KO tumors by RNA-Seq. Gene Set Enrichment Analyses (GSEA) of the differentially expressed genes of AXL KO tumors revealed a decrease in expression of genes involved in “TGF-β/EMT”, “Stem Cells/Differentiation”, and “Metastasis/Tumorigenesis”. For in vitro characterization, we used the HCC1954 cell line in which TGF-β induces AXL expression and increases their migratory/invasive behavior. Inhibition of AXL in those cells reduces TGF-β-induced cell migration, pointing out a specific role for AXL in this process. In addition, analysis of the gene expression data by DAVID (Gene Ontology) uncovered links between AXL and “Angiogenesis/Blood Vessel Morphogenesis”. Thus, immunohistofluorescence analysis suggests AXL involvement in tumor angiogenesis where AXL KO vessels appeared to be less permeable.

Conclusion: AXL is essential for breast cancer metastasis in vivo by playing a partnership role for HER2 signaling and increasing tumor vascular permeability. This indicates that AXL inhibition could be a valid approach for treatment of metastatic diseases.
REM Sleep Circuits and Respiratory Motor Inhibition
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Rapid eye movement (REM) sleep is a distinct brain state associated with dysregulation of the respiratory control system. Changes in respiratory physiology during REM sleep can cause obstructive sleep apnea (OSA) or exacerbate existing respiratory dysfunction in vulnerable populations. These pathologies ultimately stem from interactions between REM sleep and respiratory control circuits.

Objectives: We aimed to: (i) determine mechanisms of REM sleep generation, (ii) identify interactions between REM sleep circuitry and the hypoglossal motor (HM) pool, (iii) identify the major mechanism responsible for suppression of HM activity in REM sleep (a precipitating cause of OSA), and (iv) identify pharmacological strategies for reactivating the upper airway musculature throughout sleep.

Methods: Microdialysis of select drugs in freely behaving rats (12 groups, total n=122), instrumented for electroencephalographic and electromyographic recordings, was performed to modulate sites in the REM sleep and respiratory control circuits.

Results and Conclusions: REM sleep generation is suspected to require activation of the pontine subcoeruleus (SubC) by pedunculopontine tegmental nucleus (PPT) cholinergic neurons. However, we found that REM sleep is not reduced by acetylcholine (Ach) receptor antagonism in the SubC, at a concentration capable of blocking the effects of stimulating cholinergic PPT neurons. Instead we found that SubC Ach inputs act to increase the reliability and robustness of transitions into REM sleep through a positive feedback mechanism between the SubC and PPT. The PPT also controls components of the respiratory control system including the HM pool. We found that REM-sleep active PPT cells contribute to suppression of upper airway activity in REM sleep. Nevertheless, the major mechanism mediating paralysis of the pharyngeal musculature in REM sleep was still unknown. We identified this mechanism. We restored respiratory activation of the upper airway specifically in REM sleep using pharmacological blockade of muscarinic Ach receptors and downstream G-protein-coupled potassium (K+) channels at the HM pool. We also established proof-of-principle that targeted blockade of certain K channels at the HM pool could be an effective strategy for reversing upper airway hypotonia in sleep: blockade of voltage-gated and inwardly-rectifying K+ channels, but not K+ leak channels, restored upper airway activity to waking levels throughout sleep.
Assessing the LAG-3 Inhibitory Mechanism on T cell Activation
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**Background:** T cell dysfunction is implicated in many chronic diseases, including cancer and persistent infection. Chronic disease can cause prolonged immune activation, leading to immune dysfunctions such as T cell exhaustion. Exhaustion is characterized by decreased cellular proliferation, impaired effector function and expression of inhibitory co-receptors. LAG-3 is one such co-receptor and is present on multiple lymphocyte subsets when activated. LAG-3 is upregulated in several chronic diseases, including HIV infection, and can abate stimulation-induced cellular proliferation and production of IL-2, IFN\(\gamma\) and TNF\(\alpha\).

**Rationale:** T cell receptor signaling occurs via a complex multifaceted phosphotyrosine pathway, ending in general activation of the T cell. A rapid influx of calcium is critical for downstream activation of NFAT, an transcription factor necessary for complete T cell activation. LAG-3 activity leads to impaired calcium flux and reduced levels of Th1 cytokines upon T cell stimulation, but how LAG-3 does this is unknown.

**Hypothesis:** We hypothesize that LAG-3 interferes with the TCR activation pathway by inhibiting the phosphorylation of a signaling protein at, or upstream of PLC\(\gamma\)-mediated hydrolysis of PIP\(_2\).

**Approach:** If LAG-3 inhibits the phosphorylation of a signaling protein in the TCR pathway, the phosphorylation state of that protein will be lower when LAG-3 is activated. To test this, we will upregulate LAG-3 on primary T cells, then use phospho-flow cytometry to identify the earliest stage of TCR signaling that is affected by LAG-3. We will quantify the phosphorylation state of suspect TCR signaling proteins upon receptor-stimulation following LAG-3 cross-linking and antibody-mediated blocking, respectively.
Biodegradable Film for the Targeted Delivery of siRNA-loaded Nanoparticles to Vaginal Immune Cells

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Introduction: The goal of this study was to develop and characterize a novel intravaginal film platform for the delivery of small interfering RNA (siRNA)-loaded nanoparticles as a potential gene therapy for the prevention of sexually transmitted human immunodeficiency virus (HIV) infection.

Method: Poly (ethylene glycol) (PEG)-functionalized poly (D, L-lactic-co-glycolic acid) (PLGA)/polyethylenimine (PEI)/siRNA nanoparticles (siRNA-NP) were fabricated using a modified emulsion-solvent evaporation method and characterized for particle size, zeta potential, encapsulation efficiency (EE) and siRNA release. Nanoparticles were decorated with anti-HLA-DR antibody for targeting delivery to HLA-DR+ dendritic cells (DCs) and homogeneously dispersed in a biodegradable film consisting of poly vinyl alcohol (PVA) and λ-carrageenan.

Results: The siRNA-NP loaded film (siRNA-NP-film) was homogeneous, transparent, displayed suitable physico-mechanical properties, and was non-cytotoxic. Targeting activity was evaluated in a vaginal mucosal co-culture model consisting of a vaginal epithelial monolayer (VK2/E6E7 cells) and differentiated KG-1 cells (HLA-DR+ DCs). Anti-HLA-DR conjugated siRNA-NP (siRNA-NP-Ab) was rapidly released from the film and was able to penetrate the epithelial layer to be taken up by KG-1 cells. siRNA-NP-Ab demonstrated higher targeting activity and significantly higher knockdown of SNAP-23 (synaptosome-associated 23-kDa protein) mRNA and protein when compared to siRNA-NP without antibody conjugation.

Conclusion: Overall, this data suggest that our novel siRNA-NP-Ab-film may be a promising platform for preventing HIV infection within the female genital tract.
Clinical Significance and Function of L1CAM in Esophageal Squamous Cell Carcinoma
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Introduction: Esophageal squamous cell carcinoma (ESCC) is the predominant histological subtype of esophageal carcinoma in Asia. One of the primary reasons for this high-mortality rate is that ESCC is often diagnosed at an advanced stage. Therefore, the poor prognosis for most patients with ESCC has prompted the authors to seek for novel molecular prognostic markers that help identify patients at higher risk of death and improve the prognosis of patients with ESCC. L1 cell adhesion molecule (L1CAM), which belongs to the immunoglobulin superfamily and was originally identified as an adhesion molecule involved in neural development. It has recently been observed in a variety of human malignancies. In many human epithelial carcinomas, L1CAM is overexpressed and thereby augments cell motility, invasion and metastasis formation. L1CAM positive carcinomas are associated with bad prognosis. In the current study, we aimed to detect the expression of L1CAM in ESCC tissues further more, the clinical significance and function were also analyzed and discovered.

Methods: Tissue microarray and immunohistochemistry staining was used to detect the expression patterns of L1CAM in ESCC tissues. Immunofluorescence staining and Western blotting was performed to determine the distribution and expression level of L1CAM in a panel of ESCC cell lines. The xCELLigence proliferation assay is used to reveal its role in proliferation. What is more, the real-time measurement of cell migration is used to detect its role in migration.

Results: Statistical analysis showed that the expression of L1CAM in cell nucleus was significantly associated with the differentiation (P=0.016) and lymph node metastasis (P=0.000) of ESCC cells. Kaplan–Meier survival analysis revealed that over-expression of L1CAM was associated with poor survival of ESCC patient (P=0.04). L1CAM expression was detected in cytoplasm or nucleus. Knockdown of L1CAM led to decrease proliferation and inhibit migration of ESCC cells. L1CAM was highly expressed in most of cell lines and was mainly distributed in the membrane and nucleus of ESCC cells, which was consistent with what we observed in ESCC tissues. Knockdown of L1CAM led to decrease proliferation and inhabit migration of ESCC cells.

Conclusion: The expression of L1CAM can be used as an independent risk factor for judging the prognosis of patients with esophageal carcinoma. These data suggest that L1CAM may be consistent, molecular marker for early warning of the occurrence and development of esophageal squamous cell carcinoma. L1CAM can promote proliferation and migration in ESCCs.
Cord Blood TNF Alpha Levels are elevated in Newborns Exposed to Preeclampsia during Gestation

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Introduction: Preeclampsia (PE) affects up to 10% of pregnancies worldwide and is a leading cause of morbidity and mortality of both mothers and their offspring. However, few studies have investigated how placental pro-inflammatory imbalance, characteristic of PE, impacts the fetus. The objective of this study was to characterize effect of preeclampsia and gestational hypertension (GH) on health outcomes of offspring at delivery.

Methods: We performed a prospective cohort of pregnant women studied 1st (5-16 weeks of gestation) and 2nd (24-28 weeks) trimesters. Offspring were evaluated at birth. The main exposure variables were maternal blood pressure (BP) and inflammatory markers during pregnancy. Main outcome measures were birth weight and TNFα in cord blood.

Results: Among the 636 mother-newborn pairs, 18 mothers presented with PE, 25 with GH and 593 remained normotensive, healthy controls. As expected, primiparity (PE=55.6%, GH=60.0%, NT=33.1%; p=0.004) and BP at inclusion (PE=121/79 ± 6/6 mmHg, GH=120/76 ± 8/5 mmHg, NT=110/68 ± 7/6 mmHg, p<0.0001) were higher in PE and GH compared to NT participants. However, maternal TNFα levels were comparable throughout pregnancy (p>0.05). Newborns’ birth weight was smaller in the PE vs NT group (PE=3035 [2770-3394] g, GH=3285 [2930-3585] g, NT=3425 [3145-3720] g; p=0.004). Cord blood TNFα levels were significantly elevated in the PE (6.53 [4.94-8.38] pg/mL; p=0.015), but not in the GH (5.44 [3.94-6.68] pg/mL; p=0.6), group vs NT (5.16 [4.11-6.75] pg/mL). These elevated TNFα levels were associated with smaller birth weight (r= -0.45; p=0.061) and were independent from gestational week at delivery, meaning that cord blood TNFα may be a marker of severity of PE.

Conclusion: Preeclampsia increases neonatal exposure to inflammation, which is related to decreased birth weight.
Selectively Targeting RNF20-Deficient Colorectal Cancer Cells
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Introduction: Chromosomal instability (CIN) is an early event that drives the development of tumors and it is associated with virtually all tumor types. CIN is defined as an increase in the rate at which whole chromosomes or large parts thereof, are gained or lost. Genes that normally function to maintain chromosome stability are often found altered in human cancers. RNF20, a histone H2B mono-ubiquitin ligase, is one such chromosome stability gene that is mutated in various cancers. The prevalence of RNF20 mutations across multiple tumor types, and its role in homologous recombination repair make RNF20 an attractive target for therapeutic intervention. Previous groups have shown that cells harboring defects in homologous recombination repair proteins (BRCA1/2) can be selectively killed through PARP1 inhibition. As a result, we hypothesized that RNF20-depleted or -deficient cancer cells may be selectively killed in a similar manner.

Methods: We employ RNA-interference and chemical inhibitors in established cell lines to simultaneously silence RNF20 and PARP1. Following a three-day incubation period, we utilize high-content fluorescent imaging to evaluate the remaining cell numbers compared to controls. To assess apoptosis as a candidate mechanism of cell death, we employ indirect immunofluorescent imaging and activated-Caspase 3 antibodies in RNF20-silenced and control populations.

Results: We show that simultaneous silencing of RNF20 and PARP1 can specifically diminish cell numbers compared to RNF20-silenced plus GAPDH-silenced controls. In addition, diminished cell numbers were recapitulated using Olaparib or BMN673 (chemical PARP1 inhibitors) in combination with RNF20-silencing. To evaluate the underlying cause of diminished cell numbers detailed above, immunofluorescent imaging revealed an increase in activated-Caspase 3, implying an apoptotic response.

Conclusion: Our data suggest that RNF20 is a CIN gene that when silenced, can be selectively killed by either silencing or inhibiting PARP1. Using this genetic strategy we have identified PARP1 as a novel candidate drug target that warrants further pre-clinical study.
Investigating Potential Consequence of Low Level Viremia in HIV-1 Infected Children undergoing Antiretroviral Therapy

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Introduction: About 12 million people infected with HIV receives Anti Retroviral Therapy (ART). Multiple drugs are used in combination to reduce disease progression by interfering with viral replication in patients. A method to monitor treatment response is by measuring Viral Load (VL). It is considered Low Level Viremia (LLV) when a patient has VL of 50-1000 RNA copies/mL. Virologic Failure (VF) is when a patient has VL >1000 RNA copies/mL while taking the treatment. HIV infected patients with VF are unable to suppress virus completely, creating an ideal situation for the virus to mutate and promote development of drug resistance (DR). The challenges of HIV DR are most profound in children but its role in virologic failure is not clear. We hypothesize that low level viremia tends to lead to virological failure due to accumulation of drug resistant mutations in HIV-1 infected children undergoing ART. The objectives are to investigate the association between LLV and VF; detect and profile HIV DR mutations in HIV infected children with ART during the period of LLV; correlate HIV DR mutations with VF.

Methods: 80 plasma samples from HIV-infected ART-experienced children who failed first-line treatment in Nairobi will amplified by PCR, followed by Illumina Miseq sequencing. Once the data is generated, it will be processed for data analysis using an in-house HIV DR testing pipeline developed at the NML.

Significance: This study will provide wholesome evidence that LLV can lead to VF in HIV-infected patients. It will be significant in Canada and Kenya as there are common drugs and mutations. As Sub-Saharan Africa is known to be prevalent in HIV infection, we are more likely to find a variety of mutations that will help us better understand the diversity of DR. This study will promote the use of NGS technologies on HIV DR testing in Manitoba and help us improve treatment strategies in Canada.
Executive Functioning as a Mechanism to Explain the Development of Academic Achievement in Adolescents with Autism Spectrum Disorder

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**Introduction:** Executive functioning (EF) is a set of higher order cognitive processes that involve inhibition, cognitive shifting, and working memory. Executive functioning and academic achievement are related in typically developing (TD) adolescents. This relation is relevant to clinical populations that have difficulties in these areas, such as individuals diagnosed with autism spectrum disorder (ASD). This study aims to extend our understanding of cognitive and academic difficulties among adolescents with ASD by investigating the correlations of both an eye-tracking and a standardized behavioural measure of executive functioning with academic achievement.

**Method:** Data collection was conducted on 40 adolescents (20 ASD and 20 TD), 11-18 years of age. The Eyelink 1000 eye tracking system was used to collect data on saccadic eye-movements during a memory-guided eye-tracking task. Participants also completed the Trail Making task from the Delis-Kaplin Executive Function System. Both tasks assess working memory and cognitive shifting. Academic achievement was assessed using the Woodcock-Johnson III Tests of Academic Achievement.

**Results:** Partial correlations, controlling for full-scale IQ, were conducted to assess whether the percent of saccadic eye-movement errors and the Trail Making Switching score were correlated with academic achievement for both the ASD and TD groups. For the ASD group, percent of saccadic errors significantly negatively correlated with Math Calculations ($r = -.54$) and Math Fluency ($r = -.69$), and also negatively correlated with Writing Fluency ($r = -.47$) with marginal significance. For the TD group, percent of saccadic errors was only significantly negatively correlated with Math Calculations ($r = -.51$). Similarly, the Trail Making Switching Score was significantly correlated with Math Calculations ($r = .58$) and Math Fluency ($r = .53$) and also correlated with Writing Fluency ($r = .41$) with marginal significance in the ASD group. There were no correlations between this score and academic measures in the TD group.

**Conclusions:** The present study begins to provide some insight into understanding the development of academic skills through adolescence, and the cognitive mechanisms that are important to this development. Such insights will be important for developing strategies to provide support for adolescents with ASD as they transition into adulthood.
Does Intravenous Magnesium Sulfate Reduce Hospital Admission Rates in Adults With Severe Acute Asthma?

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Introduction: Asthma is a chronic respiratory disease characterized by bronchial hyperresponsiveness, airflow obstruction, and inflammation that presents with symptoms of cough, dyspnea, chest tightness, wheezing, and sputum production. Symptoms of asthma can range from mild to severe, resulting in hospitalization, intubation, and even death. In addition to inhaled beta agonists, nebulized Ipratropium, oxygen, and systemic corticosteroids, Magnesium Sulfate has been used as an adjunctive treatment of acute severe asthma exacerbations. However, despite its use, its effectiveness has been inconclusive and controversial, with previous studies showing the greatest benefit in those having severe exacerbations. This literature review was conducted to determine if Magnesium Sulfate administration reduces admission rates in adults with acute, severe exacerbations.

Methods: A literature search was conducted for research studies of adult patients who presented with an acute, severe asthma exacerbation and were treated with intravenous Magnesium Sulfate. Study dates were limited to the years 1989-2014, and were identified through PubMed, Scopus, Embase, and Google Scholar, with further studies identified through the search of reference lists of published articles. A total of 11 studies were found using the sources above. Of these 11 studies, 5 double-blind, randomized, placebo controlled trials were selected for this review.

Results: Results from the five randomized controlled trials selected were mixed, with two studies showing a decrease in admissions (one had weak evidence of a decrease), and three studies showing no difference in admission rates.

Conclusion: Intravenous Magnesium Sulfate does not appear to decrease admission rates in adult asthmatics having a severe exacerbation.
Defining the Roles of IRF-1 and IRF-7 in Anti-viral Responses
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Introduction: The interferon regulatory factor (IRF) family members are regulators of many biological processes such as cytokine signalling, immune responses, and apoptosis. Both IRF-1 and IRF-7 have been shown to be essential for anti-viral immune responses via regulating the expression of type 1 and 2 interferons and interferon-stimulated-genes. We hypothesize that IRF-1 and IRF-7 are expressed in every cell type examined (CD-4, CD-8, B-cells, NK cells, monocytes, and dendritic cells) and that increased cellular expression of IRF-1 and IRF-7 will boost anti-viral responses and reduce viral replication in infected cells. Conversely, reducing cellular IRF-1 or IRF-7 will render the cells more susceptible to infection, with the exception of HIV-1 because HIV-1 requires IRF-1 for its viral replication.

Methods: IRF-1 and IRF-7 level were examined in defined immune subtypes in blood using multi-color flow-cytometry. IRF-1 and IRF-7 levels in ex-vivo human T cells and monocytes were modulated either by transfection with siRNA or transduction with lentiviral particles encoding IRF-1 and IRF-7 cDNA. The efficiency of transactivating HIV-1 genes (ie HIV replication) was measured by p24 ELISA and Gag mRNA expression. The expression of host cell anti-viral genes were also evaluated.

Results: Preliminary data showed that prior to stimulation, IRF-1 was expressed in all cellular subtypes examined with the highest expression found in monocytes. In response to exogenous interferon-gamma (IFN-gamma) stimulation, IRF-1 protein level was increased by ~2-fold. In contrast, IRF-7 was expressed at low levels in unstimulated cells, and its expression was augmented by ~3-fold in IFN\textalpha A-treated cells. Our preliminary work showed that as little as 40\% knockdown of endogenous IRF-1 level resulted in >90\% reduction in transcription of HIV-1 genes and consequently impaired viral replication. Such modest IRF-1 knockdown had no effects on the transactivation of host anti-viral genes.

Summary: Fine-tuning the expression of immune regulators is critical for an appropriate immune response, as modest reduction in IRF-1 expression significantly impaired the transactivation of HIV genes, but not the regulation of host genes. Findings from this study will define the roles of IRF-1 and IRF-7 roles in anti-viral responses and host-viral interaction assisting in vaccine design.
Inflammatory Bowel Disease Symptoms, Religiosity, and Health Locus of Control: Belief in God is Optional, but Trust in Your Doctor is advised

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Introduction: Religion sometimes has been found to be good for physical health, but most research has been cross-sectional and only looked at one aspect of religiosity.

Method: 175 Manitoba Inflammatory Bowel Disease Cohort Study participants answered questions about religiosity, spiritual values, “health locus of control” (who they believed was in charge of their health: doctors, the self, fate, or God), and symptoms, over a decade.

Results: Did religiosity change? Yes. At baseline, 39% were religious. Of those, 31% had shifted down by year 10. At baseline, 49% were not very religious. Of those, 20% of them shifted up by year 10.

Did worsened IBD predict increased spirituality? No. IBD symptoms did not drive people to or from religion. There was no correlation between shifts in religiosity and: pain (SF-36) or symptom scores (Manitoba IBD Index) at years 1-4, or in IBD symptom shift (years 1-4 compared to years 8-10).

Was spirituality used as a coping strategy? Yes. Spiritual values gave strength to face everyday difficulties: A lot for 26%, Some: 21%, A little: 20%, Not at all: 32%. Spiritual values helped in understanding the difficulties of life: A lot, 21%; Some, 25%; A little, 21%; Not at all, 32%.

Who did participants believe was in charge of their health? Doctors were seen as the most important factor in health, with an average score of 4.2 out of 6. The self was next (3.5), then fate (2.9). God was seen as the least of the factors, with only 1.7.

Was health locus of control related to IBD symptoms? Yes. Believing that the self and doctors were in charge of health was correlated with lower levels of symptoms; belief that fate or God was in charge was neither good nor bad. Doctor health locus of control was correlated with cross-sectional IBDQ bowel symptoms $r = -.18$ ($p = .02$), showing that the more well someone was, the more they endorsed doctors as having power over their health. Self health locus of control showed a correlation of $r = -.16$ ($p = .04$). The other correlations were statistically insignificant.
Non-Linear Change in A Focused Cognitive-Behavioural Treatment for Generalized Anxiety Disorder
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Introduction: Generalized anxiety disorder (GAD) is a common and debilitating mental illness characterized by excessive worry, anxiety (American Psychiatric Association, 2013), and safety behaviours. Cognitive-behavioural treatments (CBT) target underlying mechanisms that maintain GAD symptoms. One such mechanism is intolerance of uncertainty (IU), a dispositional characteristic arising from a set of negative beliefs about uncertainty and its consequences (Koerner & Dugas, 2006). Although a CBT protocol targeting IU has demonstrated efficacy across 5 randomized controlled trials (e.g., Dugas et al., 2003) approximately 30% of individuals do not achieve full remission by posttreatment. These non-remitted individuals continue to endorse elevated levels of IU, suggesting that treatment could be further optimized. Additionally, greater efficiency and parsimony within CBT protocols has been advocated (e.g., Cougle, 2012). To address these issues, we have developed a streamlined CBT protocol for GAD targeting IU.

Method: We hypothesized that changes in IU would precede changes in GAD symptoms, specifically worry and safety behaviours. These changes were evaluated using sudden gains, a non-linear measure of rapid change between two therapy sessions. Given the treatment’s focus on IU, we also predicted that a greater number of sudden gains in IU would occur relative to sudden gains in worry or safety behaviours. Each participant (N = 7) completed 12 weekly sessions of the streamlined CBT protocol with a licensed clinical psychologist. IU, worry, and safety behaviours were assessed prior to each therapy session.

Results: Results showed that a greater number of participants experienced sudden gains in IU than in worry or safety behaviours. The majority of participants first experienced sudden gains in IU (57%), either alone or in combination with worry or safety behaviours. When a first sudden gain involved IU, second sudden gains were most likely to involve IU (50%), followed by worry (25%) and safety behaviours (25%).

Conclusion: Non-linear changes in IU tended to precede further changes in IU or change in worry or safety behaviours. Research and clinical implications will be discussed.
The Patient Medical Home: How do Canadian Primary Care Practices Measure up to its Ten Goals?

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Objective: The Patient Medical Home is a primary healthcare organizational model promoted by the College of Family Physicians Canada as a way to offer comprehensive, co-ordinated, and continuous care. Ten goals comprise the core attributes of the model. Currently there is no evaluation tool to determine accordance to the model.

Approach: We are utilizing data from the QUALICOPC study, a cross sectional study of 772 Canadian primary care practices spanning the 10 provinces and 8,332 of their patients. The data consists of information collected on the practice level, the physician level, and the patient level. The survey questionnaires have been mapped to the ten goals of the Patient Medical Home. We are using linear regression to evaluate how primary care as it is currently being practiced across the Canadian provinces relates to the Patient Medical Home.

Results: It is believed that this study will demonstrate that presently many practices across Canada operate on the PMH goals or analogous principles. The outcomes will be relevant for provinces determining primary care reform policies, to note the progress already made in the domains of the PMH goals, and the areas which need improvement.

Conclusions: The study will gain baseline measures as to how provinces compare in their ability to measure up to the goals of the PMH, as described by the CFPC. It is hoped the work will establish a novel evaluation methodology inclusive of patient perspectives of care.
Distribution, Dynamics and Function of Ryanodine Receptor Clusters at the Periphery of Ventricular Myocytes

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**Background:** Ryanodine receptors (RyRs) are intracellular calcium (Ca) release channels located in the sarco(endo)plasmic reticulum (ER/SR) membrane. The subcellular distribution of RyRs critically determines their activity and thus Ca-release profiles. Cardiac RyRs (RyR2s) are organized in highly-ordered arrays of clusters in the cell interior. This RyR2 organization is believed to mediate synchronous, stable Ca-release during excitation-contraction coupling. However, little is known about RyR2 distribution and function in the peripheral region of cardiac cells.

**Methods and Results:** Recently, our group created a knock-in RyR2 mouse model that expresses green fluorescence protein (GFP)-tagged RyR2. These GFP-RyR2 mice can be used to directly and simultaneously monitor the distribution and function of RyR2s in live cells without the use of anti-RyR2 antibodies. Total internal reflection fluorescence (TIRF) and confocal imaging of live isolated cardiomyocytes and intact hearts from GFP-tagged RyR2 mice revealed discrete GFP-RyR2 cluster distribution just beneath the sarcolemma (subSL), different from the cell interior. Electron microscopy confirmed the presence of subSL SR. Time-lapsed TIRF monitoring of these GFP-RyR2s showed cluster dynamics only along the z-axis. High-speed confocal Ca-imaging of GFP-RyR2 cardiomyocytes loaded with Rhod-2 AM further showed that Ca-release events from subSL GFP-RyR2 clusters differ from internal clusters signaling.

**Significance and Conclusion:** *Ex vivo* cellular and intact heart studies showed three distinct GFP-RyR2 distribution patterns in ventricular myocytes: i) subSL dis-registered RyR2 clusters; ii) double-rows of RyR2 clusters; and iii) highly-organized arrays of RyR2 clusters in the cell interior. Functional studies suggest that RyR2 clusters located at the cell periphery have distinct physiological roles in Ca-signaling in cardiac cells.
**Introduction:** Adolescent, low-income, or undereducated women are more likely than others to be at high risk for poor maternal and child health outcomes. Providing services in areas where risk is higher are key components of the work that public health nurses (PHNs) do to promote and protect the health of individuals, families, and communities. The purpose of this study is to explore how PHN practice affects health outcomes related to breastfeeding initiation and duration, infant immunizations, and maternal tobacco use within this population of pregnant and postnatal women negatively affected by the social determinants of health. Additionally, this study will examine how the healthcare organization of which PHNs are a part, influences their practice and ultimately the achievement of those outcomes. This study is designed to explore the real-life practice of PHNs, as it exists day-to-day amidst the influences of ever changing politics, leadership, and program demands.

**Methods:** Both quantitative and qualitative data will be used in a case study design. Three separate communities within one health authority will be examined to determine if there are differences in outcomes between the different areas, and to see how they compare with the larger population of women and children during the first two years of life. Descriptive statistics will be used to analyze this quantitative data from electronic health records. Qualitative data from PHN interviews, documents related to PHN practice, local demographics, and participant observations will be analyzed using the constant comparison method to explore themes.

**Results:** This study has not yet been completed.

**Conclusions:** Findings may demonstrate that the electronic health record database can be a source of valuable information regarding client outcomes. The views of PHNs regarding factors influencing the achievement of client outcomes will help to provide context and a better understanding of available data. Not only could this provide managers, in a publicly funded system, with information about the effectiveness of services directed towards specific populations, but it could also provide a solid foundation for future cost effective program planning.
Food Diversity in the First Year of Life and the Development of Allergic Disease in High-Risk Children

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Introduction: Mounting evidence suggests that delaying the introduction of solid food may promote allergic disease, leading to recent changes in infant feeding guidelines. We investigated the association of food diversity during the first year of life and the subsequent risk of asthma, atopy and atopic dermatitis.

Methods: We accessed data from the 1995 Canadian Asthma Primary Prevention Study (CAPPS), a multifaceted intervention study in high risk children. 545 families were recruited from Winnipeg and Vancouver and children were assessed at 1, 2 and 7 years. Families reported on the introduction of 11 foods (rice, soy, wheat, other grains, eggs, dairy, seafood/fish, peanuts, vegetables, fruits, and meat), and we defined food diversity as the total number of foods introduced. Associations between food diversity and physician-diagnosed asthma, atopic dermatitis, and atopy (determined by skin prick testing) were compared using chi-squared tests and multiple regression.

Results: The average food diversity at 6 months was 3.43 ± 2.01 foods and at 12 months was 8.28 ± 1.47 foods. More foods were introduced earlier in the control group compared to the intervention group (p<0.001) and in Vancouver compared to Winnipeg (p<0.001). Maternal food allergy (p=0.03) and exclusive breastfeeding for >4 months (p=0.02) were associated with lower food diversity. After controlling for these factors, low food diversity (0-2 foods) at 6 months was associated with lower risk of sensitization to non-food allergens at 1 year of age: adjusted OR (aOR) 0.13 (95% confidence interval (95% CI) 0.03-0.53), compared to high food diversity (5-11 foods). Low food diversity at 6 months was also associated with a reduced risk for asthma (aOR 0.40, 95% CI 0.17-0.91) and atopic dermatitis (aOR 0.12, 95% CI 0.04-0.34) at 7 years of age. Fewer associations were observed for food diversity at 12 months.

Conclusion: In this high-risk cohort, low food diversity in the first 6 months of life was associated with reduced risk of certain allergic disease outcomes. This is contrary to recent findings in a population-based European cohort, indicating that further studies are necessary to clarify the impact of early food diversity on the development of allergic disease, particularly in genetically predisposed children.
Endothelial NMDA Receptors are involved in Astrocyte-Mediated Cortical Vasodilation
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Introduction: Brain glutamatergic neurotransmission results in enhanced blood flow to active areas. This hyperemic response is mediated by neuronal N-methyl-D-aspartate (NMDA) receptors and nitric oxide (NO) produced by neuronal NO synthase (nNOS). However, we have observed that isolated middle cerebral artery segments free of neurons dilate in response to NMDA receptor agonists in a manner that requires functional endothelium and endothelial NO synthase (eNOS) (LeMaistre et al. 2012, JCBFM). We also found that direct stimulation of astrocytes in mouse cortical slices led to NMDA receptor and eNOS-dependent vasodilation (LeMaistre et al. 2013, PNAS). The current study was designed to test the possibility that NMDA receptors expressed by brain endothelial cells participate in neurovascular coupling mediated by astrocytes.

Methods: Mouse brain endothelial cells were cultured and NMDA receptor immunoreactivity was investigated. Direct visualization of NO was performed by confocal microscopy. Immunohistochemistry and immunoelectron microscopy were used to determine NMDA receptor localization in the brain vasculature. In mouse cortical slices, astrocyte-mediated vasodilation was observed using two-photon laser scanning microscopy. The effects of neuronal and endothelial NMDA receptors were separated using a Cre-Lox system to reduce expression of NR1 selectively in endothelial cells (NR1fl/fl/Tek-Cre).

Results: mRNA and immunoreactivity for the pan-NMDA receptor subunit, NR1, were detected in brain endothelial cells. These cultures produced NO in response to NMDA receptor co-agonists, glutamate and D-serine, in a manner sensitive to attenuation by glutamate and glycine (D-serine) site competitive NMDA receptor antagonists, chelation of intracellular Ca\(^{2+}\), eNOS inhibition, and endothelial-specific knockdown of NR1. NR1 immunoreactivity was localized to penetrating arterioles and capillaries with a preferential distribution to brain-facing endothelial membranes, suggesting an endothelial distribution at the neurovascular unit in close approximation with astrocyte end-feet. Direct arteriolar exposure to glutamate and D-serine, astrocytic activation by TP flash photolysis of caged Ca\(^{2+}\), and bath-delivered metabotropic glutamate receptor agonist produced increases in local arteriolar lumen diameter. Astrocytic induced vasodilation was significantly reduced in eNR1-Cre\(^{+}\) tissue and in the presence of either a glutamate site or a glycine (D-serine) co-agonist site NMDA receptor antagonist.

Conclusion: Our results support a role for endothelial cell NMDA receptors in activity-dependent brain microvascular vasodilation.
Neuroprotective Action of Neuregulin-1 (Nrg-1) Signaling Against Oxidative Stress and Neuroinflammation in Cerebellar Granule Neurons
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**Introduction:** Oxidative stress and neuroinflammation promote neurodegeneration. Cerebellum has been shown to undergo degenerative changes in neurodegenerative diseases. Neuregulin-1 (Nrg1) mediates inter- and intra-cellular communication through binding to the ErbB receptors and regulates diverse biological responses in nervous system development. Here, we investigated the role of Nrg1 in the cerebellum in neurodegenerative diseases.

**Methods:** Tissue microarray and immunofluorescence staining were used to localize Nrg1 and ErbB4 and ErB2 (Neu) receptors in the human cerebellum. Western blot analysis was used to detect pErbB4, pNeu and pAkt1/Akt1 levels in the mouse primary cultured cerebellar granule neurons (CGNs) in response to recombinant Nrg1β treatment in the conditions of oxidative stress and neuroinflammation.

**Results:** Nrg1 was found to co-localize with pErbB4 or pNeu in the human cerebellar tissue. pErbB4 and pNeu levels were elevated in rNrg1β-treated mouse CGNs. Treatment with H2O2 and LPS decreased the levels of pNeu and pAkt1/Akt1, whereas recombinant Nrg1β treatment significantly reversed pErbB4, pNeu and pAkt1/Akt1 levels.

**Conclusion:** Nrg1 signaling plays a protective role against the oxidative stress and neuroinflammation in cerebellar granule neurons in mice.
p66ShcA Promotes Breast Cancer Plasticity by Inducing an Epithelial to Mesenchymal Transition

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Introduction: Breast cancer is broken into molecular subtypes that correlate with patient prognosis. The majority of Luminal A breast tumors favor good outcome, in contrast to luminal B, ErbB2+ and basal tumors, which associate with reduced survival. However, significant heterogeneity exists even within subtypes, as a subset of luminal patients remain at an elevated risk of cancer-related death. Consequently, there is a need to identify biomarkers that predict patients at an increased likelihood of recurrence. A key process that increases the malignant potential of breast tumors is the epithelial-to-mesenchymal-transition (EMT). An EMT provides epithelial cells with properties of mesenchymal cells by reducing their ability to adhere to adjacent cells and extracellular matrix and endows them with increased motility and invasiveness.

Methods: We employed a multi-pronged approach by combining cell-based systems, mammary fatpad injection of breast cancer cells stably overexpressing p66ShcA and access to primary breast tumors to define a novel role for p66ShcA during breast cancer progression.

Results: Screening a panel of human breast cancer cell lines we discovered elevated expression of p66ShcA specifically within the basal subtype. Injection of ErbB2+ luminal breast cancer cells, stably overexpressing p66ShcA, into the mammary fat pad gave rise to tumors appearing mesenchymal in morphology relative to vector controls. Indeed, further characterization revealed p66ShcA tumors underwent an EMT distinguished by reduced expression of luminal markers: E-cadherin, claudins 3/4/7 and epithelial cytokeratins 8/18, increased expression of mesenchymal features: vimentin and smooth muscle actin (SMA), and associated with increased expression of the basal cell marker cytokeratin 14. Moreover, mRNA expression of EMT-inducing transcription factors: Slug, Twist 1/2 and Zeb 1/2 were significantly elevated in p66ShcA tumors. We also outlined a role for activated Met signalling in promoting a p66ShcA-induced EMT. Furthermore, p66ShcA is sufficient to elevate the invasive and migratory properties of ErbB2+ luminal breast cancer cells in vitro. Finally, p66ShcA mRNA expression stratifies breast cancer patients with elevated EMT markers independent of breast cancer subtype.

Conclusion: This study identifies p66ShcA as one of the first prognostic biomarkers for the identification of more aggressive breast tumors with mesenchymal properties, regardless of intrinsic subtype.
The successful transition to motherhood can be associated with the experience of infant feeding, and women's views of whether that experience has been positive or negative can shape the mothering experience. However, nurses' engagement with best practice breastfeeding promotion may elicit negative responses from women who are either unsuccessful with their attempts to breastfeed, or do not breastfeed for other reasons. For example, under the auspices of informed choice, nurses are obliged to comply with Baby Friendly expectations to disseminate evidence that pertains to the health risks of introducing formula to infants. However, in circumstances where infant formula is recommended by practitioners in order to provide crucial hydration and nourishment, the discourse surrounding risk becomes destabilized. How, then, do nurses navigate the inconsistent messages of evidence, informed choice, risk, and interdisciplinary influences that are associated with best practice? The question arises as to whether nurses are adequately prepared or supported to deal with infant feeding challenges that inevitably arise in perinatal practice settings.

The instability in nurse's preparation to support women in infant feeding challenges informs the research question: What is nurses' experience of infant feeding support? This question is addressed using a hermeneutic methodology, with the intention of understanding nurse's experiences and interpreting these experiences in the context of Canadian perinatal nursing practice. In this case, the research seeks enhanced understanding of nurses' experience with infant feeding support, including dilemmas that nurses encounter when enacting the best practice guidelines of breastfeeding promotion. Participants are comprised of Canadian perinatal nurses of diverse ethnicity, age, and experience. Interviews take place in face-to-face, skype and telephone encounters. It is expected this study may disrupt the taken for granted discourses surrounding infant nutrition and will add to the growing evidence that calls for diverse and particular approaches to infant feeding.
Prolactin–Inducible-Protein (PIP) Influences the Host Immunity by Regulating Intracellular Signaling Pathways in Macrophages

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Introduction: The human Prolactin-Inducible-Protein (PIP) is a secreted protein abundantly found in saliva and other bodily fluids that bathe various ports of pathogen entry suggesting its role in immunity. To investigate the role of PIP in host defense, we generated PIP knockout (KO) mice and assessed the development and function of immune cells. Lymphoid organs from PIP KO mice had significantly lower numbers of CD4⁺ helper T cells compared with wild type (WT) mice. These cells were impaired in their ability to differentiate into Th1 cells in vitro. Also, PIP KO mice were impaired in their ability to mount interferon gamma (IFN-γ) Th1 response, thus failed to control Leishmania major. Collectively, these results suggest that macrophages from PIP KO mice are unable to respond to IFN-γ stimulation. Since the expression of IFN-γ receptor (R) is critical for responsiveness to IFN-γ, we hypothesized that deficiency of PIP affects IFN-γR expression on macrophages, and/or impairs intracellular signaling pathways associated with IFN-γR ligation.

Methods: Splenic and bone marrow-derived macrophages (BMDMs) from PIP KO and WT mice were assessed for IFN-γR using flow cytometry. In addition, the cells were also stimulated in vitro with LPS and IFN-γ and the total cell lysates were assessed for phosphorylation of mitogen-activated protein kinases (MAPKs) and signal transducer and activator of transcription (STATs) protein by western blot.

Results: The expression of IFN-γR on macrophages from PIP KO mice was not different when compared with those from their WT counterpart mice. Furthermore, we found impaired phosphorylation of MAPKs and STATs protein in IFN-γ and LPS-stimulated macrophages from PIP KO mice. Interestingly, the expression of suppressors of cytokine signaling (SOCS) 1 and 3 proteins, known to suppress IFN-γ signaling, was higher in PIP KO macrophages compared to those from WT mice.

Conclusions: Our studies show that although deficiency of PIP does not affect IFN-γR expression on macrophages, it significantly affects intracellular signaling events associated with IFN-γR ligation. This suggest that the inability of PIP KO macrophages to kill Leishmania parasites following IFN-γ stimulation may not be due to impaired expression of functional IFN-γR, but may be related in part to impaired IFN-γ signaling.
Thioredoxin (Trx) system is a major controller of cellular redox status which regulates oxidation/reduction of thiol groups in signaling proteins involved in cell survival & proliferation. Increased availability of Trx1 has been shown to enhance survival & proliferation in neural precursor cells (NPCs). Their contribution towards repair after CNS trauma remains limited. In this study, we hypothesized that availability of Trx will optimize NPC’s contribution to repair after neurotrauma. To increase Trx tissue deposition we employed TAT peptide mediated delivery method to ensure intracellular delivery of Trx (I-Trx). A noTAT protein was used to compare the advantage of intracellular delivery, identified as Extracellular Trx (E-Trx). The effect of I-Trx and E-Trx on NPCs proliferation was quantified in cultures after local delivery. Our in vitro data indicates an increased cell proliferation in brain and spinal cord-derived NPCs in response to Trx treatment. This effect was more pronounced when cells were treated with I-Trx. Additionally, I-Trx increased oligodendrocytes in NPC differentiation. To test efficacy of Trx transduction in preclinical neurotrauma models, we used focal permanent cortical devascularization and clip compression spinal cord injury in rats. Preliminary results from focal devascularization showed an increased number of proliferating NPCs in subventricular zone. We are currently investigating effect of Trx transduction after spinal cord injury. This study represents the first application of intracellular Trx delivery for potential treatment of neurotrauma.
The Roles of NOD Receptors in Fat-Induced Beta-Cell Dysfunction
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**Background and Rationale:** Recent studies suggest intracellular nucleotide-oligomerization domain (NOD) receptors of innate immunity may play a significant role in metabolism and the development of diabetes. The bacterial cell wall components meso-glutamyl diaminopimelic acid (DAP) and muramyl dipeptide (MDP) are endogenous ligands of NOD1 and NOD2 receptors, respectively. NOD1 activation leads to a predominantly proinflammatory response, whereas NOD2 appears to have an immunoregulatory role. Saturated fatty acids can also activate NOD receptors and synergize with NOD ligands. Accordingly, recent data suggest NOD1-null mice have better glucose tolerance with high fat feeding, whereas in NOD2-null mice glucose tolerance is worse, than in controls. This suggests a β-cell role of NODs because glucose tolerance reflects β-cell performance in relation to insulin sensitivity.

**Methods:** First, NOD mRNA was assessed in mouse islets. Wild-type islets were then exposed to the NOD1 ligand FK565 and the NOD2 ligand L18-MDP in the presence or absence of palmitate (and oleate as negative control) for 48h. NOD1- and NOD2-KO islets were also exposed to palmitate. Ex vivo studies were performed in islets of wild-type and NOD2-KO mice following 96h infusion of saline or Intralipid 20%. Finally, wild-type mice were injected intraperitoneally with the NOD1 or NOD2 activator or saline 6 h before undergoing hyperglycemic clamps in vivo.

**Results and Discussion:** Mouse islets express NOD1/2 mRNA. The NOD1 ligand FK565 decreases β-cell function in vitro, whereas NOD2 ligand MDP does not have any affect. The NOD 1 ligand also synergizes with palmitate in decreasing β-cell function and the NOD2 ligand appears to prevent palmitate-induced β-cell dysfunction in vitro. NOD1-KO and, surprisingly, NOD2-KO islets are protected from palmitate-induced β-cell dysfunction in vitro. Ex vivo data also suggest NOD2 deletion protects against fat-induced β-cell dysfunction. During in vivo hyperglycemic clamp studies, the NOD1 activator decreased β-cell function, whereas the NOD2 activator did not have a significant effect on β-cell function.

These data support the notion of a detrimental role of NOD1 activation and a dose-, time- and context-dependent role of NOD2 activation in beta-cell function.
Zeb2: A Novel Regulator of Cardiac Fibroblast to Myofibroblast Transition
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Introduction: Fibroblast to myofibroblast phenoconversion is a crucial step during cardiac fibrosis. Myofibroblasts contribute to: chronic extracellular matrix remodelling and the development of scar tissue which subsequently leads to myocardial stiffening, impaired contraction, cardiac dysfunction and eventually to heart failure. Previously we showed that Meox2, a homeobox transcription factor, can inhibit myofibroblast phenoconversion by down-regulating a set of myofibroblast markers. Here we show for the first time that Zeb2, a repressor of Meox2 and an epithelial to mesenchymal transition inducer, may play a crucial role during this phenoconversion process.

Methods: Rat cardiac fibroblasts were isolated using Langendorff perfusion technique followed by enzymatic digestion. Zeb2 and Meox2 expression levels and their sub-cellular localization were determined by performing immunoblotting and immunocytochemistry. Zeb2 gain of function experiments were carried out using adenoviral construct encoding Zeb2; and protein was harvested 96h post-transduction and subjected to immunoblotting.

Results: We showed that Zeb2 expression is lower in fibroblasts but higher in phenoconverted myofibroblasts. Conversely, Meox2 expression is higher in fibroblasts than in myofibroblasts. In sub-cellular studies, Zeb2 expression was found to be higher in the nuclei of myofibroblasts whereas it is only minimally expressed in fibroblasts. Zeb2 overexpression caused an up-regulation of a set of myofibroblast markers- SMemb, ED-A fibronectin and α-SMA. In wound healing migration assay, ectopic expression of Zeb2 resulted in a less migratory phenotype- characteristic of mature myofibroblasts. We have also shown that Zeb2 over-expression causes decreased Meox2 expression in endothelial cells.

Conclusion: We will investigate the role of Zeb2 in myofibroblast proliferation, apoptosis and function in vitro. Using a rat myocardial infarct model we will determine the role of Zeb2 in cardiac fibrosis. Thus, findings from our study will contribute to our current understanding of the mechanism behind fibroblast to myofibroblast phenoconversion and may provide a basis for developing a Zeb2-based novel anti-fibrotic drug in the future.
Protein arginine methyltransferases (PRMTs) and Transcriptionally Active Chromatin Domains
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In higher eukaryotes genomes are organized into chromosomal domains. Specialized elements called barrier elements play role in preserving the domain structure, are located at the boundaries of the domains. But the mechanisms by which active chromosomal domain is maintained are still in its infancy. It was demonstrated for the well characterized chicken erythroid β-globin domain that the histone modifying enzyme protein arginine methyltransferase 1 (PRMT1), which methylates H4 at R3 producing H4R3me2a (asymmetric), plays a critical role in establishing and maintaining active histone marks. Therefore, we hypothesize that recruitment of H3 and H4 PRMTs to the barrier site and active chromosomal domains is a critical event in maintaining active domain structure because it establishes active histone marks during cellular differentiation. It is of interest as these enzymes can confer both active and inactive arginine modifications in a context dependent manner and can change the fate of chromatin structure. With the combination of Next-generation sequencing and molecular approach we have discovered and characterized active chromatin domain in G0 phase chicken erythrocyte cells. In the current study, we will be exploring the role of PRMTs in maintaining the domain conformation. Our study will provide novel insight into the mechanisms of how the chromatin modifying enzymes such as, PRMTs can regulate the higher order chromatin structure and complex regulatory network of gene expression.
Overcoming Drug-Resistant Influenza through Inhibition of M2 Ion Channel

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Introduction: Influenza virus infections lead to thousands of deaths and millions of hospitalizations each year. M2 is a virally-encoded ion channel that is required for influenza replication. However, licensed therapies such as amantadine and rimantadine, which are potent M2 ion channel inhibitors, are no longer effective due to widespread drug resistance. The emergence of resistance of influenza viruses emphasizes the importance of finding new antivirals. Almost all adamantane-resistant viruses encode a S31N mutation within M2. Thus, compounds that target both WT and the S31N mutant are highly desired. We describe novel HMA-derived compounds that inhibit wild-type and drug-resistant M2 ion channel and viruses with minimal cytotoxicity.

Methods: Conventional medicinal chemistry approaches, molecular docking and molecular dynamics (MD) simulations of drug-M2 interactions supported our design hypothesis. In-house medicinal chemistry was carried out to synthesize derivatives of our lead compounds to further investigate structure-activity relation of this novel class of compounds. We investigated M2 ion channel activity of our compounds by co-transfecting tSA-201 cells with plasmids encoding GFP and M2(S31/N31) (A/Hong Kong/1073/99(H9N2)) and recording pH-dependent ion currents in GFP-positive cells by whole-cell patch clamp electrophysiology. The potency of the inhibitors was expressed as the percentage inhibition of A/M2 current observed after incubation with up to 100 μM compounds. Antiviral activity of compounds was tested in mini-plaque assay where cell survival after infection with the virus in presence or absence of drug was correlated to the antiviral activity. Pharmacokinetic profile of lead molecules was investigated in CD-1 mice using UPLC/MS/MS tandem system that benefited from pg sensitivity and allowed the calculation of elimination half-life. Maximum tolerated dose study was performed in rats to assess cytotoxicity in vivo.

Results: The potency of the most active compound SM111 in inhibiting WT influenza virus is comparable with that of amantadine (EC50 = 5.2 ± 0.3 μM for SM111 vs. 0.4 ± 0.1 for amantadine) which correlates well with the electrophysiology assay (90% inhibition of M2 current at 100 μM). On the other hand, SM122 shows moderate inhibition of the A/M2(S31N) current (35 ± 5% at 100 μM). SM122 also inhibited the viral replication in mini-plaque assay with an EC50 of of 2.4 ± 0.5 μM against Influenza virus (A/PR/8/1934(H1N1)) bearing the S31N mutation. The pharmacokinetic study revealed a plasma t1/2 of 60
and 80 minutes for SM111, and SM122 respectively. In an attempt to quantify the maximum tolerated
dose, a cumulative dose of 7.5 or 10.8 mg/kg for SM111 and SM122 was injected over a course of 3 hours
to rats and hemodynamic and ECG parameters were monitored. Neither molecule caused significant
changes in respiration, blood pressure, cardiac function, or abnormal behaviour.

**Conclusion:** Here we described a novel class of acylguanidines that inhibit the A/M2 ion channel and
virus and are additionally stable and tolerated *in vivo*. The potency of the lead molecules in both
electrophysiology and antiviral assays in addition to PK and MTD results suggest the potency, efficacy
and safety of this novel class of compounds that can act as a platform for their further development as
potential broad-spectrum antivirals in a long-term attempt to confront influenza virus resistance.
Are Epigenetic Marks Preserved in Post-Mortem Brain Tissue? Studying the Epigenetic Effects of in utero Alcohol Exposure in Human Autopsy Brain Tissue

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Introduction: Epigenetic changes in the brain have been studied in many different animal models of in utero alcohol exposure. Variation in the circumstances before and after death (cause of death, time to autopsy, time in fixative) are some of the limitations to using human autopsy tissue. DNA is stable after death, however, the stability of epigenetic marks including histone post-translational modifications (PTMs) and DNA cytosine modifications remains to be fully investigated. Hypothesis: epigenetic marks are stable post-mortem, and can be studied in human brains that have been exposed to alcohol in utero.

Methods: Normal animals including adult rat (n=1); 1-3-day-old piglets (n=7); newborn (n=16), 10-day-old (n=16), 8-10-week-old (n=16) and 13+-week-old (n=2) mice were euthanized, their brains were removed, sectioned and subjected to artificial post-mortem delay at four time points (0, 24, 48 and 72 hours). A portion of the brains also remained at room temperature for 6-8 hours before reaching the 4 time points at 4°C. Brain samples were then frozen at -70°C for biochemical assays, or immersed in formalin, embedded in paraffin, and used for immunohistochemical study of 14 epigenetic markers (4 DNA cytosine modification, 10 histone PTM).

Results: The one rat brain, which was used for proof-of-principle, demonstrated five epigenetic markers to be stable ≥72 hours post-mortem (5caC, 5fC, 5hmC, H3K14ac, H3K27me2). The seven pig brains demonstrated similar results. At ≥72 hours post-mortem, six epigenetic marks remained stable (5caC, 5hmC, 5mC, H3K4me3, H3K14ac, H4K16ac). Those with the 6-8 hour room-temperature delay were found to not differ greatly from those stored immediately at 4°C. Preliminary mouse brain results are also correlating with the above findings. This indicates that the majority of epigenetic marks planned for study in human brains remain stable up to ≥48 hours post-mortem.

Conclusion: Preliminary data supports the hypothesis: certain epigenetic marks are relatively stable (≥48 hours post-mortem), and in some, much longer (≥72 hours post-mortem). This assures that the investigation of epigenetic marks in human autopsy material will be possible. The next part of the study is to investigate the epigenetic effects of prenatal alcohol exposure on the human brain in autopsy samples.
Phosphatidylinositol (3,4)-Bisphosphate-Binding Proteins Control B Cell Metabolism and Suppress Autoimmunity

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Introduction: Activation of the PI3K pathway generates multiple phosphoinositides including PI(3,4)P2 and PI(3,4,5)P3. While PI(3,4,5)P3 plays critical roles via binding to Btk and other effectors proteins, the function of PI(3,4)P2 in B cells remains unknown. Tandem PH domain containing proteins (TAPPs) are adaptor proteins that specifically bind to PI(3,4)P2 via their C-terminal PH domains, targeting them to the plasma membrane.

Methods: To investigate the role of TAPP binding to PI(3,4)P2 in B cell activation, we have performed studies on gene-targeted mice bearing inactivating mutations in the PH domains of both TAPP1 and TAPP2.

Results: TAPP knock-in (KI) mice exhibit hypergammaglobulinemia and develop lupus-like characteristics including anti-DNA and anti-nuclear antibodies and deposition of immune complexes in kidneys. TAPP KI mice develop chronic germinal centres (GCs) and spontaneous increase in co-stimulatory molecules such as CD80 and CD86 within spleen and lymph nodes. B cell transfer experiments indicate that elevated serum IgG results from B cell intrinsic functions of TAPP-PI(3,4)P2 interaction. B cell contribution to other autoimmune phenotypes is currently under investigation. TAPP KI B cells show elevated phosphorylation of Akt, increased metabolic activity and increased survival both in germinal centres and with stimulation in vitro. Paradoxically, TAPP KI B cells have elevated expression of the inhibitory phosphatase PTEN, an signalling adaptation previously reported in chronically stimulated B cells. Current investigations are examining B cell glycolysis, as preliminary results indicate increased media acidification rate and increased expression of glucose transporter Glut1 in TAPP KI B cells. Together our findings suggest that TAPP - PI(3,4)P2 interaction is important for regulating antigen receptor signalling via Akt, B cell metabolism and development of autoimmunity.
Approaches to Handle Implausible Diet Recalls in Surveys
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Background: The association of dietary exposures with health outcomes may be attenuated or reversed as a result of misreporting of energy intakes (EI). It is still unknown which statistical technique for handling implausible recalls can best address this problem.

Objective: This study aimed to compare 7 different approaches for dealing with implausible recalls when analyzing the association between overweight and obesity with dietary covariates.

Design: We examined data from 16,187 Canadian adolescent and adult participants (≥12 years) in the Canadian Community Health Survey 2.2. Multinominal logistic regression was conducted with overweight and obesity as outcomes of interest and dietary variables as exposures.

Results: Adult under-reporters reported substantially lower mean EI (1434 in males, 1075 in females) compared to plausible (2611 in males, and 1967 in females) and over-reporters (4483 in males, and 3267 in females) (p-trend <0.0001). Similarly, % energy from solid fat and added sugars, and energy density were higher in over-reporters compared to under- and plausible reporters (p-trend<0.001). In the basic multilevel model, EI showed a significant negative association with overweight (OR: 0.988 (0.979-0.998)), and obesity (OR: 0.989 (0.977-1.00)). After adding a variable indicating the reporting status, a significantly positive association between EI and overweight (OR: 1.037 (1.019-1.055) and obesity (OR: 1.109 (1.082-1.137) was observed (p<0.0001).

Conclusions: Simple adjustment for the reporting group using a variable for reporting status, maintained statistical power and shifted the association of dietary exposures with obesity to the expected direction.
Inhibition of Allergic Airway Hyperresponsiveness by Inhaled Simvastatin is Associated with Selective Suppression of Cathepsin Activity in the Lung

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**Background:** Increased activity of cathepsins (cysteine proteases) drives local airway inflammation and tissue remodeling associated with asthma. We have shown that low dose inhaled simvastatin (S\textsubscript{in}) prevents airway inflammation and hyperresponsiveness (AHR) in allergen-challenged mice. Here, we tested if the impact of S\textsubscript{in} on lung inflammation and AHR is associated with suppression of cathepsin activity in lungs.

**Method:** Female Balb/c mice were repeatedly challenged with inhaled house dust mite (HDM) for 2-wk with/without S\textsubscript{in} (6μg/kg) or fluticasone (10μg/kg). Another group of mice underwent 1-week of “allergen avoidance” post 2-wk HDM challenge. 48h after final HDM challenge/allergen avoidance week we measured lung function in response to inhaled methacholine (MCh) using a small animal ventilator, total and differential inflammatory cell number in bronchoalveolar lavage (BAL). All mice were injected (tail vein) with ProSense 750 FAST, a fluorescence dye selectively activated by cathepsin, and subjected to quantitative in vivo optical imaging using IVIS® Spectrum system.

**Results:** HDM challenge induced significant cellular infiltration, including >82-fold increase in total inflammatory cell number (n=10, p<0.001), and >800-fold increase in eosinophil and neutrophil number (p<0.001) compared to allergen-naïve mice. Influx of total inflammatory cells in BAL was blunted in fluticasone (54.3±11.6%; n=8; p<0.05), S\textsubscript{in} (76.2±4.2%; n=8; p<0.01) and allergen avoidance group (80.1±0.7%; n=4; p<0.01), this included blunted influx of eosinophil by fluticasone (45.0±12.9%), S\textsubscript{in} (76±4.5%) and allergen avoidance (73.7±1.6%). Similarly influx of neutrophil was also blunted by fluticasone (47.1±13.8%), S\textsubscript{in} (73.7±3.1%) and allergen avoidance (89.7±0.8%). Notably, S\textsubscript{in}, but not fluticasone or allergen avoidance, blunted HDM-induced increased airway resistance (R\textsubscript{aw}) and tissue damping (G) by 30% and 44% respectively in response to inhaled MCh (p<0.05). In vivo imaging revealed that S\textsubscript{in}, but not fluticasone or allergen avoidance, decreased lung-specific cathepsin activity ((2.0±0.5)10\textsuperscript{10} photons/sec) compared to HDM-challenged mice ((4.0±1.5)10\textsuperscript{10} photons/sec) (n= 3; p<0.05)
**Conclusion:** Sin, fluticasone and allergen avoidance significantly blunted allergen induced airway inflammation, however only inhaled simvastatin significantly improved lung function, an effect that correlated with the unique capacity of inhaled simvastatin to blunt cathepsin activity in the lungs. This reveals a new mechanism of action for statins that can positively impact inflammatory lung disorders.
The Role of Lysosomal Acid Phosphatase (Acp2) On Development of Cerebellar Granule Cells

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Introduction: The cerebellum is responsible for motor control and cognitive functions. The cerebellar cortex can be divided into three layers consist of different cell types, such as granule cells (gcs), Purkinje cells (PCs) and Bergmann glial cells. During the development, the gc precursors arise from the cerebellar rhombic lip and initially migrate over the entire subpial surface of the cerebellar primordium to form the external germinal zone. Thereafter, the gc precursors radially move to the inner granule cell layer (igl) and differentiate into gcs. In this process, sonic hedgehog (Shh) which secreted by PCs is an important promoter of gc precursors proliferation. A lysosomal acid phosphatase 2 (Acp2) mutant mouse (nax) shows a significant gcs reduction in the cerebellum. We hypothesize that the Acp2 plays a pivotal role in the proliferation of cerebellar granule cells.

Methods: We used the Acp2 mutant and the wild-type sibling mice as control in this study. Immunohistochemistry and Western blotting are used to detect the molecular expression.

Results: In the Acp2 mutant mouse the amount of gcs is greatly reduced compared with the wild-type sibling mouse. It is also revealed that the normal parallel pattern of the Bergmann glia cell fibers is substituted for the disrupted appearance. While the Shh expression is down regulated from P12, the expression of Pax6 by gc precursors in external germinal zone is down regulated at around P7 and it is maybe due to the decreased number of gcs.

Conclusion: The absence of Acp2 impacts on the gcs proliferation prior to the Shh signaling pathway to cease gcs proliferation during cerebellar development. The significant reduction in the proliferation and probably differentiation of gcs in the Acp2 mutant mouse reveals that the Acp2 may participate in the development of granule cells and has a critical role in this process.
Clinical Presentation of Malnourished Patients with Pneumonia Requiring Prolonged Ventilatory Support
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Introduction: Prolonged exposure to oxygen during ventilatory support is likely to generate physiological abnormalities in the lungs of critically ill infants with bronchopneumonia. The induced physiological changes could impact the growth of young infants and could promote morbidity and mortality of susceptible patients.

Methods: We assessed the impact of prolonged exposure to oxygen on the clinical course of infectious pneumonia, nutritional status, relative growth and lung functions of newborn and young infants who were treated for severe pneumonia and required ventilatory support (G1, N=38). Ventilated patients were studied longitudinally to assess the association of weight/height for age z-scores with blood proteins, the ferroxidase enzyme ceruloplasmin oxidase (Cer) and copper (Cu) in plasma. The relationship of prolonged ventilation with severity of infection and type of feeding compared with non-ventilated patients (G2, N=19) and healthy controls (C, N=18) was assessed.

Results: On admission patients exhibited statistically lower blood protein, Cer and Cu levels compared to C. Prolonged exposure to oxygen in G1 patients induced a steady state of hypoalbuminemia compared to zero time. The groups differed with regard to growth characteristics, with patients in G1 having consistently, but not significantly, lower z-scores than G2. Although not statistically significant, both fever and death were more common among G1 patients compared to G2 patients (p=0.14 and p=0.25 respectively). Recurrent infection rates and duration of ventilation were consistently worse among patients with indicators of malnutrition. Eighty % of ricketic patients required oxygen therapy compared to 20% who did not require oxygen therapy.

Conclusion: The sample of children with pneumonia is small and therefore caution should be exercised in making conclusion.
A Feasibility Trial of Light Therapy for Post-Treatment Cancer-Related Fatigue
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Background: Cancer-related fatigue (CRF) 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 of CRF are poorly understood and the available treatments may not provide relief. Fatigue has been identified as a significant contributor to decreased QOL, making it an important target for intervention. One approach that may be a safe and inexpensive treatment is light therapy as it has been shown to prevent the typical worsening of fatigue in patients undergoing chemotherapy. The aim of was to evaluate the feasibility of a one-month light therapy intervention in post-treatment cancer survivors with CRF.

Methods: This was a 6-week blinded randomized controlled design. Subjects were recruited from Calgary and included men and women who met criteria for fatigue and had completed cancer treatment at least three months prior. 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 every morning for 30 minutes upon waking for one month. Changes in fatigue, mood, sleep quality, and QOL were assessed using questionnaires.

Results: Eight participants were randomized (BWL=4, DRL=4). The majority of participants were women with an average age of 57 (SD=11). Time since last treatment ranged from 3 months to 5.4 years (M=26, SD=26). Participants in both groups complied with the treatment, using the light for an average of 27 days and for an average of 29 minutes per day. Preliminary analyses suggested that participants in both groups showed improvements in fatigue, sleep quality, and QOL over time.

Conclusion: These results indicate that the light treatment intervention is feasible and acceptable to cancer survivors with CRF. Participants in both groups were compliant with the protocol. Analysis of the preliminary data suggested that both groups showed improvements on outcome measures; however the sample size was too small and there was insufficient power to reach conclusions about treatment efficacy. A larger trial is currently underway which will evaluate outcomes and potential mechanisms associated with light treatment of CRF.
Polypharmacology of a Novel Bi-Functional, Phosphodiesterase 4 Inhibitor and Long-Acting β2-Adrenoceptor Agonist: GS-5759, In Relevance to the COPD Treatment

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Introduction: Global initiative of chronic obstructive lung disease (GOLD) recommends that combining different bronchodilators may improve efficacy and decrease the risk of side effects, compared to increasing the dose of a single bronchodilator. GS-5759 is a bi-functional phosphodiesterase-4 inhibitor (PDE4i) and long-acting β2-adrenoceptor agonist (LABA) that is being developed as a novel therapy for chronic obstructive pulmonary disease (COPD). GS-5759 is a unique compound because it acts simultaneously on a surface receptor (β2) and intracellular target, an enzyme phosphodiesterase 4 (PDE4). It is the only inhaled PDE4i available so far. GS5759 has similar deposition characteristics of multiple pharmacophores and hence the target tissues are exposed to both pharmacophores in a fixed 1:1 ratio. It is designed to retain the in-vitro and in-vivo activities of its individual pharmacophores.

Methods: In this study, we have compared the ability of GS-5759 to enhance anti-inflammatory gene transcription in the BEAS-2B human airway epithelial cell line with the LABA, indacaterol and the PDE4i, GSK256066. We performed luciferase reporter assays and real time PCR studies as a measure of gene induction. BEAS-2B cells were treated and harvested at different time points: 1h, 2h, 6h and 18h, to study gene induction profile by GS5759.

Results: We report that GS-5759 has similar affinities for the human β2-adrenoceptor and PDE4. We treated BEAS-2B cells with the above mentioned compounds at various time points and performed microarray analysis to understand the gene induction profiles of the novel bi-functional compound in comparison to its mono-functional parent compounds. In the microarray analysis more than 50,000 probe sets were used. Microarray analysis identified some very important cyclic AMP regulated genes: CRISPLD2, RGS2, C5aR1, CD200, SOCS3, which are anti-inflammatory and bronchoprotective. Most of these genes are induced within 2h. We selected some of these genes to perform real time PCR studies in detail to understand polypharmacology of this bifunctional compound.

Conclusion: We suggest that the ability of GS-5759 to enhance cAMP dependent gene transcription indicates that combination of an inhaled PDE4i and a LABA as one molecule, may impart clinical benefit in COPD by enhancing therapeutic Index, anti-inflammatory and bronchodilatory effects.
### Introduction
Alcohol use disorder is a global health problem affecting over 140 million people worldwide. The plasticity of brain changes associated with alcohol dependence, recovery, and different treatments are only partially understood. We compared changes in resting state functional connectivity in patients undergoing residential treatment for alcohol use disorder. Our aim was to identify changes in the resting state networks caused by chronic alcohol abuse and to study the brain’s adaptation to early recovery during different treatments.

### Methods
20 male patients (age 24-63) with alcohol use disorder (DSM-IV) were recruited 5-10 days after detoxification and scanned at the beginning and after a 21-day residential treatment. 10 healthy volunteers matched for age, handedness, and education level were scanned for comparison. The subjects were scanned using a 4.7 Tesla magnetic resonance imaging (MRI). The two scanning sessions included an anatomical scan, a resting-state functional MRI scan, and a diffusion tensor imaging scan. The functional data was analyzed using an independent component analysis with FSL and a custom MATLAB code.

### Results
Our preliminary findings revealed significant changes in several resting state networks including the core and frontal networks. In comparison to controls, patients had significant differences in functional connectivity between anterior cingulate cortex and different somatosensory, motor, visual, and association regions.

### Conclusion
These findings suggest changes in functional connections of anterior cingulate cortex in alcohol dependent patients before and after undergoing treatment. Anterior cingulate cortex is involved in modulation of execution of appropriate and suppression of inappropriate responses in not only higher order motor control and signal processing but also in reward anticipation and impulse control. Our findings, therefore, help us better understand dynamic changes in functional brain connectivity which are closely associated with addiction, craving and internal conflict resolution. They also provide evidence of dynamic brain plasticity in very early stages of addiction recovery.
Novel Function of High Mobility Group A2 (HMGA2) in Triple-Negative Breast Cancer
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Introduction: Triple-negative breast cancer (TNBC) is characterized by the lack of immunohistochemical expression of the ER, PR, and HER2 proteins. The TNBC subtype primarily occurs in younger women of African American or Hispanic descent and tumors tend to be high grade and characteristically aggressive with high recurrence, metastasis, and mortality rates. The non-histone chromatin-binding protein HMGA2 plays an important role in fetal development and carcinogenesis. Here, we examined MDA breast cancer cell extracts for formation of a complex that may reflect association between the damage responsive proteins HMGA2 and poly (ADP-ribose) polymerase-1 (PARP-1), a multifunctional and abundant nuclear enzyme known to recognize DNA lesions and promote DNA repair.

Method: RT-PCR and Western blot analysis of untreated MDA-MB-231 cells was performed to show expression of HMGA2 in the MDA cells with different HMGA2 protein levels. Incubation with methyl methanesulfonate (MMS) was performed to induce DNA damage. Immunoblotting to identify Gamma-H2AX (a biomarker for DNA double strand breaks) was done in the cell extracts. Co-immunoprecipitation experiments for PARP1 and HMGA2 were performed. Silencing of HMGA2 in the MDA cells was accomplished by siRNA. PARylation was detected by Western blot after pull-down of PARP1.

Results: We showed the expression of HMGA2 in human MDA cell lines. We discovered that HMGA2 binds to PARP1 and influences PARylation activity of PARP1. The catalytic activity of PARP-1 is activated by DNA strand breaks, and results in self-PARylation and PARylation of other PARP1-interacting proteins. Upon DNA damage we found that self-PARylation of PARP1 is reduced following HMGA2 knock-down. These results suggest a regulatory role for HMGA2 in PARP1 activity.

Conclusion: HMGA2 is a novel interaction partner of PARP1 in TNBC cells. Our data suggest that HMGA2 participates in the regulation of PARP1 catalytic activity.
Gene Regulation by FREM1 Isoform 2 (TILRR) and Its Potential Role in HIV-1 Vaginal Infection

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Introduction: FREM1 transcript variant 2 (TILRR) is a novel regulatory component, which stimulates host defense against infection through binding of IL-1R1 and TLR complex and enhancing the recruitment of MYD88 in the Ras-dependent NFκB signal transduction pathway. Our previous study has identified FREM1 as a novel candidate gene in resistance and susceptibility to HIV infection in the Pumwani Sex worker cohort (PSWC). In this study we investigated the effect of TILRR on gene expression of several important signal transduction pathways by overexpressing it in the HeLa cells.

Methods: TILRR was overexpressed in HeLa cells using eGFP tagged plasmid construct. Transfection efficiency was determined by fluorescence microscopy and flow cytometry. TILRR RNA overexpression was confirmed by qRT-PCR. The effect of TILRR on the expression of 252 genes in important signal transduction pathways was subsequently investigated by qRT-PCR with 3PCR arrays (Human signal transduction, extracellular matrix and transendothelium migration, and MAPKinase).

Results: Overexpression of TILRR significantly upregulated 64 genes, and downregulated 69 genes (p<0.001) in MAPKinase, transendothelium migration and NFκB pathways. These findings are novel. Pathway studio analysis showed that some of the most significant upregulated genes directly influence gene expression and inflammatory responses. Although how TILRR influence the expression of these genes need to be investigated, our study is the first to show that TILRR may direct influence gene expression in addition to its role in enhancing NFκB and inflammatory responses.

Conclusion: Since transendothelium migration, NFκB and inflammatory response pathways are extremely important in HIV vaginal transmission, further study of the role of TILRR in gene regulation may identify novel targets and develop intervention technology against HIV-1 vaginal infection.
Otx2 Exhibits Cell Context-Dependent Effects on Cellular and Molecular Properties of Human Embryonic Neural Precursors and Medulloblastoma Cells.

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Introduction: Medulloblastoma (MB) is the most common primary pediatric brain tumor and is divided into 4 subtypes: Wnt, Sonic Hedgehog (Shh), Group 3 and Group 4, based on different genomic alterations and gene expression profiles. This extensive heterogeneity has made it difficult to assess the functional relevance of genes to malignant progression. For example, expression of transcription factor, Orthodenticle homeobox2 (Otx2) is frequently dysregulated in multiple MB variants; however, its role may be subtype-specific. We recently demonstrated that neural precursors derived from transformed human embryonic stem cells (trans-hENs), but not their normal counterparts, resemble Groups 3/4 MB in vitro and in vivo and also exhibit >12-fold overexpression of Otx2. Here, we tested the utility of normal hENs and trans-hENs as a means of dissecting the role of Otx2 in MB using gain and loss of function studies respectively.

Methods: Following overexpression (hENs and Daoy Shh MB cells) and knockdown (trans-hENs and D283 Group 4 cells), we evaluated the effects of Otx2 on 1) cellular functions such as self-renewal, proliferation and cell migration in vitro 2) tumorigenic potential in vivo and 3) global gene expression using Affymetrix analysis. To rescue Otx2 induced cellular phenotype, we overexpressed Sox2 in Otx2+ Shh MB and hENs. Data were analyzed using the Prism 5 software and P values ≤0.05 were considered significant.

Results: Parallel experiments with MB cells revealed that Otx2 exerts tumor suppressive effects in hEN and Shh MB cells, likely by regulating self-renewal and decreasing expression of pluripotent genes such as Lin28A, Oct4, Nanog and Sox2. Concomitant to the negative regulation of hESC genes, an increase in Otx2 recruitment at Sox2 promoter was observed by chromatin immunoprecipitation assays. This was supported by overexpression of Sox2 in Otx2+ Shh MB and hENs that resulted in significant rescue of self-renewal and cell migration. In contrast, Otx2 is oncogenic and promotes self-renewal of trans-hENs and Groups 3/4 MB independent of pluripotent genes.

Conclusion: Our study demonstrates a cell context-dependent function of Otx2. It also underscores the value of hESC derivatives as developmental models of MB and for investigating key biological questions related to embryonal childhood cancers.
Cardiac fibrosis is component of a number of cardiovascular diseases, including myocardial infarction. Excessive formation of extracellular matrix (ECM) occurs in activated cardiac (myo)fibroblasts that reside in the infarct scar or those moving from adjacent viable tissue. We propose that Ski, an endogenous repressor of TGF-β1, regulates ECM remodeling and cellular motility by influencing MMP function, and specifically MMP-2. Moreover, Ski alter cardiac fibroblast motility by altering the expression of paxillin (a focal adhesion associated protein) and its kinases including FAK (Tyr 397) and PYK2 (Y402). Primary adult rat fibroblasts (P1) were subjected to either exogenous Ski overexpression by adenoviral Ski (Ad-Ski) or Ad-Lac-Z control with the multiplicity of infection (MOI) of 50 and 150. Ski overexpressing cells exhibited significantly lower MMP-2 secretion and significantly decreased MMP-2 activity as detected using gelatin zymography. Using Transwell plates, Ski overexpression was associated with a significant decrease in migration of cells in the presence of a chemoattractant in the lower well. Moreover, reduced motility of P1 cells vs. control was observed by Ski overexpression group via scratch assay. Furthermore, downregulation of paxillin was detected in Ski overexpressing cells lysates vs. Lac-Z and control samples. Both FAK (Tyr 397) and PYK2 (Y402) were decreased in Ski overexpressing cells vs control. We suggest that Ski may exert multiple effects eg, MMP2 and paxillin, which affects motility and adhesion, and thus represents a putative mechanism for modulation of myofibroblast function and cardiac fibrosis.
Gestational Diabetes Mellitus Induces Cardiovascular Defects and Mitochondrial Dysfunction in Fetal and Neonatal Offspring

Introduction: Gestational diabetes mellitus (GDM) is the most common complication of pregnancy. Children of mothers that had GDM are at an increased risk for the development of cardiovascular complications later in life. The purpose of this study was to determine the effect of GDM on fetal and neonatal offspring. We hypothesize that the hearts from offspring exposed to GDM will exhibit impaired mitochondrial respiration that is associated with altered cardiac structure and/or function.

Methods: To induce GDM, female rats were fed a high fat and sucrose diet (45% kcal from fat) to induce obesity and glucose intolerance prior to mating with lean males. Lean control dams received a low fat diet (10% kcal from fat). Maternal diets were continued during mating and pregnancy. In utero echocardiography was performed during gestation (embryonic day 18) using a Vevo 2100 ultrasound system. Fetal rat ventricular myocytes were isolated from the hearts of fetal rat offspring (embryonic day 20) and mitochondrial oxygen consumption was analyzed using a Seahorse Biosciences Extracellular Flux Analyzer.

Results: Heart weights of newborn pups from GDM dams are increased when compared to lean controls (24-hours perinatal; p<0.05). Fetal offspring exposed to GDM exhibit altered left ventricle posterior wall thickness. Mitochondrial basal and maximal respiration was reduced in fetal offspring exposed to GDM (p<0.05). Mitochondrial spare capacity was also reduced (p<0.05).

Conclusion: Our data indicate that the hearts of the offspring of GDM mothers compensate in response to metabolic distress through hypertrophic myocardial growth and altered mitochondrial function. Future work will determine whether cardiac phenotypes in the offspring are programmed in utero and whether the ability of the mitochondria to increase energy production in response to stress could compromise cardiac contractibility in the offspring as they age. These factors could put offspring of GDM mothers at greater risk of cardiovascular disease later in life.
Hepatic Stellate Cells - Tregs - PI3K Interaction: A Costly Three-Way Relationship in Visceral Leishmaniasis

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Background: Visceral leishmaniasis (VL), caused by the intracellular parasite, Leishmania, is associated with immune dysfunction in the spleen, liver and bone marrow and if not treated, can lead to death. We previously reported that mice with inactivating knock-in mutation in the p110δ gene (p110δD910A mice) are resistance to VL, due in part to impaired regulatory T cell (Treg) function. Here, we investigated the mechanism of this resistance by focusing on the role of hepatic stellate cells (HSCs), which are known to regulate Treg induction in the liver.

Methods: Parasite burden and Treg levels in the livers of L. donovani infected wild type and p110δD910A were determined at different time points. HSC numbers were detected by immunofluorescence staining and direct ex vivo flow cytometry. The ability of L. donovani to infect HSCs in vitro and in vivo was assessed via H&E staining of cytospin preparations and confocal microscopy. Infected HSCs were co-cultured with CD4⁺ T cells and their impact on Treg induction was assessed by flow cytometry. HSCs were depleted in vivo in order to determine the impact of HSCs on hepatic immunity (Treg numbers, cytokine production and parasite burden).

Results: We show that L. donovani can infect HSCs in vivo and in vitro and this infection leads to the production of proinflammatory and immunoregulatory cytokines that are known to induce Tregs. We also demonstrate that infection with L. donovani leads to dramatic expansion of HSCs in a PI3K-dependent manner, and this correlated with expansion of hepatic Tregs. We further show that L. donovani-infected HSCs can induce CD4⁺ T cells to become Tregs and this effect is dependent on signalling via the p110δ pathway of PI3K. Depletion of HSCs led to significantly less parasite burden, fewer Tregs in the liver, enhanced IFN-γ and a concomitant decrease in IL-10 production by HSCs and hepatic T cells.

Conclusions: Collectively, our results demonstrate, for the first time, the critical role of HSCs in the pathogenesis of VL, and suggest that the enhanced resistance of p110δD910A mice to L. donovani is due in part to impaired expansion and inability of their HSCs to induce Tregs.
Inhibitory Influence of Denosumab: A Monoclonal Antibody to Receptor Activator of Nuclear Factor-Kappa B Ligand (RANKL) in the Context of Experimental Colitis

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The pro-inflammatory mediator receptor activator of nuclear factor-kappa B ligand (RANKL) plays a major role in the development of rheumatoid arthritis, however its role in inflammatory bowel disease is unknown. Genome-wide association scan meta-analysis for Crohn's disease (CD) identified a variant, near the gene TNFSF11 that encodes RANKL and CD risk allele increased expression of RANKL in specific cell lines. Furthermore, enhanced RANKL can enhance osteoclastogenesis and osteoporosis is increased in CD. Therefore, this study aims to elucidate how the RANKL inhibitor (Denosumab) can be harnessed to diminish experimental colitis and ultimately serve as a safe therapeutic target in IBD.

Methods: An experimental model of CD was used. Colitis was induced by intracolonic injection of 2,4,6-dinitrobenzenesulfonic acid (DNBS, 4mg/kg) dissolved in ethanol (30%) on C57Bl/6 mice (7 weeks). One day before colitis induction, daily injection of Desnosumab (10mg/kg/day, i.p.) was initiated and continued over three days. Control mice received saline (0.9%). Disease severity index was evaluated daily after induction of colitis. At sacrifice composite macroscopic score including diarrhea, hyperemia, thickness and adhesion were evaluated. Colonic myeloperoxidase activity (MPO) and proinflammatory cytokines levels were determined.

Results: Denosumab treatment decreased the clinical disease as assessed by hyperemia and mucosal thickness and weight lose. On day 3 after colitis induction, 66% and 16% of Desnosumab-DSS-treated mice had weight loss and mucosal thickening respectively, compared to 100% and 66% of vehicle DSS-treated mice (p<0.05). Composite macroscopic score was 41% lower in Desnosumab-DSS-treated mice than in vehicle DSS-treated mice (p<0.05). All four markers including diarrhea, hyperemia, thickness and adhesion were decreased. Denosumab treatment decreased the colonic MPO activity (p<0.05). Colonic IL-1b levels decreased from 41.1±1.7 in vehicle-DSS-treated mice to 13.11±1.3 pg/mg of tissue in Denosumab-DSS-treated mice (p<0.05). Conversely, no effect was evident on colonic IL-6 levels. In control mice (without colitis) Denosumab did not affect any inflammatory marker.

Conclusions: These results support the hypothesis that preventative treatment with Denosumab modulates intestinal inflammation in a murine model of colitis. This provides a rationale for considering Denosumab as a therapy in CD.
MiR-200b and Pulmonary Development

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MicroRNAs are considered to be one of the most important epigenetic factors in development and diseases. Recently, we showed that the expression of one of these microRNAs, miR-200b is disrupted in abnormal lung development in human babies born with a hole in the diaphragm. In this study, we generated knockout mice for miR-200b to delineate the role of miR-200b in normal lung development. Additionally, we evaluated the effects of normalizing miR-200b expression ex vivo and in vivo in our rat model of abnormal lung development.

We generated knockout mice for miR-200b and evaluated the expression of miR-200b in whole embryos using the inserted lac-Z reporter. Lung branching and lung function analyses were performed on miR-200b +/-, +/- and -/- embryos and 8-week old mice, respectively. To evaluate the role of miR-200b in our rat model of lung hypoplasia, we treated the embryos with miR-200b indirectly via a tail vein injection of the pregnant rat. We compared the outcomes in the offspring of this dam with the offspring from the appropriate controls.

LacZ staining of embryos with miR-200b showed a unique expression in lung, palate and inner ear. Results from lung function studies demonstrated that miR-200b -/- mice have significantly higher lung tissue resistance and elasticity compared to miR-200b +/- or +/- littermates. Lung branching ex vivo culture showed significantly lower branching in miR-200b +/- than +/-+. In our rat model of hypoplastic lungs, morphometry and histology data demonstrate an impressive improvement in hypoplastic lungs following treatment with miR-200b mimics.

These data indicate that miR-200b plays an essential role during normal lung development and can potentially be used as a treatment for hypoplastic lung development.
Autoregulation of PilA--The Major Component of a Key Virulence Factor in *Pseudomonas aeruginosa*

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**Introduction:** *Pseudomonas aeruginosa* is an opportunistic pathogen that causes severe infections in immunocompromised hosts and Cystic Fibrosis patients. The expression of adhesive, retractile surface fibers called type IV pili (T4P) are essential to its pathogenicity. Transcription of the gene encoding the major pilin subunit, *PilA*, which comprises the majority of the pilus, is controlled by the *PilS*-*PilR* two-component regulatory system. *PilA* is imbedded in the inner membrane by its conserved N-terminus when not part of a pilus. *PilS* is a dual function sensor protein that can have both kinase and phosphatase activity against its response regulator, PilR. The 6-transmembrane (TM) topology of *PilS* suggests that unlike most sensor kinases, it recognizes an intramembrane signal—*PilA* itself. We show that direct interactions between *PilA* and *PilS* within the inner membrane at high levels of intracellular *PilA*, inhibit *pilA* transcription and identify critical residues required for the interaction and the regulation of *pilA* transcription.

**Methods:** A bacterial two-hybrid assay was used to test for protein-protein interactions between *PilS* and *PilA* or structurally related pilins. Amino acid substitutions in the conserved N-terminus of PilA and in the TM segments of *PilS* were generated using site directed mutagenesis and tested for their effect on interactions. Western blotting and a *lux-pilA* luminescent reporter assay were used to identify pilins that repress chromosomal *pilA* transcription upon overexpression.

**Results:** Overexpression of diverse *PilA* variants, each with near identical N-termini, decreased *pilA* transcription via direct interaction with the sensor during overexpression. Mutagenesis studies revealed several amino acid substitutions on both *PilA* and *PilS* that disrupt the A-S interaction and additional substitutions that impair autoregulation without disrupting the interaction. These data indicate that pilin-sensor interaction is necessary but not sufficient for *pilA* autoregulation. We also determined that intrinsic *PilS* phosphatase activity is required for the autoregulation of *pilA* transcription.

**Conclusions:** The T4P system is an attractive therapeutic target since it is important for virulence. The conserved N-terminus of *PilA* autoregulates *pilA* transcription via direct interaction with the sensor kinase *PilS*. Peptides corresponding to this short region may have potential to suppress T4P biogenesis and thus reduce *P. aeruginosa* virulence.
Introduction: Hepatitis C virus (HCV) infection is a global health challenge, with 3% of the world’s population chronically infected and at high risk to develop severe liver complications including cirrhosis and hepatocellular carcinoma (HCC). Apoptosis and pyroptosis are two forms of programmed cell death (PCD) producing different pathological outcomes. Studying induction of these forms of PCD by HCV will help in understanding the development of liver complications.

Methods: Huh-7.5 cells were infected with the JFH1T HCV. Cell viability and proliferation were measured by MTT and CFSE assays, respectively. DNA fragmentation was assessed by DNA laddering and by detecting hypodiploid cells in the propidium iodide (PI) stained population by flow cytometry. Apoptosis was assessed by detecting cleaved-PARP (cPARP)-positive cells and by the effect of inhibiting caspase-3. Bystander apoptosis was detected by staining the infected Huh-7.5 cell population with anti-HCV core, followed by PI to detect hypodiploid cells, and confirmed by co-culturing permissive and non-permissive cells that had been inoculated with virus, then analyzed for the presence of cPARP in the S29 cell population. Induction of pyroptosis was tested by staining the cells with caspase-1-specific FAM-YVAD-FMK-FLICA and by testing the effect of inhibiting caspase-1 on the number of hypodiploid cells.

Results: HCV Infection reduced the viability of Huh-7.5 cells and their proliferation rate. PI staining and DNA laddering assays showed that HCV induces DNA fragmentation, a characteristic of both apoptosis and pyroptosis. HCV infection increased the number of cPARP-positive cells, and inhibition of caspase-3 resulted in a significant decrease in hypodiploid cells, indicating that HCV infection induces apoptosis. Hypodiploid cells were detected in both HCV core-positive and -negative populations, and cPARP-positive cells were found in both Huh-7.5 and S29 cell populations. These two findings demonstrate the induction of bystander apoptosis. Virus infection increased the number of active-caspase-1-positive cells and inhibiting caspase-1 resulted in a significant reduction in the number of hypodiploid cells, providing evidence for the induction of pyroptosis.

Conclusion: HCV infection induced different forms of PCD including: apoptosis, bystander apoptosis and pyroptosis. If we understand how HCV can do this we will uncover mechanisms of liver disease and HCC development.
Genomic Non-B DNA Motifs Significantly Influence Integration Site Selection of Latent HIV-1 and Potently Inhibit Gene Expression: Implications for Cure-Focused Antiretrovirals
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Introduction: In human immunodeficiency virus type 1 (HIV-1) infection, HIV-1 permanently incorporates its genome into the host genome in a process called integration. This leads to life-long infection accompanied by a period of latency. A cure for HIV infection will require elimination of latently-infected cells since these cells can re-initiate systemic infection at a later time. We previously showed that HIV-1 preferentially integrates near non-B DNA motifs (nBDMs). nBDMs are secondary structures in our genome formed by specific nucleotide sequences which exhibit non-canonical DNA base pairing. We also identified the strongest hotspot to date for retroviral integration sites in mice, which contained a nBDM sequence. Given these findings, and that some nBDMs inhibit gene expression, we hypothesized that nBDMs play a key role in attracting HIV-1 integration and contribute to latency by inhibiting HIV-1 gene expression.

Methods: Integration sites were isolated by ligation-mediated PCR and sequenced using high-throughput Illumina MiSeq. We designed a bioinformatics pipeline utilizing BEDTools, BEDOPS, BLAT and Biopython to map integration sites to the genome and nBDMs. Hotspot sequences were cloned into episomal plasmids and integration in human cells was measured using quantitative PCR (qPCR). The impact of nBDMs on HIV-1 latency was assessed by cloning nBDMs near the promoter of an HIV-1 provirus and measuring HIV-1 gene expression by qPCR and Western blotting.

Results: Bioinformatics analysis of integration site datasets from evolutionarily diverse retroviruses revealed significant enrichment of integration sites in or near nBDMs. All retroviruses except HIV-1 showed a strong preference for integration directly within nBDMs, whereas HIV-1 preferred integration near nBDMs. Introduction of the murine hotspot sequence into human cells resulted in a 10-fold increase in integration, demonstrating that the factor dictating integration in/near nBDMs are not species-specific. Interestingly, incorporation of a nBDM near the HIV-1 promoter potently inhibited HIV-1 gene expression.

Conclusion: Our data demonstrate that nBDMs are novel factors that significantly influence retroviral integration site targeting. We also identified nBDMs as a potential new factor that contributes to HIV-1 latency by potently inhibiting proviral gene expression. Our data will help inform the design of future experiments in HIV-1 eradication research.
Emergence and Molecular Characterization of Nap4 *Clostridium difficile* Healthcare-Associated Infections in Canada

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**Introduction:** *Clostridium difficile* is an important nosocomial pathogen and the leading cause of antibiotic-associated diarrhea. Through the Canadian Nosocomial Infection Surveillance Program (CNISP) a steady increase in *C. difficile* infections (CDI) associated with the NAP4 strain has been observed, from 7.4% of all tested isolates in 2004 to 19.5% in 2011.

**Methods:** From March 01-April 30 of each surveillance year, CNISP sites submitted stool samples from healthcare-associated CDI (HA-CDI) cases for *C. difficile* isolation, PFGE analysis using the SmaI restriction enzyme, ribotyping by capillary gel electrophoresis, and antimicrobial susceptibility testing. Growth of the isolates was monitored over 24 hours by measuring the OD₆₀₀ every 2 hours and spore counts were conducted after 48 hours.

**Results:** From 2004-2011 there were 332 available HA-CDI cases associated with NAP4. From available data, the 30 day all-cause and attributable mortality in patients with NAP4 CDI was 8.5% and 1%, respectively. Patients requiring colectomy or admission to the ICU as a direct cause of NAP4 were both below 1%. The average age of a patient infected with NAP4 was 53 years old and 23.5% of patients were under 18 years of age. Antimicrobial susceptibility testing revealed that 37% of the NAP4 isolates were resistant to clindamycin. All NAP4 isolates were susceptible to metronidazole, vancomycin, and tigecycline. The 2 predominant ribotypes associated with NAP4 were 020 (42%) and 014 (26%). There were two major NAP4 PFGE clusters and the predominant PFGE types from each cluster were 0023 and 0033. Between 2004 and 2011 PFGE type 0033 increased from 0% to 42% amongst all tested isolates whereas PFGE type 0023 decreased from 56% to 12% over this same time period. In comparison to a selection of PFGE type 0023 isolates, PFGE type 0033 was found to have a significantly greater growth rate in the exponential phase and an observable increase in sporulation capacity.

**Conclusion:** This investigation provides insight into the increasing prevalence of NAP4 in Canada. We are currently performing whole genome sequencing to further characterize these strains and to better understand the evolution and dissemination of NAP4 across Canada.
Introduction: While there has been some progress, Indigenous peoples living in rural and remote areas and requiring renal care continue to experience systemic and economic barriers to care. A comprehensive literature search in access to renal care for Indigenous populations revealed a gap in the literature.

Methods: Scopus and Google Scholar were the academic databases used. Google was used for environmental searches. 143 citations were relevant to the study. In a qualitative relocation study, we interviewed 29 Indigenous patients and family members of patients and 17 providers and administrators about their experience with relocation for dialysis.

Results: Satellite dialysis has been heralded as a solution to rural and Indigenous disparities in the literature, showing seemingly positive outcomes for rural communities in Manitoba and elsewhere. It is the predominant model of rural dialysis in Canada. However, there are many infrastructural issues in satellite dialysis: poor water and power supply in Indigenous communities, non-Indigenous and itinerant staffing, and lack of connection, organizing, and engagement between the community and the satellite units. With poor primary care, the number of Indigenous people with End Stage Renal Disease (ESRD) will continue to grow. Combined with systemic poverty and racism, this lack of primary care leads to other poor health outcomes such as high rates of diabetes. These co-morbidities make it less likely that a patient can receive care at a satellite dialysis unit. Coupled with limited capacity and infrastructure in satellite dialysis units, it is likely that more and more patients will continue to relocate for dialysis.

Initial qualitative analysis of interviews revealed that the system is much more fluid than the literature suggests. Patients move between satellite units, relocate back and forth from their community to an urban centre, and may even forgo dialysis to die in their community. Relocation can be traumatizing, creating loneliness, heavy burdens on family members, and loss of community.

Conclusion: The literature has overlooked the experience of relocated patients, focusing on comparison of cross sectional outcomes of urban and rural dialysis units. We hope to shed light on these patients’ experience and on the complexity of rural indigenous ESRD.
Evidence for Dissociable Neurocognitive Systems Subserving Emotion Recognition and Emotional Intensity Encoding

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Introduction: It is often thought that neurocognitive systems devoted to emotional expression recognition are intertwined with other critical aspects of emotional encoding, such as emotional embodiment or simulation. In contrast, some theories have suggested that the valence of an emotional object is represented independent of the level of arousal. We tested the hypothesis that affective priming through emotional auditory cues would modulate the perceived intensity of an emotion, it would have no impact on attention to diagnostic facial features or emotion recognition time.

Methods: To help determine the extent to which the neurocognitive systems for emotion recognition and emotional intensity perception are dissociable, we examined the relationship between emotional expression recognition, attention to diagnostic facial features, and perceived emotional intensity. Participants performed an emotional facial expression recognition task while task-irrelevant emotional auditory vocalizations (known to modulate perceived intensity of facial expressions) were played.

Results: As expected, emotional sounds modified the emotional intensity rating of the faces. However, whereas the relative proportion of gaze on diagnostic features was significantly correlated with the time taken to identify the emotional expression, they were unrelated to emotional intensity.

Discussion: The results support the idea of dissociable neurocognitive systems for emotional intensity perception and expression recognition, and highlight some limitations of relying on emotional expression recognition as a more general metric of emotional encoding.
In Vitro Effects of G-CSF, GM-CSF, and M-CSF Treatments on Expansion and Suppressive Function of Murine Bone Marrow-Derived Myeloid-Derived Suppressor Cells

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Introduction: Myeloid-derived suppressor cells (MDSCs) are a heterogeneous population of immature myeloid cells that can suppress the adaptive and innate immune responses and upregulate other downregulatory elements of the immune system via inhibition of T cell proliferation and activation. During allogeneic peripheral blood hematopoietic stem cell transplantation (HSCT), donors are treated with G-CSF to mobilize and expand HSC. We have shown that G-CSF mobilization also expands MDSC populations in vivo and in vitro. These MDSCs, with known immunosuppressive activity, may help to suppress graft-versus-host disease (GVHD) in allogeneic transplant recipients. GVHD is a common complication following allogeneic stem cell transplantation in which graft CD8+ and CD4+ T cells recognize recipient tissue antigens as foreign and launch an immunological response against the recipient. Treatment with MDSCs could help to dampen this immune response in GVHD.

Methods: To test the ability of other myeloid GF to maintain, expand and improve function of bone marrow (BM)-derived MDSC we cultured murine BM with G-CSF, GM-CSF, and M-CSF in two-fold dilutions at concentrations of 10 to 80 ng/ml. Cells were counted and MDSC measured by flow cytometry at day 4. CFSE-labeled bone marrow was treated with these GF to analyze bone marrow cell and MDSC subset proliferation at day 4. Suppressive function of the in vitro growth factor-generated MDSCs was measured by inhibition of third party CFSE-labeled splenocyte proliferation assay with co-culturing of GF-treated murine BM.

Results: Four days of culture bone marrow cells with 10ng/ml GM-CSF, 20ng/ml G-CSF or M-CSF generated high numbers of live MDSC, of which GM-CSF stimulation generated the highest number of MDSC. MDSC induced by different GF can suppress the proliferation of T lymphocytes in dose-dependent manner, of which M-CSF induced MDSC showed greatest suppressive functions.

Conclusion: GM-CSF treatment had the greatest impact on MDSC cell number in culture, but M-CSF had a strong suppressive effect on the proliferation of murine splenocytes. Both were superior to the clinically used G-CSF. GM-CSF or M-CSF, used in production of HSC product or post-transplant, may be a new tool for the prevention and treatment of GVHD.
Although it is known that *Trypanosoma congolense* infection is associated with excessive production of pro-inflammatory cytokines in mice, the signaling pathways leading to the production of these cytokines remain unknown. In this study, we investigated the innate receptors and intracellular signaling pathways that are involved in *T. congolense*-induced pro-inflammatory cytokine production in macrophages. We show that the production of IL-6, IL-12 and TNF-α in macrophages *in vitro* and *in vivo* following interaction with *T. congolense* is dependent on phosphorylation of mitogen-activated protein kinase (MAPK) including ERK, p38, JNK and signal transducer and activation of transcription proteins (STAT). The production of proinflammatory cytokines and phosphorylation of MAPK and STAT was significantly inhibited by specific MAPK and STAT inhibitors, confirming the involvement of these signaling molecules in this process. We further show that *T. congolense* induced pro-inflammatory cytokine production is triggered by the ligation of Toll-like receptor 2 (TLR-2) and is dependent on signals mediated via MyD88. Collectively our findings provide a mechanistic insight into the activation of innate immune system leading to the production of proinflammatory cytokines by *T. congolense*. Understanding the innate immune receptors and signaling molecules involved in *T. congolense* infection may help to identify novel targets for immunomodulation aimed at regulating the disease.
Studies on the Roles of Viral Envelope Surface Proteins in Cell Attachment and Entry
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Introduction: Type 1 viral fusion proteins, which include the influenza haemagglutinin and the HIV GP120 protein, as well as the filovirus glycoprotein (GP) spike, are a group of trimeric surface glycoproteins that are responsible for binding to the host cell receptor and subsequent membrane fusion. Hypothetical mechanisms of membrane fusion have been proposed, but many details regarding entry and fusion remain unknown. Filoviruses, such as Ebola virus, are enveloped viruses, which have an external layer of GP spikes on their envelope. The GP spike contains the receptor binding domain which in its cleaved form binds to the cellular receptor Niemann-Pick C1 (NPC1).

Methods: This project will study the interaction of GP with NPC1, and the interaction of the virus and host cell membranes, using a virus-like particle (VLP)/liposome model system. Liposomes will be composed of lipids that resemble the human endosomal membrane with NPC1 attached to their surface, and VLPs will be composed of Ebola viral protein VP40, and the spike protein, GP. Once interaction has been established, intermediate fusion structures will be captured using cryo-electron microscopy.

Results: To date, Ebola VLPs have been constructed by co-transfecting 293T cells with expression vectors harboring the Ebola virus proteins of interest (VP40 and GP). Work is still being completed on optimizing the transfection protocol to result in high levels of expression to produce VLPs at a high enough concentration to be easily viewed in the transmission electron microscope. We are currently in the preliminary stages of liposome preparation.

Conclusion: Upon completion, this project will enable us to clearly understand the roles that envelope surface proteins play in cell attachment and entry during a viral infection. We will also be able to elucidate the possible triggers involved in how virus-to-cell fusion occurs. By determining how the GP spike protein is involved in the fusion process, new avenues for drugs and antibodies to combat this deadly virus may be revealed.
The Effect of Musical Training on Multisensory Reaction Times
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Introduction: Our perception of the world is based on the combination of multisensory cues provided by our senses. Recent studies have suggested that musical training can have an influence on these multisensory interactions. However, these investigations often use musically-related methodologies. Moreover, results are often analysed with musical training as a binary construct, which ignores the possibility of control group participants having limited but significant musical experience. In this present study, we look at audiotactile reaction times of non-musical stimuli in relation to self-reported musical training scores for all participants.

Methods: Individuals with long-term musical training were paired with control group participants and completed a musical sophistication questionnaire to assess their level of musical training. A non-musical audiotactile reaction time task was used. Participants were presented with 50ms white noise bursts, 50ms vibrotactile stimulations, or both simultaneously, and had to respond as quickly as possible. Each of the three stimulation type was randomly presented 180 times.

Results: Analysis of preliminary results suggest that musicians have statistically significant faster reaction times for tactile stimuli \((p=0.031)\), but not for auditory \((p=0.211)\) or audiotactile \((p=0.200)\) stimuli. Furthermore, reaction times for each modalities were correlated with self-reported musical training scores and found significant negative correlation between musical training scores and tactile reaction time \((r=-0.426, p=0.044)\) but not for the auditory \((r=-0.290, p=0.130)\) or audiotactile \((r=-0.242, p=0.175)\) reaction times.

Conclusion: Previous investigations have revealed enhanced audiotactile interactions in musicians. The present data suggest that these results could be explained by superior unimodal tactile ability in musicians, which might have an influence on multimodal perception. This is in line with neuroimaging studies that have revealed an increased cortical representation of the fingers for musicians. Moreover, statistically significant correlations between tactile reaction times and musical abilities suggest that exposure to any musical training can decrease tactile reaction times. These data, which suggest for the first time a correlation between exposure to musical training and enhanced multisensory ability, highlight the importance of considering musical training as a spectrum instead of a binary construct.
A How-To Guide for Planning Hospital-to-Home Care Transition Interventions: Findings and Implications of a Realist Synthesis

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Introduction: Individuals transitioning home after a hospitalization are at heightened risk for medication errors, functional decline, re-hospitalization and admission to institutional care. The goal of this study was to synthesize evidence on which care transition activities benefit this population and how activity outcomes are affected by co-existing activities and context.

Methods: The results of a scoping review of care transition intervention studies were summarized using the realist synthesis technique. Through both qualitative and quantitative analyses, we examined how and why more than 40 different care transition activities (e.g. medication reconciliation, tele-health monitoring) achieved their outcomes. We looked at variation in activity mechanisms across different target populations in the presence of coexisting intervention activities and across contexts. Contextual variables included location of the intervention and organizational characteristics. The results of this synthesis are summarized in a cohesive program theory for care transition interventions, with examples of key activity-mechanism-outcome relationships provided.

Results: Most of the reviewed studies employed similar transition activities but differed significantly in how they identified their target populations, the timing of activities (pre- or post-discharge), and the type of health care provider responsible for care delivery. These factors had significant effects on whether activities achieved desired outcomes. Similarly, the context created by shared electronic medical records, program champions at the organizational level and financial incentives for success modified the effect of intervention activities across studies.

Conclusions: This study leverages the differences in care transition intervention characteristics across studies to produce actionable outputs. The specific activity-mechanism-outcome relationships (and their modification with contextual factors) identified are relevant to decision-makers and managers seeking to improve care transition interventions in their unique healthcare context.
Mechanism of Damaged Peroxisome Degradation – Developing a Novel Therapeutic Treatment for Peroxisome Biogenesis Disorders
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Introduction: Peroxisome Biogenesis Disorders (PBDs) are a group of autosomal recessive disorders that have a prevalence of 1:50,000. Currently there is no cure, and PBDs are often fatal within the first year of life. PBDs are thought to be the result of a biogenesis defect in the formation of functional peroxisomes. This results in effects such as a decrease in peroxisome number, and the accumulation of very long chain fatty acids (VLCFA), since these are oxidized exclusively in peroxisomes. 90% of PBDs are due to mutations encoding for the peroxisomal AAA ATPase complex (PEX1, PEX6 and PEX26), which is responsible for removing ubiquitinated proteins from the peroxisomal membrane. Recently we have shown that ubiquitinated peroxisomes are removed from the cell by pexophagy, the specific degradation of peroxisomes by the autophagy pathway. Thus, we hypothesized that the decline in peroxisomes found in PBD cases caused by AAA ATPase mutations may not be a defect in peroxisome biogenesis, but instead due to an increase in pexophagy.

Methods and Results: Using fluorescence microscopy and immunoblotting analyses, we have found that depleting cells of PEX1 or PEX26 using RNAi in HeLa cells results in: 1) a decline in peroxisome number, 2) a disruption of matrix protein import (reflective of peroxisome function), and 3) an accumulation of ubiquitinated proteins. Furthermore, we found that the loss of peroxisome number is due to an increase in pexophagy, as depleting the cells of NBR1 (required for pexophagy) results in a restoration of peroxisome number. We further found that treating PEX1-mutated PBD patient cells with FDA-approved autophagy inhibitors restored peroxisome number (based on fluorescence microscopy and immunoblotting) and function (based on LC-MS/MS analyzing levels of VLCFA) without negatively effecting cellular viability long-term.

Conclusion: There is currently no treatment for PBDs, and since the intermediate and milder phenotypes cause a progressive loss of clinical function over time, these patients would benefit from therapeutic interventions that restore peroxisomal function. Our findings suggest that modulating autophagy activity may be a viable treatment for PBDs to increase both peroxisome number and function, and prevent or ameliorate the development of PBD phenotypes.
Inflammatory Oxylipin and Monocyte Responsiveness in Fatty Liver Disease

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Introduction: Non-alcoholic fatty liver disease (NAFLD) is the most common liver disease and affects 20-30% of people worldwide. The annual incidence of this disease is 10% and still rising. NAFLD ranges from simple steatosis to non-alcoholic steatohepatitis (NASH) which can progress to cirrhosis and liver cancer. However, the mechanisms of simple steatosis progression towards NASH are poorly understood. The pathogenesis of NAFLD is known strongly associated with type II diabetes (T2D). In preliminary studies, liver enzymes and oxylipins, serum fatty acid metabolites, were evaluated in T2D and clinically obese cohorts. T2D patients compared to obese controls showed elevated pro-inflammatory oxylipin 20-hydroxyeicosatrienoic (HETE) levels and decreased anti-inflammatory oxylipin 11-hydroxyeicosapentaenoic acid (HEPE) levels. Liver enzyme ALT levels indicate an association with 20-HETE (n=8, r=0.659, p=0.07) and with 11-HEPE (n=8, r=-0.749, p=0.03) levels, suggesting an interaction between these oxylipins and liver function. In addition, Pilot data from TLR4 stimulated PBMC suggested a link between TLR responsiveness and inflammatory oxylipin synthesis. We hypothesize that oxylipin levels correlate with liver damage in NASH. Furthermore, that NASH associates with altered monocyte responsiveness, oxylipin and pro-inflammatory cytokine expression.

Methods: PBMC were isolated from (n=6) simple steatosis patients and (n=6) NASH patients. PBMC were stimulated with lipopolysaccharide (LPS) and Pam3csk4 for 24 hours. Supernatant IL-1b, IL-10 levels were examined by ELISA. Toll-like receptors TLR2, TLR4 levels and chemokine MCP-1 receptors CCR1, CCR2 expression was evaluated in monocytes by flow cytometry. Associations with clinical data (biopsy scores, serum liver enzymes etc.) will be evaluated between simple steatosis patients and NASH patients. Further, plasma oxylipin, cytokine, adiponectin, leptin and M30 keratin 18 levels (a marker of hepatocyte death) will also be compared between simple steatosis patients and NASH patients. We will also evaluate oxylipin and cytokine expression levels in TLR stimulated cells. Results will be compared to clinical parameters.

Discussion: Understanding the relationship between serum oxylipin levels, monocyte responsiveness and liver damage may reveal immune processes in the progression from NAFLD to NASH. Serum oxylipins could potentially influence monocytes remodeling towards either pro-inflammatory or anti-inflammatory activity. Ultimately understanding how oxylipin levels influence monocyte behavior may help control fatty liver disease or lead to a non-invasive way to diagnose NASH.
The Effect of Layer-By-Layer Assembly Coating on the Proliferation and Differentiation of Neural Stem Cells

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Introduction: Single-cell encapsulation with layer-by-layer (LbL) self-assembly is to compartmentalize cells at single-cell level. This shell-like structure formed by biocompatible materials (gelatin and alginate) endows protective effect for cells encapsulated inside. In addition, since the materials contain functional groups such as amine groups and carboxyl groups, it is convenient for biological regulators to be conjugated with the materials. The loaded regulators are supposed to be gradually released and thus exert their effects. This technique provides a novel strategy to support the transplanted neural stem cells (NSCs) to survive the harsh conditions in the lesion area by addressing not only mechanical isolation but also nutrients delivery.

Methods: NSCs were coated with gelatin and alginate solutions alternatively to form the LbL encapsulation. Transmission electron microscope (TEM) and fluorescent reagents were applied to show the LbL encapsulation. Immunofluorescence and western blot were used to compare the cellular properties (viability, proliferation and differentiation) of NSCs in both control and encapsulation groups. Insulin-like growth factor-1 (IGF-1) was further loaded on alginate and its improvement effects for NSCs were detected.

Results: NSCs were successfully encapsulated according to TEM and fluorescent images. The natural properties (viability, proliferation and differentiation) of LbL-NSCs were not significantly affected. IGF-1 which was conjugated on the materials could be released in the medium, and in addition, had a more sustained profile under pH 6.5. Furthermore, the loaded IGF-1 greatly enhanced the proliferation of encapsulated NSCs and the viability of NSCs in acidic environment.

Conclusion: We have for the first time developed the single-cell encapsulation model on NSCs by LbL self-assembly. This encapsulation model not only retained the properties of NSCs but also regulated the cellular function by delivering nutrients in hazardous conditions. This technique will also be applied in a wide range of basic biological studies and clinical translational studies.
**Introduction:** Medulloblastoma (MB) is the most common malignant primary brain tumor in children. Despite improved clinical outcomes, children with MB often suffer from consequences of treatment such as surgery, chemotherapy and radiation. MB is currently classified into 4 distinct molecular subtypes based on genomic alterations, gene expression profile, response to treatment and cell of origin; Wnt, Sonic Hedgehog (SHH), Group 3, and Group 4. This extensive heterogeneity has revealed a critical need for subtype-specific, functionally validated biomarkers and therapeutic strategies.

**Methods:** Here, we employed an unbiased high throughput flow cytometry screen to identify differentially expressed cell surface markers in high vs. low self-renewing SHH MB phenotypes. The top 25 differentially expressed markers were refined by evaluating levels in SHH relative to the other molecular variants in 3 transcriptome datasets, representing 548 patient samples. To evaluate the effects of specific biomarkers on cellular properties, we employed gain and loss of function studies, cellular assays and in vivo experiments to measure sphere forming and self-renewal capacities.

**Results:** From a screen of 242 cell surface markers, 25 showed differential expression between higher vs. lower self-renewing MB cells. 4 of these cell surface markers were determined to be differentially expressed between the Shh variant and the other 3 MB subtypes: CD271 (p75NTR, NGFR), CD106/CAM1, CD171/NCAM-L1, EGFR. Stable overexpression of CD271 results in generation of larger tumorspheres, but lost of self-renewal capacity compared to controls. Using CD271 shRNA knockdown and a CD271 inhibitor (ɣ-secretase inhibitor), we show a reversal of the cellular phenotype seen in CD271 overexpression cells.

**Conclusion:** The restricted self-renewal capacity, combined with changes in tumorsphere size, suggests that CD271 may amplify progenitor cells. This work highlights an integrated approach for identification and subsequent functional validation of novel cell surface markers representing diverse MB phenotypes. Due to the cellular heterogeneity between MB subgroups, identification of TPC populations for specific MB variants will be crucial for the design of next generation targeted therapies.
MiR-221/222 Target Notch3 and Promote the Epithelial-to-Mesenchymal Transition in Breast Cancer
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Background: Breast cancer is the most common malignancy as the cause of cancer-related deaths in women. Death from breast cancer primarily results from cancer cells invading surrounding tissues and metastasizing to distal organs followed by formation of secondary tumors. The process of epithelial–mesenchymal transition (EMT) has been shown to be the pivotal mechanism contributing to cancer metastasis. Notch family, ERα signaling and miR221/222 have been proven to play an important role in the process of EMT. We had proven that Notch3 inhibit EMT via directly bind to ERα promoter, up-regulating ERα as well as E-cadherin expression. MiR-221 and miR-222 are frequently overexpressed in Notch3 and ERα negative breast cancer cells and are associated with increased malignancy. However, little is understood regarding their relationship and the mechanisms of miR-221/222 and Notch3.

Method and result: Using RT-PCR and Western blot, we demonstrated a negative correlation between Notch3 and miR-221/222 in breast cancer cell lines. To identify miR-221 and miR-222 targets, we performed a bioinformatics search (Pictar) for putative mRNA targets of both miRNAs. Among the candidate targets, 3’-UTR of human Notch3 contained regions that matched the seed sequences of hsa-miR-221 and miR-222. Transfected miR-221/222 mimic in MCF7 cells resulted in down-regulation of Notch3 protein level as well as ERα and the epithelial marker. Known-down miR-221/222 by miR-221/222 inhibitor in MDA-MB-231 cell resulted in restoration of Notch3 protein level and epithelial marker. In Luciferase reporter assay, we verified that miR-221 and miR-222 directly target notch3-3’UTR and decrease the protein expression of it. In transwell assay, we found that miR-221 and miR-222 enhance cell migration and invasion by blocking Notch3-mediated EMT in breast cancer cells.

Conclusion: Our group is the first one who identified the interaction of miR221/222/Notch3/ERα link to EMT in breast cancer: MiR-221/222 directly targeting Notch3 3’-UTR, resulted in down-regulating Notch3, ERα and E-cadherin protein levels, and finally induced the epithelial to mesenchymal transition (EMT) in breast cancer.
Interactions between Quorum Sensing and Phenylacetic Acid Metabolism in Cystic Fibrosis Pathogens

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Introduction: The majority of cystic fibrosis (CF) deaths are due to lung failure caused by bacterial infections. The CF lung is a nutrient rich environment for growing microbes facilitating polymicrobial disease. For one important CF pathogen, *Burkholderia cenocepacia*, the genes of the phenylacetic acid (PAA) catabolic pathway are induced in CF-like conditions and the presence of PAA itself can also induce this pathway. Previous work in our lab provided evidence of a direct link between QS-regulated pathogenic responses and PAA metabolism in *B. cenocepacia*. Interestingly, PAA inhibits certain quorum sensing (QS)-regulated virulence traits in another CF pathogen, *Pseudomonas aeruginosa*. These findings indicate that PAA may be acting as a signal in the polymicrobial interactions of CF pathogens. In order to understand the role PAA plays in the virulence of CF pathogens we must investigate which pathogens are producing PAA in the CF lung.

Methods: Using the KEGG database CF pathogens were analyzed for their potential for PAA production. Cultures of *P. aeruginosa* PAO1 were grown in NGM to high density (OD$_{600} \geq 1.5$) and the supernatants were extracted using ethyl acetate. The extractions were filtered, dried and suspended in methanol at a concentration of 2 mg/ml for analysis by 1H-NMR and HPLC. *Aspergillus fumigatus* UAMH 10899, a clinical CF isolate, was grown for seven days in PDB and the culture supernatants were extracted and analyzed.

Results: *A. fumigatus* was identified as a potential PAA producer based on the KEGG database. Although *P. aeruginosa* was not, PAA production was demonstrated in a recent publication. Testing showed that *P. aeruginosa* PAO1 does not release PAA in NGM even when supplemented with its precursor phenylalanine. *A. fumigatus* does not produce PAA in PDB.

Conclusion: While *P. aeruginosa* and *A. fumigatus* do not produce PAA in the media tested, further work is required to test for production in carbon-limited defined mineral media, designed to increase the production of PAA, and synthetic CF media. By investigating CF pathogens for their ability to produce, degrade and sense PAA, this work will allow the examination of the role of PAA in the QS-regulated virulence of CF pathogens.
RNA-Sequencing in Ethanol-Exposed Differentiating Brain-derived Neural Stem Cells: A Genome-Wide Study to Identify Biomarkers for Fetal Alcohol Spectrum Disorders (FASD)

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Introduction: Alcohol (ethanol) exposure during pregnancy leads to a range of childhood neurodevelopmental disorders referred to as Fetal Alcohol Spectrum Disorders (FASD). In Canada, approximately 1% of children are born with FASD, while its prevalence is as high as ~11% in Manitoba. The annual cost associated with FASD in Canada exceeds over $5 billion. This high prevalence and economic impact of FASD emphasizes the urgent requirement of understanding molecular mechanisms of ethanol toxicity as well as identifying biomarkers for early detection of these devastating disorders.

Objectives: The current study is aimed to investigate the adverse effects of ethanol on genome-wide gene expression patterns during differentiation of neural stem cells into neurons, astrocytes and oligodendrocytes, the three major cell types of the brain. This gene expression data will then be used will be validated at multiple levels and are aimed for identifying FASD biomarkers.

Methods: In this study, we used a previously established neural stem cell system to model three ethanol conditions, namely; binge ethanol exposure, chronic ethanol exposure and ethanol withdrawal. Total RNA extracted from control and ethanol-treated cells were subjected to genome-wide transcriptome analysis by RNA-sequencing (Genome Quebec). The obtained results were further analyzed by Ingenuity Pathway Analysis (IPA) to identify mechanisms of ethanol action and biomarkers.

Results: Our genome-wide transcriptome analysis has shown altered gene expression specific for different modes of ethanol exposure. The IPA analysis has identified several biosynthesis pathways as targets of ethanol during neural stem cell differentiation.

Conclusion and future directions: Our findings on the effect of ethanol on genome-wide gene expression will significantly contribute to the understanding of molecular mechanisms of ethanol toxicity in embryonic brain cells. The target genes with the potential of being used as biomarkers found in differentiating neural stem cells will be validated in a mouse model of FASD and post-mortem human brain samples through active collaboration within the CIHR team. New target genes of ethanol found in our study will lead to establishment of novel therapeutic biomarkers for FASD and help in early diagnosis of this devastating childhood disorder.
Achieving Nurse Leadership Assimilation of Business Intelligence

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Introduction: Despite increased adoption of health information systems (HIS) in clinical practice, the assimilation of Business Intelligence (BI) for day-to-day health system management remains limited. BI can be defined as, “the use of information and specialized analytical tools to enable informed decision making in a variety of organizational contexts” (Foshay & Kuziemsky, 2013, p.20). BI assimilation, frequently identified as an expected benefit of HIS implementation, is achieved when BI is used to fully support organizational strategic and tactical decision making. Increasing BI Assimilation in the health care sector presents an opportunity to more fully realize the benefits of significant investments in HIS being made by health organizations. More importantly, it is integral to achieving overall improvements in the efficiency and effectiveness of health service delivery. Despite these benefits, preliminary reviews of the literature have identified few academic studies exploring BI adoption and assimilation in the health sector; and even fewer that examine use of BI by nursing managers. Nursing leaders are a particular focus for this research given their unique position with oversight for a large portion of human resource budgets within health organizations and the direct impact nursing staff have on the patient experience. With increasing health system costs and complexity BI Assimilation, with its potential to assist health system leaders in day-to-day decision making, is an important area of focus.

Methods: Given the emerging state of work in this area and in preparation for a dissertation on this topic, a systematic scoping literature review will be undertaken to identify existing studies in this area and refine a conceptual framework for measuring assimilation of BI into the organizational practices of nursing leaders and managers.

Conclusion: The author will present the results of this literature review along with relevant research questions and related factors such as data quality, data governance, and patient privacy.
Introduction: Physicians must possess a clear understanding of human anatomy in order to effectively practice medicine. Traditionally, medical schools have utilized a dissection-based approach for educating medical students about human anatomy. In recent years, there has been a growing trend towards the use of a prosection-based approach to teaching, as well as the use of 2-D and 3-D imaging and learning resources. While the literature indicates that each of these methods of delivery for human anatomy education has associated strengths and weaknesses, there is poor agreement among medical educators about the most efficient or effective way to educate medical students. The purpose of this investigation was to examine how the method of educational delivery would influence student’s attitudes and perceptions about learning musculoskeletal anatomy.

Methods: During the 2014-15 academic year, University of Manitoba medical students in the class of 2018 were taught musculoskeletal anatomy using a prosection based teaching model, while students in the class of 2017 were taught musculoskeletal anatomy using a traditional dissection approach. All other aspects of the musculoskeletal anatomy curriculum were the same, including total contact hours. All students were asked to complete a standardized human anatomy education survey in order to evaluate the impact of 6 different methods of teaching on student’s ability to accomplish 8 specific learning objectives.

Results: A total of 91 medical students (class of 2017 = 37, class of 2018 =54) completed the survey. Data suggested that students believed that learning musculoskeletal anatomy was best facilitated through the use of cadaveric specimens, medical imaging and case based learning. More specifically, students ranked cadaveric specimens as the method of delivery that best accomplished 5 of the 8 learning objectives. When comparing cadaveric dissection against and prosection-based learning, students ranked cadaveric dissection as higher on 4 of the 8 learning objectives.

Conclusion: This investigation provides valuable information about which methods of delivery medical students believe are most effective for learning musculoskeletal anatomy. The results will be used to guide future curriculum development and the implementation of different educational delivery methods within the musculoskeletal anatomy course.
Ultrasonic Evaluation of Antiangiogenic Therapy on Patient-Derived Renal Cell Carcinoma Xenograft Tumors in the Chicken Embryo Model

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Introduction: Assessing patient-specific drug resistance is a promising application of the avian embryo patient-derived xenograft model. However, conventional methods of monitoring, such as tumor-take rates and light microscopy, do not provide sufficient detail for in-depth therapy evaluation. Ultrasonic monitoring permits non-destructive longitudinal evaluation of tumor growth, progression, and perfusion in the chorioallantoic membrane (CAM) xenograft ex ovo model.

Methods: A subset of tumor cells (RCC243) was isolated from a patient-derived parental renal carcinoma cell line (RCC22). On embryonic development day nine (EDD-9), cells were mixed 1:1 with Matrigel and 10 µL was deposited into a pierced opening of the CAM surface (8 animals). Half of the embryos were treated topically every two days with 10 µL (10 mM) of TAK-441, a Hedgehog pathway inhibitor. Three-dimensional B-mode and contrast-enhanced images were acquired using a Vevo 2100 ultrasound system (VisualSonics Inc.) equipped with a 20 MHz linear array. On EDD-18, the CAM vasculature was cannulated and 50 µL of microbubble contrast medium (2 x 10⁹ microbubbles/mL) was injected intravenously. Perfusion imaging was performed using a destruction-reperfusion protocol after the contrast agent had reached a steady-state concentration. Digital radio-frequency contrast images were exported, tumor volumes manually segmented, and the time-kinetics of the contrast agent wash-in was assessed using MATLAB (The MathWorks Inc., Natick, MA) to determine blood perfusion metrics (blood volume, velocity, and flow).

Results: Hedgehog pathway inhibition of RCC243 tumors via TAK-441 therapy produced a significant decrease in mean tumor volume (vehicle: 187.68 ± 69.55 mm³ vs. treatment: 78.94 ± 52.35 mm³; p = 0.047) and blood flow (vehicle: 645.7 ± 261.5 mm³/min vs. treatment: 190.8 ± 133.4 mm³/min; p = 0.049). There was a non-significant trend of reduced blood volume and flow velocity in the treated tumors.

Conclusion: This proof of principal study shows that tumors implanted in a chick CAM model can be imaged using high-frequency ultrasound and quantitative measures of tumor volume and perfusion can be obtained. This procedure could be useful for patient specific drug sensitivity evaluation, enabling selection of individualized treatments.
A Novel Chimeric Anti-HMGB1 Antibody Therapy Attenuates Paracetamol-Induced Liver Injury

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Introduction: High mobility group box 1 (HMGB1) is a prototype for a group of endogenous inflammatory mediators known as damage-associated molecular patterns (DAMPs). HMGB1 has been shown to be a key regulator of many inflammatory diseases of both sterile and infectious origin and of chronic and acute nature. Therapeutic monoclonal antibodies have a greater clinical value than polyclonal antibodies. Monoclonal antibodies can be further developed by substituting mouse constant domains to humans ones and thus rendering them more suitable for future clinical exploration. The aim of this study was to investigate the effect of a known therapeutic murine monoclonal anti-HMGB1 antibody (m2G7) alongside with a novel chimeric anti-HMGB1 monoclonal antibody (h2G7) and to determine its therapeutic mechanism of action in a model of acetaminophen (APAP) intoxication.

Methods: h2G7 was generated by subcloning the variable domains of m2G7 into a plasmid encoding human IgG1 constant domains. Affinity studies were performed by Biacore. Mice (C57BL/6) were challenged with 530mg/kg APAP by intraperitoneal (IP) injection, followed by a single antibody injection (IP, 300 μg/mouse) at 2hr post-APAP, and euthanized at 10hr post-APAP. Serum levels of HMGB1, ALT and miR-122 were measured to determine severity of APAP intoxication. Serum MCP-1, CXCL1, TNFα and histological staining were used to define the inflammatory contribution. Anti-HMGB1 antibody with the inability to bind and activate the complement system was generated by site-directed mutagenesis (K322A mutant). Antibody with reduced binding and ability to activate Fc receptors was generated by endoglycosidase (endoS) treatment.

Results: Chimerization of 2G7 did not affect the specificity but generated a small increase in antigen affinity (Kd: 170nM vs 130nM). Antibody treatments did not affect liver glutathione (GSH) content as compared with APAP+Controls, indicating no differences in abilities of neutralizing APAP toxicity. Anti-HMGB1 treatment significantly attenuated serum ALT, miR-122 and chemo/cytokine increases seen with APAP+Controls. Liver histology revealed reduced inflammation in anti-HMGB1 treated animals. A K322A
mutation abrogated the binding to C1q. Aglycosylation of h2G7 reduced binding to the lectin *Lens culinaris* agglutinin (LCA) and recombinant FCγRI. K322A mutant antibody and endoS treated h2G7 displayed similar hepatoprotective and anti-inflammatory effects as h2G7.

**Conclusion:** This study is the first to generate a novel chimeric anti-HMGB1 antibody and to validate the beneficial effects of HMGB1 blockade in a model of APAP intoxication. We could also define that the therapeutic mechanism of action is likely due to antigen neutralization in this model. This study provides evidence that h2G7 is suitable for further clinical development.
Calpain-1 and -2 are ubiquitously expressed intracellular Ca$^{2+}$-dependent proteases that are implicated in a wide range of functions, from cell migration and survival signaling to mitogenesis. They share a common 28-kDa regulatory subunit encoded by capns1, which is necessary for their activities; so genetic disruption of capns1 compromises expression of both calpain-1 and -2. Knockout, or knockdown model studies support the role of calpain-1 and -2 in PI3K/AKT signaling, survival in response to challenge with TNF-α and staurosporin, changes to downstream cyclin-dependent kinase inhibitor p27$^\text{kip1}$, as well as acquired trastuzumab (Herceptin) resistance in Her2$^+$ breast cancer cells. The regulation and subcellular localization of calpain involves activation by Ca$^{2+}$, which enables cleavage of its many substrates. Cleavage is limited and often occurs in peptides that link distinct protein domains; thus calpain cleavage modulates, rather than abolishes, substrate functions.

Here we show that RNAi-mediated knockdown of capns1 in the MDA-MB-231 human basal breast cancer cell line disrupts calpain-1 and-2 activities, and this is associated with attenuated tumor growth and metastasis in an orthotopic mouse xenograft model. A conditional capns1 knockout mouse mammary epithelial tumor cell line was also developed from a tumor isolated from a capns1 floxed MMTV-Neu compound transgenic mouse. Orthotopic engraftment of these mammary tumor epithelial tumor cells into Rag2$^{-/-}$; IL2R$^\gamma$-/- mice revealed that Cre-mediated disruption of capns1 (and loss of calpain-1 and -2 activities) was associated with loss of tumorigenesis. These observations argue that calpain-1 and -2 promote tumor growth and metastasis in vivo. Ongoing studies are aimed at identifying calpain substrates associated with these alterations in tumorigenic potential, and signaling pathways that might be targeted to synergize with calpain disruption in future therapeutic applications.
The Impact of a Severe Psychiatric Illness on a Cancer Diagnosis, Treatment and Survival
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Background: Why do people with a severe psychiatric illness (SPI) have drastically lower life expectancies than the general population? Worse mortality has been attributed to an increased burden of cardiovascular disease, diabetes, respiratory and other diseases. The relationship between an SPI and worse outcomes from cancer is less established. Individuals with an SPI are potentially at an increased risk for an unknown or incurable stage cancer, non-receipt of cancer treatment and worse overall survival through a number of patient/illness factors (e.g. socioeconomic status), provider factors (e.g. diagnostic overshadowing, suboptimal treatment) and healthcare system factors (fragmented healthcare). Better information is needed about the presence and size of associations between SPI and survival and the underlying mechanisms that lead to worse outcomes.

Methods: This thesis will investigate the relationship between SPI and selected cancer outcomes in a population-based, retrospective cohort study designed to use administrative healthcare data. The study population will include individuals diagnosed with colorectal cancer in Ontario between 01/01/2007 and 12/31/2013. Hospitalization data, physician billing data and emergency room visit data, cancer registry data and other national and provincial healthcare datasets housed at the Institute for Clinical Evaluative Sciences will be combined. In phase I, the association between SPI status and cancer stage at diagnosis using and the association between SPI status and receipt of cancer treatment will be investigated separately using multiple log-binomial regression. In phase II, the association between SPI status and overall survival from cancer will be evaluated using Cox-proportional hazards and Aalen additive hazards regression. In phase III, the mediating effects of stage and treatment on the relationship between SPI status and overall cancer survival will be investigated using causal mediation analysis methods for time to event data.

Results: Pending

Conclusions: The findings of this thesis could help researchers, clinicians and policy-makers better understand the association between SPI status and stage of cancer at diagnosis, receipt of cancer treatment and overall survival. The results could reduce health disparities for this vulnerable population by helping target further research and develop interventions to reduce excess mortality from cancer in individuals with an SPI.
The Functional Role of Prenyltransferases in COPD vs Non-COPD Patients Exposed to Cigarette Smoke

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**Introduction:** Chronic obstructive pulmonary disease (COPD) is a type of lung disease characterized by poor airflow. The main symptoms of COPD include shortness of breath, persistent coughing and sputum production. The mechanisms behind COPD are partly attributed to airway remodeling and alveolar destruction principally caused by cigarette smoke exposure. Our previously work using primary cultured lung fibroblasts show that simvastatin inhibits the expression and release of airway remodeling proteins which cause excessive extracellular matrix (ECM) thickening. The molecular mechanisms underlying these effects are linked to a special class of three enzymes collectively called prenyltransferases (PTs), which catalyze covalent conjugation of isoprenoids to specific proteins to facilitate their anchoring to cellular membranes. It has been shown that selective inhibition of one PT member, can mimic these anti-fibrotic effects of simvastatin in primary cultured human airway smooth muscle cells from non-COPD patients. However, the functional role of PTs in COPD patients has never been investigated. Our objective is to assess the role of PTs in COPD and whether circumventing PTs by statins alters key biomarkers of COPD pathogenesis.

**Methods:** Using our collection of primary human lung cell cultures derived from lung biopsies from ex-smokers, lung fibroblasts from COPD and non-COPD patients were profiled. To mimic cigarette smoke exposure in an *in vitro* setting, COPD and non-COPD cultures were pretreated with simvastatin or vehicle-control and exposed to fresh CSE and thereafter profiled for changes in PT transcript, protein, and enzymatic activity. Known COPD biomarkers will survey the downstream effects of both simvastatin and/or CSE treatments. These studies will be complemented by assessing subcellular protein localization using high-resolution spectral confocal microscopy.

**Results:** Exposing our cell cultures to CSE showed a visual decrease in cell density, however, our gene expression analysis of the prenyltransferases showed no large differences between COPD and non-COPD cell lines. Western blot and enzymatic activity still need to be assessed to understand effects of CSE and simvastatin on protein expression and PT activity.
**Conclusion:** These experiments will establish whether PTs are differently activated in ex-smokers with or without COPD in response to CSE, and does inhibiting their effects provide protection against the airway remodeling in COPD. Prenyltransferase inhibitors are already in various stages of clinical trials for their effects in chronic illness to reduce cell proliferation – PT inhibition could very well provide the targeted approach needed to mitigate the effects of COPD.
MicroRNAs Induced Early In Prion Disease May Have Neuroprotective Function
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**Background:** The molecular process governing synaptic dysfunction and neuronal loss during prion-induced neurodegeneration remains largely unknown. Investigating molecular changes that occur early in disease, when the infectious prion protein is beginning to accumulate, may hold promise in identifying these disease-related pathways. To this end, we performed high-throughput transcriptomic and miRNomic temporal screens on a neuronal-rich brain region (CA1 hippocampus). From this screen, we identified the induction of a neuroprotective response that occurred during pre-clinical disease which diminished as disease progressed. Similarly, 7 miRNAs followed the same pattern of expression as the neuroprotective gene signature. In fact, 3 of these miRNAs have known neuroprotective functions. We hypothesized that the remaining 4 deregulated miRNAs also contribute to this neuroprotective process initiated and data for one of these miRNAs, miR-26a-5p, will be presented.

**Methods:** Real-time PCR and *in situ* hybridization for the 4 candidate miRNAs (miR-16, miR-26a, miR-140 and miR-146a) was used to further validate the expression levels of these miRNAs in prion infected CA1 hippocampal regions. To identify potential effects of these miRNAs on neuronal morphology, gain-of-function studies for miR-26a-5p was performed in primary mouse hippocampal cultures. MiRNA target prediction programs in association with mRNA data were employed to discern neuronal-specific genes that were regulated by these miRNAs. Luciferase reporter assays are currently underway to validate the top candidate targets.

**Results:** We confirmed the upregulation of miRNA-26a in early prion-induced neurodegeneration using real-time PCR and *in situ* hybridization techniques. Primary mouse hippocampal cultures were established in the laboratory and validated to produce long-lived, viable neurons. Gain-of-function studies for miR-26a-5p revealed that this miRNA enhancing dendrite arborisation and spine density. Based on bioinformatic predictions, targets of this candidate miRNAs were strongly associated with neuronal function. Currently, the top candidate genes are being screened by luciferase reporter assays.

**Conclusions:** We have shown that numerous miRNAs may help mediate a neuroprotective program long before clinical symptoms were apparent in an animal model of prion disease. We believe that miR-26a also exhibit neuroprotective properties and the contribution of this microRNA to this protection remain the focus of further study.
Retinoic Acid Improves Vitamin A Homeostasis, Reduces Adipocytes Hypertrophy and Induces Beige-Brown Fat in Obese-Diabetic Mice

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Introduction: We have previously demonstrated a retinoic acid (RA) treatment in diabetic model rodents, reduces serum ROL, RBP4, glucose levels, insulin resistance, body weight and visceral fat, despite isocaloric food intake.

Aims: To determine whether RA affects: 1) Endogenous vitamin A levels and expression of genes from vitamin A metabolism - uptake (STRA6), transport (RBP4,CRBP,CRABP), storage (LRAT), oxidation (RDH,RALDH), catabolism (CYP26A1 and CYP26B1) and nuclear receptors (RAR,RXR) in subcutaneous fat (SF), visceral fat (VF), skeletal muscles (SM) and liver (LIV). 2) Expression in SF, of genes from mitochondria-genesis (PGC1α) and energy metabolism (UCP1) specific for beige-brown fat. 3) Morphology of adipocytes in SF.

Methods: Female 9-week-old B6.V-Lep/J ob/ob mice (n=16) obese and insulin resistant, divided in two groups, a group (n=8) treated with 100 μg of atRA dissolved in 100 μl corn oil (vehicle) daily (~ 2μg/gbw/day) by stomach intubation for 16 days, and a group (n=8) with vehicle alone. Gene expression (mRNA) was evaluated by RT-PCR, RBP4 by Western Blot, vitamin A by HPLC, and fat morphology by histological study.

Results: RA treatment resulted in: 1) tissue specific expression for vitamin A metabolism genes in SF, VF, SM, and LIV 2) normalization of ROL concentrations in SF, VF and SM and RE in SM and LIV with increased total vitamin A (ROL+RE) in SF, VF and LIV ; 3) normalization of RBP4 protein levels in SF, VF, LIV; 4) increased expression of PGC1α and UCP1 in SF; 5) reduced adipocytes size in SF.

Conclusions: The vitamin A metabolism genes are tissue specific affected by RA, improving on vitamin A homeostasis. Lower body weight despite isocaloric food intake, increased expression of PGC1α and UCP1 genes and reduced adipocytes sizes of RA treated animals suggests higher basic metabolism (like in beige-brown fat).
The Effect of Vitamin D Supplementation on Fall Prevention in the Elderly
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**Background:** Falls in the elderly is a significant public health problem due to their high prevalence of 30% in those over 65, many of which result in fractures and soft tissue injuries, longstanding pain, functional impairment, reduced quality of life, and increased mortality. The elderly population is more at risk for vitamin D deficiency, and there is increasing evidence for supplementation and reduction in the rates of falls and fractures.

**Objective:** The objective of this paper is to critically appraise five key articles with the aim to answer the question: ‘What is the relationship between vitamin D supplementation in the elderly and fall prevention?’

**Methods:** A literature search of PubMed, EMBASE and CINAHL was conducted and five key articles were selected based on relevance and date of publication. Study findings were also considered when selecting articles so as to provide a complete depiction of the variability of results on this topic.

**Results:** Out of the five key studies, one looked at the effect of oral and parenteral megadose vitamin D supplementation in the elderly, and showed prevention of falls and improving functional mobility. The second study showed a correlation between vitamin D serum concentrations and cognition and falls, but no correlation with motor measures. A third study showed a positive association between serum 25(OH)D levels and physical performance in the elderly. A fourth study found positive associations between vitamin D insufficiency and impairments in factors which predispose the elderly to fall. The last study identified a positive role of vitamin D in balance control, which could affect falling in the elderly. Based on these findings as well as the quality of study designs and methods it would appear as though vitamin D supplementation in the elderly plays an important role in fall prevention.
Evaluating PARP1 as a Synthetic Lethal Interactor of RAD54B in Colorectal Cancer

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Introduction: Colorectal Cancer (CRC) is the second leading cause of cancer death in Canada. A limitation of most therapeutic approaches is that their cytotoxic effects are not restricted to cancer cells, and unwanted side effects occur within normal tissues. Accordingly, identifying novel therapeutic strategies and drug targets to better target and combat the disease are highly warranted. Synthetic lethality is a strategy that targets and kills cancer cells with mutations specific to cancer cells and is therefore expected to minimize side effects. Synthetic lethality is defined as a rare lethal combination of two independently viable mutations that holds tremendous promise in treating cancer. In this study, we investigate the potential synthetic lethal (SL) interaction between RAD54B and PARP1. RAD54B normally functions in homologous recombination repair and mutations have been identified in CRC as well as various other cancers such as breast, prostate, and lung.

Methods: To validate PARP1 as a SL interactor of RAD54B, we employed an established RNAi-based screening platform, along with a RAD54B isogenic CRC cell model. Conceptually, a SL interaction will result in fewer cells within RAD54B-deficient cells compared to RAD54B-proficient cells following PARP1 silencing. To further validate PARP1 as a candidate therapeutic target, cells were treated with PARP1 inhibitors (Olaparib and BMN673) to confirm preferential killing within the RAD54B-deficient cells.

Results: Following PARP1 silencing by either pooled or individual siRNA duplexes, quantitative imaging microscopy revealed a statistically significant decrease in the mean number of RAD54B-deficient cells relative to controls. Preliminary results from the Olaparib and BMN673 treatments also appear to show preferential killing within the RAD54B-deficient cells relative to controls.

Conclusion: Collectively, these data suggest that RAD54B and PARP1 are SL. They further suggest that PARP1 may be a candidate lead target in CRCs (or other tumors) with RAD54B-defects.
Enterovirus D68: Lessons from a North American Outbreak
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**Introduction:** Enterovirus D68 (EVD68) infection is often associated with respiratory symptoms, but is known to occasionally involve the central nervous system. Though identified in 1962, EVD68 was not recognized as a prominent Enterovirus serotype until 2006. Since, it has caused numerous outbreaks, and has steadily appeared in surveillance around globally. In 2014 a large outbreak occurred in Canada and the United States. Of 2600 specimens received by the CDC in Atlanta, 36% were positive for EVD68. The outbreak included at least 13 deaths and substantial morbidity. Only 22 complete genome sequences were available in public databases at the beginning of 2015. In the study described here, all publicly available EVD68 full genome sequences and 39 additional Canadian sequences are compared. This study will provide insight for EVD68 evolution and pathogenicity.

**Methods:** The National Microbiology Laboratory (NML) received 282 EVD68 positive samples from across Canada between August and October 2014 during a North American EVD68 outbreak. A full genome sequencing assay was developed with long range rtPCR and overlapping EVD68 primers. Select sequences were chosen for full genome analysis based on VP1 sequence analysis. We also used 20 years of Enterovirus surveillance to select representative pre-2014 EVD68 samples from the National Centre for Enteroviruses (NCEV) repository. Sequences were aligned using the ClustalW algorithm within the NML’s Galaxy platform. EVD68 alignments were analyzed using maximum likelihood phylogenetic trees and Bayesian Evolutionary Analysis Sampling Trees (BEAST).

**Results:** BEAST analysis of EVD68 highlights two major lineages, which diverged from a common ancestor in roughly 1990. Phylogenetic analysis using a maximum likelihood method confirms several clades within each lineage. Two closely related clades contain the vast majority (>98%) of 2014 EVD68 outbreak isolates, while the other isolates are found in the other lineage. One clade in particular contains exclusively 2014 outbreak isolates and represents roughly 80% of all outbreak isolates.

**Conclusions:** EVD68 has emerged to become a prominent human pathogen. Two closely related clades of EVD68 have been implicated as the cause of the 2014 North American Enterovirus outbreak. Continued surveillance will determine whether EVD68 continues its prominence in North America.
Predictors of Appropriate Shocks Following Device Replacement in Primary Prevention Implantable Cardioverter Defibrillator Patients

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Introduction: Implantable Cardioverter Defibrillators (ICDs) are implanted for primary prevention of sudden cardiac death in patients with reduced left ventricular ejection fraction (LVEF). Guidelines are based on trials with follow-up periods of 2-4 years. Contemporary ICDs have a lifespan of 5-7 years. The ongoing risk/benefit profile beyond this time span is unknown.

Objectives: The purpose of this study was to determine if the incidence of appropriate shocks after replacement could be predicted by a history of appropriate shocks or by the LVEF at the time of replacement.

Methods: Single-centre, retrospective review of 69 consecutive patients who had a primary prevention ICD and a replacement between January 2005 and December 2014. Patient characteristics, LVEF at implant and replacement and the rates of therapies and complications were collected. Recovery of LVEF was defined as LVEF > 35% and an increase of ≥10%. Predictors of appropriate therapies after replacement were explored by logistic regression analysis.

Results: Mean age at replacement was 66 +/- 12 years. Males represented 84% of patients. Mean follow-up time was 7.9 +/- 2.3 years overall and 2.5 +/- 1.5 years after replacement. Mean LVEF at replacement was 30 +/- 13%. LVEF Recovery occurred in 19 Patients (28%) and none of these patients received an appropriate shock after device replacement. The incidence of appropriate shocks was 25% (17 patients) prior to replacement and 28% (19 patients) following replacement. Complications requiring surgical re-intervention occurred in 11 patients (16%). Results of regression analysis are shown in the table.

Conclusions: In primary prevention ICD patients, an appropriate shock prior to device replacement is predictive of appropriate shocks following replacement and recovery of LVEF is protective. ICD replacement is associated with a high incidence of complications requiring surgical intervention.
Monolysocardiolipin Acyltransferase-1: A Cardiolipin Remodeling Enzyme that May be used to Treat Barth Syndrome.

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**Background:** Barth Syndrome (BTHS) is a rare but serious X-linked genetic disorder caused by TAZ gene mutations. The TAZ gene codes for an enzyme called tafazzin (TAZ). This enzyme's function includes remodeling the polyglycerophospholipid, cardiolipin (CL), with specific acyl groups. Symptoms of BTHS patients include cardiomyopathy and neutropenia. Studies show that the acyl groups on CL can also be remodeled by a mitochondrial enzyme called monolysocardiolipin acyltransferase-1 (MLCL AT-1).

**Objectives:** 1.) Examine the effects of tafazzin alteration \textit{in vitro} and \textit{ex vivo}, and 2.) determine the effects of MLCL AT-1 expression in BTHS lymphoblasts.

**Methods:** Cell lines used include Epstein-Barr virus transformed lymphoblasts from healthy and BTHS patients. Our experiments also include using a doxycycline-induced \textit{Taz} knockdown mouse model. TAZ and MLCL AT-1 gene expression were analyzed in these cells via RT-PCR. Western blot analysis was used to analyze TAZ and MLCL AT-1 protein expression in these cells. MLCL AT-1 enzyme activity assays as well as phosphorous mass analysis (to determine CL mass) were also performed. Blue native polyacrylamide gel electrophoresis (BN-PAGE) was used to analyze mitochondrial supercomplexes in the cell lines as well as in tissues from wild-type and \textit{Taz} knockdown mice. Mitochondrial function was analyzed in healthy and BTHS lymphoblasts using a Seahorse XF 24 Extracellular Flux Analyzer.

**Results:** MLCL AT-1 gene expression increased when TAZ was knocked down in healthy lymphoblasts. In BTHS lymphoblasts, TAZ protein expression is significantly reduced. Expression of MLCL AT-1 is able to increase MLCL AT-1 enzyme activity and CL mass in healthy and BTHS lymphoblasts. Mitochondrial supercomplex formation is decreased in BTHS lymphoblasts. In \textit{Taz} knockdown mice, supercomplex formation is also disturbed. Mitochondrial function is altered in BTHS lymphoblasts, but the use MLCL AT-1 is able to improve this effect.

**Conclusion:** MLCL AT-1 expression may serve as a potential therapeutic approach to treat BTHS.
The long-lived human immunodeficiency virus (HIV) pandemic and the current Ebola virus (EBOV) outbreak are two significant threats to global public health. EBOV causes a highly lethal hemorrhagic fever, for which there are no licensed treatments and vaccines. On the other hand, HIV infects and destroys CD4+ T cells, causing acquired immunodeficiency syndrome, which leads to deadly secondary infections. Developing effective vaccines and treatments for HIV has been extremely challenging because of its high mutation rate.

We propose to develop a vaccine against both viruses using a live-attenuated HIV backbone in which the HIV envelope protein is functionally replaced by the EBOV glycoprotein (GP) to shift the tropism away from CD4+ T cells and toward antigen presenting cells (APCs), while inducing different immune responses compared to wild-type HIV infection. Three chimeric viruses were rescued by transfection of 293T cells with DNA clones containing truncations of EBOV GP in place of HIV gp120 within the NL4-3 backbone. Tropism of the chimeric viruses will be evaluated by kinetics studies in CD4+ T cells, and APCs such as monocytes, macrophages, and dendritic cells, isolated from human and murine peripheral blood mononuclear cells.

For vaccine studies, BALB/c mice will be systemically immunized with candidates that are able to infect APCs and unable to infect CD4+ T cells. The B cell response will be analyzed by quantifying the total anti-EBOV and anti-HIV IgG antibodies in complement-inactivated sera by ELISA using plates coated with EBOV GP or heat-inactivated purified HIV-1 particles, respectively. Neutralizing serum antibodies will be measured by neutralization assays. The T cell response will be measured by ex vivo re-stimulation of splenocytes with HIV and EBOV peptides followed by quantification of activated IFN-γ-producing CD8+ T cells by ELISPOT.

The study of this novel vaccine approach for the prevention of EBOV and HIV infection can generate substantial knowledge on the span of immune responses initiated upon shifting the delicate balance of HIV replication away from CD4+ T cells to APCs. Importantly, this approach may one day serve as a cost-effective, dual-acting vaccine to provide protection in the ongoing battles against EBOV and HIV.
Diagnostic and Prognostic Significance of Serum and CSF MMP-9, CCL2, Svcam-1 and Tjs in Leukemia CNS Metastasis
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Introduction: Metastasis to the central nervous system (CNS) is the primary obstacle in leukemia treatment. Matrix metalloproteinase-9 (MMP-9), chemokine ligand 2 (CCL2), soluble vascular adhesion molecule-1 (sVCAM-1) and Tight junction proteins (TJs) play crucial roles in tumor cell adhesion, motivation and survival, but their roles in leukemia CNS metastasis remain to be elucidated.

Methods: MMP-9, CCL2, sVCAM-1 and TJs in paired CSF and serum samples collected from 33 leukemia patients with (n=12) or without (n=21) CNS metastasis were measured by ELISA. Other risk factors related to CNS leukemia (CNSL) prognosis were also analyzed.

Results: MMP-9_{CSF}, sVCAM-1_{Serum}, ZO-1_{CSF} and ZO-1_{Serum} were significant higher in the CNSL group than in the non-CNSL group (p<0.05). Positive correlations were observed between ZO-1_{CSF} and CCL2_{Serum}, ZO-1_{CSF} and CCL2_{CSF}, ZO-1_{Serum} and CCL2_{CSF}, and ZO-1_{Serum} and MMP-9_{CSF}. Patients with higher levels of CCL2_{CSF} and sVCAM-1_{Serum} had shorter event-free survival.

Conclusion: Tight junction proteins may be good predictors of leukemia CNS metastasis. MMP-9, CCL2 and sVCAM-1 in the CSF may play crucial roles in predicting the possibility of leukemia metastatic CNS and the outcome of CNSL patients.
**Introduction:** Diffusion-weighted magnetic resonance imaging can be used to determine axon diameter distributions in brain tissue. Current methods are able to distinguish medium sized axons in human brains, but not smaller sized axons and suffer from errors because of their fitting routines. We are using oscillating gradients in order to probe smaller axon diameters in mouse brains. We vary the frequency of these oscillating gradients from very small to very large in order to infer very small axon diameters.

**Methods:** Monte Carlo computer simulations were conducted using several distributions of non-overlapping parallel cylinders surrounded by extracellular water with lattice periodicity. This geometry aims to model the axon environment in healthy white matter regions. A cosine gradient spin echo sequence was used to generate 100 signals with different cosine frequencies (from .05 to 1 kHz) and gradient strengths (from 0 to 909 mT/m). Frequencies and gradient strengths were chosen to be clinically feasible. Simulations were run with 114688 particles and 42000 time steps. The cylinders were impermeable and water diffused within and outside the cylinders. The simulations were programmed in CUDA C/C++ and run on a HP Z240 workstation containing an Intel® Xeon® Processor E5-1650 6-core 3.20GHz CPU. The HP Z240 workstation contained two graphics cards, a Tesla C2075 (Fermi 2.0) graphics card for dedicated CUDA computation and a Quadro 600 (Fermi 2.1) graphics card handling the display. The mean signal was fit to the AxCaliber analytical model using least squares minimization.

**Results:** For many simulated geometries, the fitted data agree fairly well with the input model, allowing the extraction of axon diameters in the range of 0.5 $\mu$m to 4.5 $\mu$m. In certain situations, the fitted model is quite poor, leading to inaccurate diameter estimates.

**Conclusion:** This work is the first step toward combining oscillating gradient measurements with axon diameter distribution models to infer distributions of small axon diameters in tissues. Further studies will be directed towards determining of the range of feasible diameter sizes necessary for the model to accurately work. Distributions of non-parallel axons and more diffusion gradient directions will be needed to make a more complete model.
Introduction: Activation-induced deaminase (AID) is a DNA mutator necessary for antibody diversification during an immune response; however, AID activity is tightly regulated to prevent oncogenic side effects. Though AID is actively imported into the nucleus, where it mutates single stranded DNA, its nuclear access is restricted by CRM1-mediated nuclear export and by an uncharacterized mechanism of cytoplasmic retention. With the hypothesis that cytoplasmic retention limits AID activity, we set out to characterize its mechanism and biological relevance.

Results: By comparing AID homologs and molecular modeling we define a structural motif in AID that dictates the strength of cytoplasmic retention and we demonstrate a critical role for the translation factor eEF1A1. Cytoplasmic retention of AID variants correlate with their binding to eEF1A1 while inhibiting eEF1A1 prevents this interaction and causes AID nuclear accumulation. The interaction of AID or tRNA to eEF1A1 is mutually exclusive, suggesting a mechanism for regulating cytoplasmic retention during protein translation. Moreover, the interaction of AID with eEF1A1 is inversely correlated with its binding to HSP90. Despite both interactions stabilizing cytoplasmic AID, eEF1A1 inhibition increases class switch recombination (CSR), while HSP90 inhibition reduces it. This distinguishes two complexes of AID, which sequentially stabilize immature AID (HSP90) then store functionally mature AID (eEF1A1) in the cytoplasm. We further show that cytoplasmic retention of AID can limit oncogenic cMyc-IgH chromosomal translocations. On the other hand, while we find that cytoplasmic retention and nuclear export are complementary in excluding AID from the nucleus, CRM1 inhibition does not increase CSR while eEF1A1 inhibition does.

Conclusion: We conclude that eEF1A1 is essential for restricting nuclear access of mature AID, thereby limiting CSR and oncogenic side effects. We also integrate multiple regulatory pathways of AID into a coherent model.
Assessment of Soluble Prorenin Receptor as a Prostate Cancer Biomarker
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Background: Prostate Cancer (PC) is the most common male malignancy and is the leading cause of cancer-related death among men (Canadian Cancer Statistics 2013). Unfortunately, the serum levels of the prostate-specific antigen (PSA), which is the only current PC screening tool, can vary independently of the presence of PC, leading to misdiagnosis of 60% of PC cases.

Rationale: The phosphatase and tensin homolog (PTEN) gene is a tumor suppressor gene that is deleted or mutated in most PC cases. PTEN-loss is linked to PC metastasis through allowing secretion of multiple pro-metastatic factors. The detection of soluble pro-metastatic factors secreted as a result of PTEN loss may be utilized as a new screening tool for studying metastatic PC. PTEN regulates the (PI3K)/Akt/mTOR signaling pathway. The PI3K/Akt/mTOR pathway promotes protein synthesis and cell growth through the mTORC1, and inhibits cell death through the phosphorylated form of the protein Akt (p-Akt). Loss of PTEN regulation leads to over-activation of the PI3K/Akt/mTOR pathway resulting in uncontrolled cell growth and rapid proliferation. We used a metastatic PC cell line (LnCap) that lacks PTEN to examine secreted factors regulated by PTEN, utilizing the 2D-DiGE approach. Mass Spectrometry analysis revealed [pro]Renin Receptor ([p]RR) as a PTEN-regulated protein. As a validation to our approach, PSA was down-regulated the most after re-expression of PTEN, as PTEN is known to regulate PSA.

[p]RR is a membrane protein that is cleaved, leaving behind a transmembrane domain and an intracellular domain at the C-terminus side. After cleavage, the soluble extracellular N-terminus fragment of [p]RR (s[p]RR) is secreted and can be detected in the blood.

[p]RR promotes angiogenesis and is overexpressed in glioblastoma cells. Also, [p]RR is a chaperone to the Vacuolar-ATPase pump (V-ATPase). V-ATPase maintains acidity of lysosomes by pumping protons into the lumen of the vacuoles. V-ATPase activity is critical in cancer because many tumor cells secrete pH-dependent lysosomal enzymes to digest the extracellular matrix (ECM) for subsequent invasion.

Mitochondrial Bioenergetics in Platelets of Healthy Subjects with a Family History of Diabetes

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Introduction: Platelets may serve as an amenable tissue for detection of mitochondrial dysfunction, a plausible early biomarker of disease. Previous studies have demonstrated that platelets from patients with type 2 diabetes had lower mitochondrial membrane potential, membrane fluidity and higher adenosine tri-phosphate content and oxidative stress than that of healthy subjects. The impact of family history of diabetes on mitochondrial bioenergetics in platelets of healthy subjects remains unknown.

Methods: We investigated the impact of family history of diabetes or heart disease or dyslipidemia on mitochondrial bioenergetics in the intact human platelets from 48 (16 males and 32 females) healthy fasting donors by determining the oxygen consumption rates in intact platelets using a highly sensitive Clark type oxygen electrode oxygraph-2k and analyzed by Datlab (Oroboros, Innsbruck, Austria).

Results: Mitochondrial bioenergetics profiles in the healthy subjects were not significantly affected by age, gender or body mass index. However, healthy subjects with a family history of diabetes alone had significantly lower basal, maximal and ATP-linked respiration and significantly high non-mitochondrial respiration levels when compared to healthy subjects without a family history of diabetes, heart disease or dyslipidemia (p<0.05 or 0.01).

Conclusion: The results suggest that platelet mitochondrial bioenergetics can be conveniently assessed using platelets from peripheral blood. Mitochondrial bioenergetics profile was not significantly affected by age, gender and body mass index in healthy subjects. Healthy subjects with a family history of diabetes exhibited early detectable abnormalities in their platelet mitochondrial bioenergetics profile. The assessment of mitochondrial bioenergetics may help to identify clinically healthy subjects with early signs of mitochondrial dysfunction.
Antenatal Glucocorticoids lead to Multigenerational Programming of Adult Behaviour and HPA Function via Paternal Transmission

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Introduction: Synthetic glucocorticoids (sGC) are administered to pregnant women at risk for preterm labour. Animal studies have shown that sGC modify the fetal brain epigenome and have multigenerational effects on behaviour via maternal transmission. Multiple course antenatal sGC has been associated with increased activity, impaired attention and altered hypothalamic-pituitary-adrenal (HPA) function in young children. It is currently not known whether multigenerational influences of sGC on behaviour and HPA function can be transmitted via the paternal germ line. We hypothesized that prenatal treatment with sGC would result in paternal transmission of: increased locomotor activity, reduced attention and suppressed HPA function in adult F2 guinea pig offspring.

Methods: Pregnant guinea pigs received 3 courses of either saline or betamethasone (sGC) in late gestation, and then delivered undisturbed (term ~69 days). Adult F1 male offspring were mated with control females to produce F2 offspring. Adult male and female F2 offspring underwent behavioural and HPA testing: open field (OF) locomotor activity; 24-hour activity; prepulse inhibition (PPI)-attention; saliva collected-HPA function.

Results: In F2 males, sGC significantly modified the profile of locomotor activity in the OF (P<0.05). sGC resulted in a one hour phase advance in 24h-activity in males (P<0.05), and increased 24h-activity with significant interaction with reproductive cycle in females (P<0.05). sGC resulted in increased PPI (P<0.05) for both sexes; in females the effect was only observed during the estrous phase. In females, sGC resulted in an increased HPA response to stress (P<0.05); there was no effect of sGC in males.

Conclusion: This is the first study to show paternal transmission of altered stress-related behaviours and HPA function to adult F2 offspring following antenatal sGC treatment. Male F2 offspring were more sensitive to the effects of sGC on locomotor activity than females. Interestingly, there were stage-of-cycle-dependent effects of sGC on 24hr-activity and PPI in F2 females, indicating that sGC programming interacts with sex hormones to affect behaviours across generations. Unexpectedly, sGC resulted in an increase in attention in 2nd generation offspring and increased HPA function in females following paternal transmission. This study indicates germ-line transmission sGC-mediated programming across generations, which may involve epigenetic mechanisms.
Semaphorin 3E Plays a Critical Role in Allergic Asthma through Modulation of Dendritic Cell Functions

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Background: Semaphorin 3E (Sema3E), originally identified as a neuronal chemorepellent, is involved in regulation of cell migration, proliferation and angiogenesis. However, role of Sema3E in allergic asthma has remained to be addressed.

Results: Herein, we demonstrate that expression of Sema3E is suppressed in human asthma and mouse model of the disease. Sema3e−/− mice show increased airway hyperresponsiveness, remodeling and Th2/Th17 inflammation through enhanced recruitment of pulmonary CD11b⁺ dendritic cells (DC) than the WT controls upon house dust mite (HDM) challenge. Adoptive transfer of pulmonary CD11b⁺ DC from Sema3e−/− mice into WT littermates increases HDM-induced airway inflammation. Sema3E role in allergic asthma is mediated by regulation of CCR7 expression, Rac1 GTPase activity and actin rearrangement in DC. Treatment with Sema3E protected mice from allergic asthma via regulation of DC-mediated T cell responses.

Conclusion: Together, these findings suggest that Sema3E may attenuate allergic asthma deficits by alteration of DC functions which provides new insights into its role in immunity.
**Adiponectin Deficient Mice Display Hyperglycemia, Hyperlipidemia and Glucose Intolerance during Pregnancy**

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**Introduction:** Gestational diabetes mellitus (GDM) affects 5-10% of all pregnancies. Epidemiological evidence suggests that low levels of adiponectin during pregnancy could be an independent risk factor for GDM. Adiponectin is a fat tissue-derived hormone that acts to improve the sensitivity of tissues to insulin. We hypothesize that adiponectin deficiency during pregnancy contributes to GDM. Furthermore, we suggest that increasing adiponectin levels in the bloodstream restores normoglycemia during pregnancy.

**Methods:** To test the hypothesis that adiponectin deficiency contributes to GDM, we assessed several parameters of glucose as well as lipid homeostasis, including glucose tolerance, insulin tolerance, insulin secretion capacity and also insulin, free fatty acid and triglyceride levels in pregnant adiponectin knockout (strain B6;129-Adipoq\textsuperscript{tm1Chan/J}) and wild type mice. In order to observe whether adiponectin supplementation improves insulin sensitivity and glucose homeostasis during pregnancy, adiponectin-/- mice were administered an adenovirus vector expressing either GFP (controls) or adiponectin (Ad-APN).

**Results:** In the third trimester of pregnancy, adiponectin knockout mice exhibited significantly higher fasting blood glucose (8.3 +/- 0.2) than wild-type controls (4.55 +/- 0.4). Similarly, pregnant adiponectin null mice exhibit elevated fasting serum insulin and impaired insulin tolerance relative to wild type controls. Adiponectin-/- mice have higher serum triglyceride and ~5-fold higher free fatty acid levels during pregnancy. In addition, adiponectin knockout mice displayed impaired glucose tolerance, which could be due in part to the reduced capacity of islets isolated from pregnant mice to secrete insulin in response to high glucose concentrations. Administration of Ad-APN to pregnant adiponectin-/- mice increased adiponectin levels in the circulation and improved their glucose tolerance compared to Ad-GFP adiponectin-/- mice. Body weights and food consumption during pregnancy were not significantly different between the genotypes, or following Ad-APN administration.

**Conclusion:** Our results show that adiponectin deficiency during pregnancy disrupts glucose homeostasis and is associated with hyperlipidemia that is characteristic of GDM. Furthermore, we show that increasing adiponectin in the circulation improves glucose tolerance and reduces fasting blood glucose in pregnant adiponectin-/- mice.
Myocardin Regulates Mitochondrial Function to Prevent Programmed Cell Death during Cardiac Development

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Introduction: Twelve newborns each day are diagnosed with congenital heart defects, a disease linked to aberrant programmed cell death, and associated with infant mortality. Although advances in medicine have improved infant survival, the underlying transcriptional mechanisms regulating cell death in the heart requires further investigation. We hypothesize that myocardin, a nuclear transcriptional co-activator, regulates mitochondrial function to prevent programmed cell death during cardiac development.

Methods: Using a cell and molecular approach, data was obtained from the cardiac H9c2 culture model, where cells were genetically manipulated by transfection. Programmed cell death, elicited by ionomycin treatment or adrenergic stimulation, was determined by cell viability assays, where >300 cells were counted for each condition and compared to vehicle treated control cells (n=3). Mitochondrial function was assessed by fluorescent imaging, and gene expression was determined by qPCR and protein immunoblot. One-way anova determined multiple comparisons between groups and student t-test compared mean differences.

Results: Ionomycin and adrenergic stimulation reduced cell viability by 25% (p<0.02) and 55% (p<0.001), respectively. In addition, expression of myocardin abrogated cell death induced by these experimental treatments (p<0.01). Furthermore, ionomycin and adrenergic stimulation triggered mitochondrial dysfunction, which was restored by myocardin expression. Mechanistically, we found that myocardin promoted cell survival through transcriptional activation of microRNA-133a by 1.55 fold compared to control cells (p<0.05) and reduced protein expression of a death gene called Nix. The survival phenotype of myocardin was found to be activated by a histone deacetylase kinase, called salt-inducible kinase-1 (SIK1), that increased microRNA-133a gene expression by 1.60 fold (p<0.05). Finally, we demonstrate that SIK1 is inhibited during adrenergic-induced cell death by direct phosphorylation, resulting in disruption of myocardin activity.

Conclusion: Our data supports the hypothesis that myocardin activation prevents Nix-induced mitochondrial dysfunction to promote cardiac cell survival during development.
Microrna-301a Alters Dicer Expression in both Primary Human Atrial Cells and Bone Marrow-Derived Mesenchymal Progenitor Cells: Implications for Cardiac Fibrosis

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Introduction: It has been previously established that there are multiple cell types that can contribute to cardiac fibrosis including both atrial fibroblasts (AFs) and bone marrow-derived progenitor cells (MPCs). We have previously shown that MPCs display a myofibroblast phenotype in vitro which is linked to altered microRNA(miR)-301 expression, a miR affiliated with maintaining proliferation in numerous cell types. We have also shown that miR-301a influences a dichotomous phenotype in primary human MPCs isolated from patients undergoing open heart surgery. The objective of this experiment was to further understand how this phenotype change could be influenced so we performed a microarray analysis investigating potential targets of miR-301a. One of the most exciting targets was Dicer which is responsible for activating miRs, therefore altering protein expression and ultimately, influencing phenotype.

Methods: As both MPCs and AFs display a dichotomous phenotype where each cell type displays a phenotype that pathologically contributes to fibrosis, we transfected both MPCs and AFs with miR-301a. Both MPCs and AFs were also isolated from patients undergoing open heart surgery. We performed qRT-PCR and Western blot analysis to investigate Dicer expression and expression of pro-fibrotic markers.

Results: Our study has found that miR-301a transfected MPCs and AFs have significantly reduced expression of Dicer. In addition, there have decreases in both the mRNA and protein levels of collagen I and non-muscle myosin IIA, both expressed in myofibroblasts, the cell type predominantly responsible for causing cardiac fibrosis.

Conclusion: These results provide insight into a potential cellular mechanism that influences the pro-fibrotic phenotypes of AFs and MPCs which could be caused by changes in Dicer, the key mechanism in activating micro-RNAs.
Perinatal Antibiotic Treatment Alters Offspring’s Gut Microbial Profile Predisposing Them to Experimental Colitis

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Background: The use of antibiotics in the perinatal period is associated with delayed microbial colonization. Postnatal maturation of the immune system is largely driven by exposure to microbes hence, the nature of the intestinal colonization may be associated with the development of childhood diseases that may persist to adulthood. Therefore, we have explored whether prenatal antibiotic therapy (ATB), can increase offspring’s susceptibility to experimental colitis by modifying the gut microbial colonization.

Methods: Pregnant C57Bl/6 mice were treated with cefazolin at 160 mg/kg bw or with saline starting six days before due date. At 7 weeks male offspring’s from the two groups received 4 % dextran sulfate sodium (DSS) in drinking water for 5 days. Disease activity index, histology, colonic interleukin (IL)-6, IL-1β and serum C-reactive protein (CRP) were determined. From colon and fecal samples the V3-V4 region of bacterial 16SrRNA was amplified and subjected to Illumina sequencing. Alpha diversity was calculated using Chao 1 and beta diversity was determined using QIIME. Differences at genus level were determined using partial least squares discriminant analysis (PLS-DA), and phylogenetic investigation of communities by reconstruction of unobserved states (PICRUSt) was used for bacterial functional predictions.

Results: Prenatal ATB increased the onset of clinical disease as assessed by stool consistency, weight loss and rectal bleeding. On day 5, macroscopic and histologic scores were significantly increased. Colonic IL-6 was increased, but IL-1β level was not modified. Conversely, in ATB-DSS group CRP level was significantly decreased. In colitic mice compared to the control group, ATB decreased bacterial species richness in fecal samples but not in the colon, and bacterial community composition differed between the groups in both sample types, although the mother further influenced this. PLS-DA analysis revealed an association of specific taxa with ATB-DSS or control-DSS at lower taxonomic levels. Also, there were differences in microbial functional pathways in both fecal and colonic samples.

Conclusions: These results support the hypothesis that prenatal antibiotherapy modulates offspring’s intestinal bacterial colonization and susceptibility to develop colonic inflammation in a murine model of
colitis. Furthering our understanding of the impact of prenatal antibiotherapy on gut bacterial colonization and susceptibility to colitis may guide future interventions to restore physiologic intestinal colonization in offspring born by antibiotic-exposed mothers.
Adiponectin Deficient Mice Have Reduced Fat Mass on a Control Diet but Not When Challenged With a High Fat Diet

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Introduction: Adipose tissue functions as a fat depot and also secretes various adipokines that regulate whole body metabolism via paracrine and endocrine mechanisms. The most abundant adipokine secreted from adipose is adiponectin, which exhibits both potent anti-inflammatory and anti-diabetic properties. However, in obesity, adipose tissue expansion results in decreased adiponectin secretion. The objective of our study was to determine if the absence of adiponectin affects fat mass using a whole body adiponectin knockout (APN-KO) model.

Methods and Results: Male 8-week old APN-KO and C57BL/6 (control) mice were fed a high fat diet (n=10/group) for 12 weeks to induce obesity. APN-KO and C57BL/6 mice fed a low fat diet (n=10/group) were used as lean controls. After 12 weeks on the high fat diet, the APN-KO mice weighed significantly more than the C57BL/6 mice (45.1±1.3 g vs 40.1±1.1 g, p=0.0008) but the epididymal fat pad weight was less (5.98±0.42 g/100g bwt vs 7.24±0.35 g/100g bwt, p=0.035). There were no differences in the final body weights between genotypes fed the low fat diet, but there were significant reductions in both visceral (3.57±0.43 g/100g bwt vs 7.37±0.32 g/100g bwt, p<0.0001) and subcutaneous fat pad weights (1.44±0.14 g/100g bwt vs 2.52±0.12 g/100g bwt, p<0.0001) in APN-KO mice relative to the C57BL/6 mice. These findings were confirmed by whole body composition analysis using EchoMRI™ which showed a reduction in %fat mass in APN-KO in comparison with the C57BL/6 mice fed a low fat diet for 12 weeks (11.5±1.5% vs 24.7±1.2%, p<0.0001) with a concomitant increase in %lean mass (88.65±2.4% vs 71.83±1.2%, p<0.0001). However, there were no differences in overall %fat mass and %lean mass between genotypes fed high fat diet.

Conclusion: The decreased fat mass in lean adiponectin deficient mice indicates that adiponectin has a potential role in regulating fat pad development under healthy conditions. Increased adipocyte size rather than increased number correlates with higher metabolic risk and so the morphometric analysis of the adipose tissue depots for hypertrophy and hyperplasia will further validate the potential role of adiponectin in adipose tissue development and associated metabolic complications.
Introduction: As many as 74% of residents in long-term care (LTC) are thought to have swallowing difficulties. Food intake may also be affected by fatigue. As fatigue sets in during mealtimes, the strength of the tongue may decline. In this pilot study, we explored the relationship between tongue strength and time to complete a meal.

Methods: The IOWA oral performance instrument was used to collect maximum anterior isometric tongue-palate pressures from 12 LTC residents (5 male; mean age: 85, range 65-99). Residents were also screened for dysphagia with applesauce and a water swallow test. Each resident was observed at three different meals, and length of time to eat the meal, amount of food eaten and overt swallowing issues were recorded.

Results: Residents who displayed swallowing difficulties at mealtimes had significantly lower tongue strength (mean:17 kPa, 95% confidence interval: 7-27) than those without swallowing difficulties (mean: 37 kPa, 95% CI: 29-44; p=0.005). Those with lower tongue strengths took significantly longer to complete meals (p < 0.05). Longer mealtimes were associated with reduced daily food intake (r² = 0.39). Tongue strength was not predictive of performance on the water screen and the water swallow test result showed poor sensitivity (25%) but good specificity (85%) for predicting which participants would display mealtime difficulties.

Conclusion: In seniors in long term care, reduced tongue strength is associated with longer meal times and the presence of swallowing difficulties. Further exploration of these relationships is warranted. Investigation of tongue strengthening interventions would be beneficial for promoting food intake.
A Novel Treatment Option for Glioblastoma: The Right Combination and Timing Counts
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**Introduction:** Temozolomide (TMZ) is an alkylating drug commonly used in glioblastoma (GB) treatment. TMZ-induced DNA damage is repaired by Base Excision Repair (BER) mechanism. We previously showed that the stem cell factor/non-histone AT-rich chromatin binding HMGA2 (High Mobility Group AT-hook-2) plays important roles in BER and contributes to cancer chemoresistance.

**Methods:** We employed RT-PCR for mRNA detection, Western blot, immunofluorescence and immunohistochemistry for protein detection in patient GB cells (PBS-10 and PBS1), mouse GB cells and human GB cell lines (U251 and U87). Patient cells were isolates from surgical GB tumor tissues. GFP-positive NF53 mouse GB cells were derived from a mouse GB model. WST and caspase 3/7 assays were used to measure cell survival and cell death, respectively.

**Results:** We observed exclusively nuclear HMGA2 expression in the patient and mouse GB (stem) cells, and human GB cell lines. Presence of HMGA2 significantly decreased recruitment of DNA damage response marker γH2AX, indicating reduced TMZ-induced DNA damage in HMGA2-expressing human and mouse GB cells. HMGA2 knockdown markedly increased the number of DNA strand breaks assessed by enhanced γH2AX nuclear foci, reduced cell survival and increased caspase 3/7-mediated GB cell death. Hence, HMGA2 protected GB cells from TMZ-induced damage. Dovitinib, a multi-kinase inhibitor and DNA minor groove binder, was found to compete with and attenuate HMGA2-mediated chemoresistance. Dovitinib reduced the activation/phosphorylation of oncogenic protein STAT3 at Tyr705 and down-regulated STAT3-coordinated Lin28A and HMGA2 expression, a pathway known to be involved in GB stem cell-like behavior and invasiveness. Indeed, this coincided with the decrease in survival and number of tumor spheres formed. Intriguingly, Dovitinib also downregulated key BER proteins. Hence we formulated a sequential therapy where GB cells were pretreated with Dovitinib followed by TMZ and Dovitinib on consecutive days, which resulted in a significant increase in GB cell killing when compared to simultaneous treatment with the two drugs or use of single drugs (p<0.001).

**Conclusion:** We identified HMGA2 as a novel protective factor against alkylating DNA stress in GB. Our results point towards the development of a new sequential therapeutic strategy using existing FDA-approved drugs that can significantly enhance GB cell death.
Reparative Fibrosis is Impeded In MK5 Deficient Mice Following Myocardial Infarction
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Background: The adverse cardiac remodelling that occurs following left ventricular (LV) myocardial infarction (MI) contributes to the impaired function and heart failure that develops after MI. As MK5 mRNA is highly expressed in heart and we have shown previously that reactive (interstitial) fibrosis is reduced in heterozygote MK5-deficient (MK5⁺/-) mice, MK5 may play a role in cardiac remodelling. Following an MI, the infarcted myocardium undergoes wound healing and accelerated matrix deposition. This reparative fibrosis is a critical component of cardiac wound healing.

Purpose: The present study was to determine if reparative fibrosis secondary to MI is impeded by MK5 haploinsufficiency.

Methods: Twelve week-old MK5⁺/- and wild-type littermate (MK5⁺⁺) mice underwent ligation of the left anterior descending coronary artery (LAD). Sham mice underwent the identical procedure but the coronary artery was not occluded. LV structure and function were assessed before and 7 days post-LAD by transthoracic echocardiography (Echo). Scar size was assessed by both magnetic resonance imaging (MRI, before and 8 days post-LAD) and Masson Trichrome staining. Mice were sacrificed 8 or 21 days post-surgery (n=24-34).

Results: Eight days post-LAD, survival rates for MK5⁺⁺ and MK5⁺/- mice did not differ significantly. In contrast, survival rates did differ over 21 days: the median survival of MK5⁺/-LAD mice being 9 days post-LAD. Echo revealed similar increases in LV end diastolic diameter, myocardial performance index, and wall motion score index in MK5⁺⁺-LAD and MK5⁺/-LAD mice compared to their respective sham mice. In contrast, the incidence of scar rupture was higher in MK5⁺/-LAD compared to MK5⁺⁺-LAD mice (30% vs. 22%). MRI indicated similar scar size in MK5⁺⁺-LAD and MK5⁺/-LAD mice 8 days post-MI. Histological analysis revealed a significant decrease in the percentage of infarction in MK5⁺/-LAD mice compared to MK5⁺⁺-LAD mice. Cardiomyocyte diameter and scar area did not significantly differ between the 2 ligated groups. Surprisingly, angiogenesis in the peri-infarct zone was significantly greater in MK5⁺/-LAD compared to MK5⁺⁺-LAD mice.

Conclusion: MK5 may play a role in scar maturation following myocardial infarction.
Role of Hedgehog Interacting Protein (Hhip) in Pancreatic β Cell function in Diabetes

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Introduction: Pancreatic beta (β) cell dysfunction plays a key role in the development of diabetes. Hedgehog interacting protein (Hhip) expression is essential for proper pancreatic morphogenesis and loss of Hhip function results in 45% reduction of islet mass and decreases β cell proliferation activity by 40%. The present pilot study aimed to establish a linkage between Hhip expression and β cell function in vitro.

Methods: Mouse pancreatic β cell line (MIN6 cell) was studied. The expression of mouse Hhip gene promoter activity (pGL4.20 containing nucleotides N-1542/+9 of mHhip gene promoter), Hhip mRNA and protein expression in normal and high glucose milieu as well as TGFβ1/NF-kB signaling on Hhip expression were assessed accordingly.

Results: Under normal glucose condition (5mM D-Glucose), rHhip stimulated insulin secretion in a time- and dose-dependent manner. Recombinant human active TGFβ1 (rTGFβ1) augmented mouse Hhip promoter activity, mRNA and protein expression (both intracellular and soluble forms) via phosphorylation of Smad 2/3 signaling. Vice versa, recombinant Hhip (rHhip) increased TGFβ1 gene expression. Moreover, transient transfection of pcDNA 3.1/NF-kB/p65 significantly up-regulated Hhip gene expression.

Conclusions: Our data suggested that Hhip regulates insulin secretion in pancreatic β cell via TGFβ1 and NF-kB signaling pathway and this phenomenon might be exaggerated in diabetic condition.
Melanoma Cells Respond Rapidly to CTL Attack

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Introduction: Cytotoxic T-lymphocytes (CTLs) can infiltrate and kill tumors. Despite successful CTL responses induced by immunotherapy, cancers frequently relapse. While much research focuses on improving CTL-based anti-tumor therapy, not much is known about how tumor-cells initially react to CTL attack. We have setup an in vitro co-culture system to study the reactions of melanoma cells during the first hours-to-days of exposure to CTLs.

Methods: Human melanoma-specific CTL clones, isolated from blood of melanoma patients, were co-cultured with low-passage melanoma cell lines derived from patient surgery specimens. Protein expression was determined by flow cytometry at four time-points (up to 72h) of co-culture. At 24h, mRNA profiling was performed using microarrays and the NanoString technology.

Results: In this system, CTLs exert substantial immune pressure on melanoma cells. Most tumor cells die but some cells persist in presence of CTLs, similar to what is observed in situ. As expected, the protein expression of IFNg-inducible genes such as PDL1 increased, whereas the expression of the target antigen decreased, validating our experimental system. Subsequently, a genome-wide microarray screen revealed >800 differentially expressed genes in CTL-exposed compared to untreated tumor-cells. These genes are implied in antigen presentation, interferon signaling and cell communication. The top candidate genes were chosen for in-depth mRNA-based evaluation, to assess whether changes are antigen-specific or dependent on CTL / tumor cell interactions. Indeed, presence of antigen-specific but not of irrelevant control CTLs induced most of the observed changes, which could also be mimicked by CTL-derived cytokines IFNg and TNFa.

Conclusion: Our findings point towards a dynamic interplay between CTLs and melanoma-cells in situ, driving resistance mechanisms involving microenvironmental factors and cells. We now focus on functional studies of the most promising candidate factors. The outcome of this project will contribute to our understanding of immune mechanisms in cancer progression, and may help improving therapeutic strategies.
Donepezil in Severe Alzheimer’s Disease: A Review of Treatment Considerations with Disease Progression

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Introduction: Alzheimer’s disease (AD) is a progressive neurodegenerative disorder that affects 35 million people worldwide, with the prevalence expecting to double by 2030. Currently available treatments are not curative, but merely stabilize or reduce the rate of decline in behavior, activities of daily living, and cognition. Patients with severe AD, defined as an MMSE score of <= 12, require extensive help with daily activities, show severe memory impairment and major personality and behavioral changes. Donepezil is one of only two drugs currently approved for use in severe AD. It is prescribed as 5mg/day, 10mg/day, and a controversial newly FDA approved 23mg/day dose. This review will discuss the risks, benefits, and current recommendations for donepezil use.

Methods: Relevant articles concerning the efficacy and side effects of various doses of donepezil (Aricept) use in severe Alzheimer’s Disease were identified using PubMed. The database search was performed using the MeSH terms “donepezil” AND “Alzheimer Disease” AND “severe”, with search limits set for studies in English on humans. Articles that were selected contained primary research from Clinical Trials, Multicenter Studies, and Randomized Controlled Trials on the use of donepezil in patients with severe AD. Three double blind, parallel-group, placebo-controlled, randomized studies, one post-hoc analysis and one sub-group analysis of original papers meeting the same standard were selected for review.

Results: Donepezil benefits patients in areas of cognition and global function in patients with severe AD. The most consistent improvement was seen in SIB scores. Incremental benefits of treatment were observed with increasing dose in patients with more advanced baseline disease. However, more patients discontinued their treatment due to an adverse event (AE) in the donepezil group vs. placebo, and AE’s occurred more frequently and with increasing severity as dose increased.

Conclusion: Donepezil treatment shows small but measurable benefits in severe AD. Clinicians must weigh these benefits against the possible AE’s when determining the appropriate course of therapy, as recommendations for discontinuation of cholinesterase inhibitors in advanced AD remain unclear and vary with different guidelines.
Introduction: Repeated exposure to ethanol (EtOH) in mice produces behavioural sensitization, a progressive and enduring increase in its locomotor-stimulating effects. We have recently found that mice resistant to developing EtOH sensitization have decreased levels of trkB mRNA throughout the brain, suggesting a critical involvement for TrkB receptor signaling in this behavior. We have also found that sensitized mice show greater levels of pCREB in the nucleus accumbens (NAc) compared to resistant mice and saline controls. Given that pCREB changes in the NAc induced by other sensitization-producing drugs has been associated with structural changes in accumbal medium spiny neurons (MSNs), our finding raises the possibility that EtOH sensitization may also involve structural changes in this neuronal population. Therefore, the overall goal of the present study was to determine whether sensitization to EtOH is like that of other drugs, requiring TrkB signalling and involving NAc MSN spine density changes.

Methods: To this end, male DBA mice received 5 biweekly EtOH (2.2g/kg, i.p.) or saline injections, after pretreatment with the TrkB receptor antagonist ANA-12 (0 and 0.5mg/kg, i.p.) or saline injections, after pretreatment with the TrkB receptor antagonist ANA-12 (0 and 0.5mg/kg, i.p.). In a separate experiment, brains were removed for the analysis NAc MSN spine density using diolistic labeling.

Results: Results showed that ANA-12 did not block the development of EtOH sensitization. Surprisingly, sensitization to EtOH was not associated with changes in NAc MSN dendritic spine density, although there was greater concentration of stubby spines in mice resistance to sensitization.

Conclusion: These results suggest that the neurobiology underlying EtOH sensitization is distinct from that of other sensitization-inducing drugs and that mice failing to sensitize might be more susceptible to the excitotoxic effects of EtOH.
Sepsis, a systemic immune response to severe bacterial infection, is characterized by whole-body inflammatory state as a result of exaggerated immune response to severe bacterial infection. It is well established that regulatory T cells (Tregs) maintain immune homeostasis and help to prevent excessive immune activation. We recently showed that depletion of Tregs leads to susceptibility and acute death to a non-lethal dose of lipopolysaccharide (LPS) or *E. coli* challenge. Here, we investigated the role of Tregs in sepsis using mice with an inactive knock-in mutation in the p110d isoform of PI3K (p110d KI mice), which constitutively have fewer numbers of Tregs compared to their wild type (WT) counterpart mice. We found that p110d mice are highly susceptible to LPS challenge, and this susceptibility was accompanied by greater influx of neutrophils in peritoneum and blood. In addition, neutrophils from p110d mice produced greater amounts of pro-inflammatory cytokines and myeloperoxidase, and displayed increased survival compared to those from WT mice. LPS-induced mortality in p110d mice was abrogated either by reduction of neutrophil numbers using anti-GR1 antibody or adoptive transfer of WT Tregs. Using both *in vitro* and *in vivo* approaches, we further show that Tregs affect neutrophil survival and their production of proinflammatory cytokines. Collectively, our results demonstrate that Tregs regulate neutrophil function and survival and that diminished Treg numbers enhances neutrophil activity and survival, leading to mortality following LPS challenge.
Introduction: Although some studies indicate that the interaction of CD40 with CD40L is critical for IL-12 production and resistance to cutaneous leishmaniasis, others suggest that this pathway may be dispensable. In order to further understand the role of CD40 and CD40L interaction in cutaneous leishmaniasis we compared the outcome of *L. major* infection in both CD40 and CD40L deficient mice.

Methods: Wild type, CD40 and CD40L deficient mice in the resistant C57BL/6 background were infected with *Leishmania major* and treated with or without recombinant IL-12 (rIL-12) for the first two weeks. Lesion development, parasite burden and immune response were determined at different time points. In vitro culture assay was also used to determine the ability of antigen presenting cells to produce IL-12 independent of CD40 and CD40L. Levels of cytokines were measured in cell culture supernatant by enzyme linked immune sorbent assay.

Results: We show that although both CD40 and CD40L KO mice are highly susceptible to *L. major* infection, treatment with rIL-12 during the first 2 weeks of infection causes resolution of cutaneous lesions and parasite growth. Interestingly, while treated CD40 KO mice remained healed, developed long-term immunity and were resistant to secondary *L. major* challenge, treated CD40L KO reactivated their lesion following cessation of rIL-12 treatment. Disease reactivation in CD40L KO mice was associated with impaired IL-12 and IFN-γ production and a concomitant increase in IL-4 and IL-10 production by cells from lymph nodes draining the infection site. We show that IL-12 production by dendritic cells and macrophages via CD40L-Mac-1 interaction is responsible for the sustained resistance in CD40 KO mice following cessation of rIL-12 treatment. Blockade of CD40L-Mac-1 interaction with anti-Mac-1 monoclonal antibody (mAb) led to spontaneous disease reactivation in healed CD40 KO mice, which was associated with impaired IFN-γ response and loss of infection-induced immunity following secondary *L. major* challenge.

Conclusion: Collectively, our data reveal for the first time a novel role of CD40L-Mac-1 interaction in IL-12 production and development as well as maintenance of optimal Th1 immunity in CD40 deficient mice infected with *Leishmania major*. 
Identifying the correlates of innate protection against Human Immunodeficiency Virus is an important goal for development of effective anti-HIV therapies and vaccines. Not all exposures to HIV lead to infection, a small group of female commercial sex-workers in Nairobi have remained HIV exposed but seronegative (HESN) in spite many years of high risk sex work.

The innate immune system is at the interface between the host’s immune system and initial contact with HIV. The work presented here, centres around a comparison of Toll-like receptor responsiveness of peripheral blood mononuclear cells (PBMCs) from two groups of female commercial sex workers to: TLR4 (bacterial cell wall lipopolysaccharide-LPS), TLR7 (Imiquimod) and TLR8 (single stranded RNA-ssRNA). The results demonstrated that the PBMCs of HESN were hypo-responsive to TLR4 and TLR7 stimulations, but hyper-responsive to TLR8 through ssRNA analogous to HIV’s genetic material. This was evidenced by the lower cytokine responses to both TLR4 and TLR7 stimulations, but higher TLR8 responses in HESN PBMCs. The ‘dichotomy’ in TLR responsiveness of the HESN PBMCs was linked to differential expression of TLR7 or TLR8 and activation of cognate TLR signalling pathways with ligation. The dichotomy of between TLR7 and TLR8 responses had consequences on the ability of HIV to infect target cells in PBMCs, where pretreatment of PBMCs with ligand specific for TLR7 enhanced susceptibility to infection by primary HIV isolates, while stimulation of TLR8 reduced susceptibility of HIV targets cells in PBMCs, more so in HESN when compared to controls. The differences in outcomes of the in vitro HIV infection assays following the activation of TLR7 or TLR8 in PBMCs, was linked to the distinct cytokine profiles generated by either stimulation. Where TLR7 stimulations produced robust type I IFNs responses without proinflammatory cytokine responses TNF-α and IL-12, while TLR8 stimulations led to type II IFN responses accompanied by robust proinflammatory responses. These results demonstrate that as non-stimulated immune cells of HESN have lower or ‘quiescent’ activation state, their immune systems can generate more potent responses to ssRNA analogous HIV’s genetic material capable of limiting low dose HIV infection in vitro.
Background: Bam32, a 32 kDa adaptor molecule, plays an important role in B cell receptor signalling, T cell receptor signalling and antibody affinity maturation in germinal centres. Since antibodies against trypanosome variant surface glycoproteins (VSG) are critically important for control of parasitemia, we hypothesized that Bam32 deficient (Bam32−/−) mice would be susceptible to T. congolense infection. Methodology/Principal Findings: We found that T. congolense-infected Bam32−/− mice successfully control the first wave of parasitemia but then fail to control subsequent waves and ultimately succumb to their infection unlike wild type (WT) C57BL6 mice which are relatively resistant. Although infected Bam32−/− mice had significantly higher hepatomegaly and splenomegaly, their serum AST and ALT levels were not different, suggesting that increased liver pathology may not be responsible for the increased susceptibility of Bam32−/− mice to T. congolense. Using direct ex vivo flow cytometry and ELISA, we show that CD4+ T cells from infected Bam32−/− mice produced significantly increased amounts of disease-exacerbating proinflammatory cytokines (including IFN-γ, TNF-α and IL-6). However, the percentages of regulatory T cells and IL-10-producing CD4+ cells were similar in infected WT and Bam32−/− mice. While serum levels of parasite-specific IgM antibodies were normal, the levels of parasite-specific IgG, (particularly IgG1 and IgG2a) were significantly lower in Bam32−/− mice throughout infection. This was associated with impaired germinal centre response in Bam32−/− mice despite increased numbers of T follicular helper (Tfh) cells. Adoptive transfer studies indicate that intrinsic B cell defect was responsible for the enhanced susceptibility of Bam32−/− mice to T. congolense infection. Conclusions/Significance: Collectively, our data show that Bam32 is important for optimal anti-trypanosome IgG antibody response and suppression of disease-promoting proinflammatory cytokines and its deficiency leads to inability to control T. congolense infection in mice.
Airway Epithelial Cells Induce Changes in Airway Smooth Muscle Cell Phenotype

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Rationale: Airway smooth muscle (ASM) plays a critical role in modulating airway diameter and its mass is increased in the airways of asthmatic subjects. Airway smooth muscle cells (ASMCs) are considered to exist in one of two phenotypes at a given time. These two states include cells that are actively proliferating, or those that are expressing proteins of the contractile apparatus. One stimulus previously described to induce ASMC proliferation involves co-culturing ASMCs with airway epithelial cells. We wished to examine whether ASMCs cultured in the presence of epithelial cells reduces the contractile phenotype, and to understand how this phenotypic switch occurs.

Methods: Primary ASMCs, normal human bronchial epithelial cells (NHBE), or the cell line BEAS-2B were utilized. Epithelial cells cultured on Transwell® permeable supports were placed in culture with ASMCs for 24 hours before calcium responses to 1μM histamine were measured by fura-2 imaging. Calcium release was used as an indicator of force. Proliferation was measured by examining the incorporation of bromodeoxyuridine (BrdU) into the ASMCs. Protein and RNA was collected for analysis by Western blot and qPCR. Micro-RNA micro-array was performed by Exiqon (Copenhagen, Denmark). Small molecule inhibitors and siRNA were used to target COX-2.

Results: ASMCs co-cultured with epithelial cells demonstrated a reduction in calcium release after stimulation with 1μM histamine. Co-cultured cells had reduced mRNA of the contractile co-transcription factor myocardin and the contractile apparatus mRNA for calponin, which was confirmed by western blotting. We detected an increase in COX-2 mRNA suggesting a possible mechanism of PGE2 related reduction in calcium release. Inhibition of COX-2 in ASMCs using indomethacin (3μM) restored intracellular calcium release levels to control values after incubation with conditioned medium of epithelial cells. ASMC micro-RNA miR-210 was induced by co-culture, and an increase in the pro-proliferative co-transcription factor Elk-1 was evident by qPCR. A reduction in the miR-210 target, Max-binding protein (MNT), a transcriptional repressor, was observed at the mRNA level after co-culture.

Conclusions: Airway epithelial cells cause a reduction in agonist-induced ASMC calcium signaling in vitro that is not transcriptionally regulated. Further examination of COX-2 products may provide explanations for this observed phenotype.
Introduction: Traditionally, large and companion animal models have been used to study joint replacement components in vivo. However, these studies are costly, requiring that animals be housed in special facilities, which are not available at all institutions. A small animal model, such as the rat, would be ideal in the early stages of research.

Objective: To create a novel rat hip replacement system based on micro-CT derived anatomical measurements that will allow for in vivo testing of functional implant properties in a traditional basic sciences laboratory setting.

Methods: A database of n=25 previously-acquired micro-CT volumes of male Sprague-Dawley rats (390-610g) were analyzed to obtain spatial and angular relationships of several anatomical features of the proximal rat femora. Mean measurements were used to create a novel rat-hip implant prototype in computer-aided design software with four different sizes: 0.85*mean (small), 0.9*mean (medium), mean (large) and 1.1*mean (extra-large). Implants were then 3D printed in 316L stainless steel. Next, the prototypes were cleaned and polished using a custom-made jig to smooth the articulating surface. Finished components were sterilized and used as part of a toolkit for a pilot study of hip replacement in n=2 live rats (900g and 500g). Micro-CT imaging and X-ray fluoroscopy were performed post-operatively at 1 day, 3 weeks and 12 weeks to evaluate the position of each component within the bone and joint space, and to observe rodent gait.

Results: Installation of components was successful and both animals were recovered from surgery, thus we report the first successful hip hemi-arthroplasty in a rat model. Micro-CT revealed at day 1, each implant was situated within the medullary of the femur with no evidence of fracture or luxation from the acetabulum. Fluoroscopy at day 1 showed both animals ambulating on their affected limbs. The same observations were made at 3 and 12 weeks for rat 1. However, rat 2 showed evidence of implant migration at 3 weeks, leading to implant migration and disarticulation with the acetabulum. A collar will be added to new prototypes to prevent migration of the implant in future studies.
**Role of Thioredoxin Reductase in Regulation of Apoptosis and Autophagy in SH-SY5Y Cells**

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**Introduction:** Oxidative stress is characterised by an elevated level of reactive oxygen species (ROS) that can directly oxidize proteins, unsaturated fatty acids and DNA thereby inducing apoptosis during various stressful conditions like ischemia/reperfusion and can also trigger autophagy. Autophagy is a lysosomal degradation pathway aimed at recycling damaged cellular organelles in order to fulfill the energy demand during diverse stress and physiological conditions. Prolonged and severe oxidative stress provokes apoptosis. Thiol containing antioxidant proteins like glutathione and thioredoxin have an inhibitory control over ROS. The role of Thioredoxin reductase (TR1), an enzyme that keeps thioredoxin in its active form, in cell growth and development has been largely investigated; however its involvement in the interplay between apoptosis and autophagy is yet to be uncovered. We hypothesize that TR1 plays a central role in regulation of autophagy and apoptosis.

**Methods:** SH-SY5Y cells were treated with Auranofin, a specific inhibitor of TR1, for either 6 or 24 Hr. Cell viability was measured using WST-1 assay and western blotting was used to determine the expression of apoptotic and autophagic protein markers.

**Results:** Pharmacological inhibition of TR1 by Auranofin caused a dose dependent cell death in SH-SY5Y neuronal cell lines, which was blocked by the autophagy inhibitor 3-Methyladenine but not Rapamycin, an autophagy inducer. Western blotting revealed that TR1 inhibition induces classical type II cell death evidenced by accumulation of LC3-II and other autophagy related proteins and simultaneous activation of caspases (3 and 9) and PARP-1. In addition, we also observed that inhibition TR1 induces expression of ATF6 and IRE1 demonstrating involvement of endoplasmic reticulum stress and unfolded protein response in this cell death cascade.

**Conclusion & future directions:** These results suggest that TR1 is required for promotion of cell survival during autophagy, and its inhibition will promote apoptosis. Using gain/loss of function studies, we are currently exploring the molecular mechanisms by which TR1 regulates the cell death/survival machinery.
Clinical Effectiveness and Timing of Influenza Immunization in Cancer Patients Receiving Chemotherapy

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Background: Patient with cancers that are either being treated with chemotherapy or are immunocompromised due to the nature of their malignancy are at increased of developing influenza complications. While the Influenza vaccination has been shown to be safe in immunocompromised patients there are currently no guidelines and inconsistent recommendations on when to give the vaccine to immunocompromised patients with cancer. The review sought to assess the proper time course in which it should be given.

Methods: A literature search for studies that looked at humoral seroconversion after vaccination and clinical effectiveness of the influenza vaccination in immunocompromised cancer patients. The review included patients with solid tumors, hematological malignancies, and patients that were post hematopoietic stem cell transplant.

Results: Patients vaccinated early in their chemotherapy cycle (Day 4-5) versus late in their chemotherapy cycle (Day 16) had a greater serological response to the influenza vaccination. Conclusion: Overall antibody response to the influenza vaccination is adequate in chemotherapy patients and has been shown to be not only safe and effective, but should be generally be given in the early in relation to the chemotherapy cycle. While there is limited data that supports this and more data must be collected, the clinician should consider the proven benefits in influenza immunization when seeing patients being treated with chemotherapy.
Leukocyte activity is controlled by balancing activating and inhibitory signals in order to generate effective immune responses while avoiding tissue damage and autoimmunity. Here we examine an important regulatory circuit in B cells that involves the lipid phosphatase SHIP, a regulator of the PI3K signaling pathway. The classic view holds that SHIP is recruited to the inhibitory receptor FcγRIIB when it is cross-linked to the B cell receptor (BCR), and it subsequently mediates the dampening of BCR-derived signals in this context. Indeed disruption of both FcγRIIB and SHIP is associated with autoimmunity. However, SHIP also regulates signaling in the absence of FcγRIIB engagement. It is not currently understood how SHIP localizes to the plasma membrane in the case of BCR ligation alone or how this differs mechanistically and functionally from the classic case of co-ligation. Using confocal microscopy, we compare the localization dynamics of wild type and mutated EGFP-tagged SHIP after BCR ligation alone versus co-ligation with FcγRIIB. We demonstrate that SHIP is significantly recruited to the plasma membrane under both stimulation conditions. The SH2 domain and C-terminal regions of SHIP are both required, as is the kinase activity of Syk. Further, fluorescence recovery after photobleaching experiments reveal a distinction in the mobility of SHIP-EGFP at the cell periphery depending on the stimulation. Taken together, our results suggest that SHIP activity is controlled not simply by the magnitude of membrane recruitment as previously hypothesized, but rather by the mode of recruitment and, likely, by the signaling complexes that are formed around this enzyme.
Evaluation of Label-Free Mass Spectrometry Approaches to Characterize Factors in Cervicovaginal Mucosa Important for Host Immunity

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Introduction: The female genital tract mucosa is the first site of contact during heterosexual exposure to HIV. Recent evidence indicates that HIV acquisition is associated with mucosal inflammation but the mechanism of this is not well understood. Advances in mass spectroscopy have allowed for unbiased evaluation of immunological systems, capable of detecting many hundreds to thousands of proteins per sample. Here we evaluated and optimized mass spectrometry-based methods to characterize immune factors found at mucosal surfaces in female genital tract samples.

Methods: Cervicovaginal lavage (CVL) samples from 45 women were trypsin digested and analyzed using label-free approach with a Q-Exactive orbitrap mass spectrometer. Data was imported into Mascot and Scaffold software for protein identifications, and quantification was performed using Progenesis. Biofunctional analysis of protein lists was determined using a combination of DAVID (Database for Annotation, Visualization and Integrated Discovery) and Ingenuity Pathway Analysis software.

Results: A total of 1030 proteins were found with high confidence of 2 or more unique peptides, 801 which had a covariance <25% among reference samples. The biofunctions associated with this list of 801 proteins included acute inflammation (p=3.1E-20), epidermis/ectoderm development (p=1.2E-14), and regulation of metabolic protein process (p=1.2E-12). Top canonical pathways included acute phase response signaling (p=2.15E-30), LXR/RXR activation (p=5.86E-25), and remodeling of epithelial adherens junctions (p=5.89E-16). Proteins we have previously been unable to detect in CVL, associated with immune cells, such as BAP31, a B cell receptor and NCF2, a neutrophil intracellular protein.

Conclusion: This is the highest number of protein factors identified in cervicovaginal mucosal samples. Many factors not previously seen in CVL, including cell surface markers and intracellular factors, associated with specific immune cells were detected. Epithelial barrier function and immune responses in the female genital tract are important considerations for HIV susceptibility. This preliminary data shows that many immunological processes are captured by label-free mass spectrometry, and will be further utilized to understand mechanisms of inflammation associated with HIV acquisition.
Cartilage Specific Deletion of Mitogen Inducible Gene 6 in Mice Increases Articular Cartilage Thickness in Late Adulthood

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Introduction: Osteoarthritis (OA) is a degenerative joint disease which is characterized by the loss of articular cartilage. Mitogen-inducible gene 6 (Mig-6) negatively regulates epidermal growth factor (EGFR) signalling which is important in cartilage homeostasis (Appleton et al, 2010). Loss of Mig-6 in mouse cartilage results in increased articular cartilage thickness and formation of chondro-osseous nodules in the knee (Pest et al, 2014). However, it is unclear if these anabolic effects persist with age, or if they can be induced post-natally.

Purpose: To determine 1) the persistence of anabolic effects of Mig-6 loss in the cartilage of aged mice and 2) the effects of deleting Mig-6 from post-natal mouse cartilage in vivo.

Methods: Selective knockout of Mig-6 in cartilage was achieved through the use of the Cre-lox system, by breeding “floxed” Mig-6 (Mig-6fl/fl) mice to either animals with Col2-Cre or tamoxifen inducible Col2-CreER. Col2-Cre mice were aged 21 months to examine joints late in life. Cre-mediated recombination in Col2-CreER mice was induced by 5 day tamoxifen injection in 3 week old mice with vehicle controls. Col2-CreER mice were assessed at 12 weeks of age. MicroCT was used to examine joints for ectopic calcified nodule formation. Histological stains and immunohistochemistry was used to evaluate cartilage anabolism and molecular changes.

Results: Anabolic increase in the cartilage thickness of various joints was observed at the age of 21 months in Mig-6 knockout mice (KO, Mig-6fl/fl;Col2-Cre+/−) when compared to Control mice (Mig-6fl/fl;Col2-Cre−/− or Mig-6fl/+; Col2-Cre−/−). Ectopic chondro-osseous nodules were identified only in the knee joints and spines of KO mice by microCT evaluation and histology. Post-natal deletion of Mig-6 did not induce increased cartilage thickness, chondrocyte proliferation or ectopic nodule formation. PCR and immunohistochemistry confirmed that recombination in a sub-population of chondrocytes did occur.

Conclusions: Under these conditions, post-natal deletion of Mig-6 did not induce anabolic effects in cartilage. However, the anabolic effects of Mig-6 loss in mouse cartilage can persist late into adulthood, though the exact mechanism is still unclear. Mig-6 and the EGFR signalling pathway may be promising targets for therapeutic interventions which induce cartilage anabolism and repair in OA diseased tissue.
The cytokine, interleukin (IL)-27, activates immune cells during adaptive immune responses; however, the precise mechanisms and cellular processes targeted by IL-27 in innate immunity are not well understood. During bacterial infection, TLR4 triggering by lipopolysaccharide (LPS) induces IL-27 production by monocytes and macrophages. We have shown that IL-27 can prime monocytes for LPS responsiveness by enhancing surface TLR4 expression and intracellular signaling, providing a feedback loop whereby LPS induces IL-27 release, and IL-27 enhances LPS responses. This feedback loop could result in damaging inflammatory responses but may also provide enhanced control of inflammatory processes induced by bacterial pathogens. Thus, it is critical that the interaction between IL-27 and LPS is delineated. A key element in the LPS response of human monocytes is activation of the inflammasome, thus, we investigated the extent to which IL-27 modulates LPS-induced inflammasome activation in monocytes. The inflammasome is a multimeric protein complex that is induced by two signals: lipopolysaccharide (LPS) and adenosine triphosphate (ATP). Together they function to cleave pro-IL-1β into its active form, the potent, pyrogenic cytokine IL-1β. We show that priming of primary human monocytes with LPS and IL-27 followed by ATP stimulation significantly upregulates inflammasome activation and IL-1β secretion compared to that of LPS and ATP alone. Furthermore, we demonstrate that IL-1β production is dependent upon activation of the inflammasome and requires engagement of the ATP purinergic receptor, P2X7R. Hence, IL-27 is capable of increasing the proinflammatory capacity of human monocytes via enhancing inflammasome activity.
FASD Model: Biochemically Mimicking Alcohol Exposure in C57bl/6 mice using Gsc Promoter Driven Cyp26A1 cDNA.

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Prenatal alcohol exposure resulting in Fetal Alcohol Spectrum Disorder (FASD) is the most common cause of neurodevelopmental impairments in the western world, with an estimated prevalence of 1-2% in Canada. FASD, is a spectrum of disorders, including Fetal Alcohol Syndrome (FAS), partial FAS, and alcohol related neurodevelopmental disorder (ARND). It is well established in rodent and *Xenopus* models of FASD that even a single exposure to alcohol during the earliest stage of gastrulation is sufficient to induce the developmental defects associated with FAS. It is our hypothesis that acute ethanol exposure overwhelms the aldehyde metabolic enzymes that would also normally convert retinol (Vitamin A) to retinoic acid (RA); and moreover, it is the reduction of RA levels during gastrulation that drive the cranio-facial defects associated with FAS. To test our hypothesis, a murine model was established to biochemically mimic the alcohol effect *in vivo* using a genetically engineered mouse expressing Cyp26A1-eGFP from the endogenous *Goosecoid* (Gsc) promoter. The Gsc promoter is utilized to dictate spatio-temporal expression of the Cyp26A1-eGFP cassette during development *in vivo*. Cyp26A1, the normal down regulator of endogenous RA, mimics the reduced RA levels induced by acute alcohol exposure at early gastrulation. Gsc:Cyp26A1-eGFP allele mice were derived by germline transmission and F1 mice were born with a normal Mendelian ratio of het:wt (0.95, n=127). Newborn F1 mice were phenotypically assessed (blinded) for cranial-facial defects characteristic of FASD features that include: a less well defined philtrum and flatter upper lip, compared to normal pups. Pups were later genotyped at weaning and analysis shows 88% (15/17) of mutant mice assessed had a discernable FAS phenotype. 100% of wild-type mice had been assessed as normal (14/14).
Regulation of Airway Hyper-responsiveness and Inflammation by an Innate Defence Regulator Peptide

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Asthma is a chronic inflammatory disease affecting more than 3 million people in Canada with an annual economic burden of more than $1 billion. Airway inflammation is reported with a decreased expression of small molecules known as host defence peptides (HDPs) that are known to control both inflammation and infections. Some HDP sequences have been used as templates to design short synthetic peptides known as innate defence regulator (IDR) peptides. Previous studies have shown that IDR peptides can control both infections and inflammation in infectious diseases. In this study, examined the effects of exogenous administration of an IDR peptide in a murine model of house dust mite (HDM)-challenged allergic asthma. The peptide by itself did not induce any pro-inflammatory cytokines e.g. TNFα, IL-4, or IL-13 either in the bronchoalveolar lavage fluid (BALF), serum or in the lung tissue. Lung mechanics measured by a flexiVent™ small animal ventilator showed that administration of the peptide suppressed airway hyperresponsiveness (AHR) in the HDM-challenged mice. Further studies demonstrated that the peptide significantly reduced the production of the major basic protein (MBP) in the lung tissues of HDM-challenged mice. As MBP activity results in increased lung contraction and AHR, our results suggest that the IDR peptide improves AHR by decreasing MBP production. The peptide also significantly suppressed the production of IL-33 and neutrophil infiltration in the lungs. As IL-33 and neutrophils in the lungs are steroid-resistance mediators, our results suggest IDR peptides may counter steroid-resistance.
The Expression of Immune Regulatory Genes was Elevated in Women who Exhibit Natural Resistance to HIV, but the Responsiveness of These Genes to Exogenous IFN-g was Reduced in These Women

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Introduction: Some women in the Majengo Commercial Sex Worker Cohort in Nairobi, Kenya are highly exposed to HIV, but remain seronegative (HESN). We have identified several key regulatory genes in the immune regulation pathway that are highly expressed in HESN peripheral blood mononuclear cells (PBMC) using RNA-sequencing. Our recent transcriptomic data led us to hypothesize that increased expression of immune regulatory genes in HESN would contribute to the immune quiescence observed in HESN. This study validated the quantitative expression of these immune regulatory genes in HESN and in control PBMC using absolute quantitative PCR (qPCR).

Methods: Ex-vivo PBMC samples from HESN women (n=17) (active in sex-work, with known exposure to HIV+ clients, but remain HIV-negative for >7 years at the time of sampling), and non-HESN HIV- women (n=16) (active in sex work and HIV-negative, but did not meet the epidemiological definition of HESN) enrolled in the Majengo cohort were collected for RNA isolation. The baseline RNA levels of several immune regulatory genes in ex-vivo PBMC were quantitated using qPCR. To assess the responsive potential of these genes, ex-vivo PBMC were stimulated with exogenous IFN-γ, since these genes have been shown to be responsive to IFN during antiviral response.

Results: The expression of CCR4, CCR7, CD28, ICOS, IL4R, ITK, and MAP3K1 genes in PBMC were significantly higher in HESN women, ranging from 1.4 to 3.0 fold higher expression. Conversely, the level of IL6ST mRNA was lower in HESN by 3.0 fold. The expression of these genes after IFN-γ stimulation was similar in both HESN, and non-HESN HIV- control groups. However, the responsiveness of ICOS, IL32, IL6ST, IRS2, and MAP3K1 genes to IFN-γ was significantly lower in HESN women by ~50%, in comparison to the control group.

Conclusion: PBMC in HESN women had increased baseline expression of immune signalling genes compared to non-HESN controls, suggesting the potential to rapidly induce immune signalling, and, a readiness to combat infection. Further, the reduced responsiveness to IFN-γ observed in HESN cells may suggest a strict regulatory environment of these genes to prevent undesirable excess immune-activation that may allow HIV replication.
Diabetic ketoacidosis is a life threatening complication of diabetes, with profound implications for patients and the healthcare system. Effective treatment of DKA depends on early diagnosis and initiation, followed by timely and accurate monitoring of the disease process. Although it is a common pathology, it is still a relatively scarce presentation in the emergency department making it difficult for physicians, nursing staff, residents, and students to stay current with best practice guidelines. A considerable amount of active research has caused new understanding of pathology and allowed treatment strategies to change consistently in recent years. Constant influx of new evidence has caused several changes recommended practice, making it difficult for healthcare staff to keep up. Herein we review epidemiology, pathology, and currently recommended diagnostic and treatment practices. Recommendations presented here under the guidance of the Canadian Diabetic Association's Clinical Practice Guidelines, and were used to construct an evidence based best practice protocol. This protocol may be used by healthcare staff in the emergency room and inpatient wards. The use of protocols in the treatment of DKA have been shown to reduce hospital stay while improving patient safety. It is our hope that the protocol presented here will be adopted by Seven Oaks General Hospital and implemented into their electronic order sets. Future studies on the effects of this protocol's implementation may prove valuable in optimizing DKA management in Manitoba.
Expression of Ebola Virus NP, VP24, and VP35 Generates Fragile Nucleocapsid-like Structures

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Filoviruses were named after their thread-like appearance in the transmission electron microscope. As filamentous viruses, the protein coat that protects their single-stranded, negative-sense RNA genomes must be flexible, but strong. As a result, Ebola and Marburg viruses construct relatively complex helical nucleocapsid structures. Five of the 8 viral proteins encoded by these viruses are components of the helical nucleocapsid; however, only the nucleoprotein (NP), VP24, and VP35 are required to assemble nucleocapsid-like structures in transfected cells. The protein-protein interactions and stoichiometry of these 3 proteins, as well as a fourth nucleocapsid protein VP30 and the viral matrix protein VP40 within Ebola and Marburg virus nucleocapsids are not well understood.

Heterologous expression of Ebola virus NP, VP24, VP30, VP35, and VP40 produced nucleocapsid-like structures as previously reported. Intriguingly, thin-section electron microscopy and cryo-electron tomography demonstrated tightly coiled nucleocapsid-like structures within cells; however, upon purification these structures appear to unwind. These structures are the correct diameter of the Ebola virus nucleocapsid, but have a helical pitch twice that of the native nucleocapsid. Various methods of purification have been attempted to protect the integrity of these nucleocapsid-like structures. Detergent and hypotonic lysis buffers, as well as freeze-thaw methods have not succeed. Our results suggest that Ebola virus nucleocapsids are more fragile than originally thought and may require additional viral factors, such as secondary structures within the RNA genome, to support assembly.

Ebola virus infection can cause severe haemorrhagic fever, with a case-fatality rate ranging from 50-90%. The current Ebola virus epidemic in Western Africa is suspected to have infected close to 25 000 people and has resulted in the death of at least 10 000. Although vaccines are currently undergoing clinical trials, there is still no treatment for individuals infected with Ebola. The rational design of anti-viral drugs that can target essential aspects of the Ebola virus lifecycle, such as the assembly of its nucleocapsid, requires greater understanding of this virus.
The Dual Nature of a Cellular Protein DREF in Adenovirus Life Cycle: A Facilitator and Restrictor of Viral Growth

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Introduction: The adenovirus E1A gene is the first gene expressed upon viral infection. E1A has two major functions in the infected cell. Firstly, E1A remodels the intracellular environment to allow for viral replication. Secondly, E1A is the major transactivator of viral early gene expression and a co-regulator of a number of cellular genes. E1A carries out its functions predominantly by binding to cellular regulatory proteins and altering their activities. The unstructured nature of E1A enables it to bind to a variety of proteins and form new molecular complexes with novel functions. The C-terminus of E1A is the least-characterized region of the protein, with few known binding partners. Here we report the identification of cellular factor DREF (ZBED1) as a novel and direct binding partner of E1A.

Methods: To assess the contribution of DREF to the viral life cycle we examined virus growth in HT1080 cells after siRNA treatment of DREF. We also assessed viral early and late gene expression in infected HT1080 cells after DREF knock-down. To determine DREF localization during infection we used immunofluorescence in infected HT1080 cells. We examined sub-cellular distribution of DREF during infection together with PML and the viral DNA binding protein, which labels viral replication centers. Furthermore, we analyzed DREF co-localization with E1A in HT1080 cells. Lastly, DREF SUMOylation was analyzed by immunoprecipitation in HT1080 cells infected with wild-type hAdV or control virus expressing no E1A. DREF was immunoprecipitated and SUMOylation was detected using anti-SUMO antibody.

Results: Our studies identify a dual role for DREF in the viral life cycle. DREF contributes to the activation of viral gene expression from all viral promoters early in infection. Unexpectedly, it also functions as a growth restriction factor for adenovirus as knockdown of DREF enhances virus growth and increases viral genome copy number late in the infection. We also identify DREF as a component of viral replication centers. E1A was found to affect the subcellular distribution of DREF within PML bodies and enhances DREF SUMOylation.

Conclusion: Our findings identify DREF as a direct E1A C-terminus binding protein and provide evidence supporting a role for DREF in viral replication.
The Role of Novel Subset of Mesencephalic Derived Neurons in Cerebellar Nuclei Development

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Introduction: The cerebellum functions in motor coordination and also implicated in non-motor behaviors including emotion and cognition. Purkinje cells (Pcs) are the sole output of the cerebellar cortex and they project to the cerebellar nuclei (CN). The CN provide the main output of the cerebellum. During cerebellar development the CN neurons and Pcs are the earliest born among the different neuronal subtypes. However, they are generated from two distinct germinal zones: the ventrally located ventricular zone, which produces Pcs and the dorsally located rhombic lip, which produces large CN neurons. We found a new subset of the neurons derived from mesencephalon and seems play an important role in cerebellar development.

Methods: This study utilized whole mount/section immunohistochemistry, western blotting and primary dissociated cerebellar and embryonic cultures to examine the origin and role of a new subset of CN neurons.

Results: It is believed that the isthmus organizer is a signaling center and district mesencephalon from romboencephalon. Our results showed that a subset of CN neurons, which are immunopositive for α-Synuclein (SNCA) and Otx2 (a mesencephalic derived cell marker), originate from the mesencephalon and migrate to the rostral end of nuclear transitory zone. SNCA and p75 neurotrophin receptor double immunostaining suggests that these cells are derived from neural crest and form a combination of neurons and nerve fibers that terminate to the subpial surface of putative lobules VI/VII. Interestingly, the SNCA+/Otx2+/p75+ cells which divide the cerebellar primordium into rostrodorsal and caudoventral compartments, undergo programmed cell death due to activation of caspase signaling pathway.

Conclusion: The temporary present of mesencephalic derived early CN neurons in the nuclear transitory zone suggest a regulatory role as a “transient signaling center” that may play as an intrinsic organizer during early cerebellar development.
Stearoyl-CoA Desaturase 1 has a Critical Role in the Regulation of Adipocyte Lipid Handling and Metabolism

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Introduction: With obesity, adipocytes accumulate large amounts of triacylglycerol (TAG) as a means of storing excess energy. The accumulation of TAG occurs concomitantly with increased expression and activity of stearoyl-CoA desaturase 1 (SCD1). SCD1 is rate-limiting for the conversion of dietary and endogenous saturated fatty acids (SFAs) into monounsaturated fatty acids (MUFA), specifically, palmitate (PA) to palmitoleate (PMA) and stearate (SA) to oleate (OA). Changes in SCD1 activity therefore alter SFA/MUFA ratios, which can affect adipocyte function and signalling pathways. Ultimately, these changes can have significant ramifications on the development of obesity-related complications. Although reduced whole-body SCD1 activity has been associated with improved insulin sensitivity and decreased body weight, the effects of altered SCD1 activity in adipocytes remain poorly characterized. We therefore examined how SCD1 inhibition influences adipocyte metabolism in order to further our knowledge of this key enzyme and its influence on human health.

Methods: Murine 3T3-L1 adipocytes were treated with a SCD1-specific inhibitor (10nM). SCD1-inhibited adipocytes were also treated with either ethanol (i.e., the control condition), or 250μM PA, SA, PMA or OA for 48 hrs. FA composition, gene expression, protein content and cytokine secretion were examined by gas-chromatography (GC), qRT-PCR, Western blotting and multiplex immunoassays, respectively.

Results: SCD1 inhibition reduced total TAG and phospholipid (PL) content in parallel with the down-regulation of genes involved in TAG and PL biosynthesis. These changes suggest possible implications regarding the adipocyte's ability to store excess energy. Treating SCD1-inhibited adipocytes with PA revealed an adaptive response in adipocytes. Specifically, elongase 6 activity was up-regulated, converting PA to SA. SCD1 inhibition also increased inflammatory cytokine secretion (e.g., IL6 and CCL5), and the subsequent treatment with SFAs augmented IL6 and MCP1 secretion beyond the levels observed with SCD1 inhibition alone. Although inflammatory cytokine secretion was increased, common markers of cellular stress (i.e., JNK, ERK1/2, STAT3, p38) appeared to be unaltered.

Conclusion: Reduced SCD1 activity significantly impacts lipid handling in adipocytes, as well as alters gene expression and cytokine secretory profiles.
Investigation of the Role of HIV Envelope Protein in HIV-1 Transcription and Underlying Mechanism

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Introduction: HIV-1 latency is a main obstacle in HIV-1 eradication. However, these latent reservoirs can be reversed by reactivating viral transcription and then be killed by viral cytopathic effect or host immune responses. Therefore, understanding the underlying mechanisms of the HIV-1 latent reservoirs' reactivation is essential for HIV curing. HIV-1 envelope protein mediates the HIV fusion with cell membrane and induces a series of downstream signaling pathway by binding to CD4/CCR5/CXCR4 receptors, which is involved in cell adhesion, actin cytoskeleton modulation and cellular transcription. The goal of this study is to investigate the role of envelope protein in HIV transcription in HIV infected cells.

Results and conclusions: To demonstrate whether HIV envelope can increase the HIV-1 LTR derived viral transcription, X4 and R5 HIV-1 Envelope proteins were used to treat TZMb-1 cell, which is integrated with the luciferase gene under control of the HIV-1 promoter. Results showed that HIV-1 Envelope protein (Env) can significantly increase the luciferase expression and this effect was CD4, CXCR4 or CCR5 specific. By using real-time PCR technique, we further demonstrated that Env enhanced the luciferase gene transcription. The same effect of Envelope was observed in HIV-1 latent infected cell line J-Lat 6.3 that contains GFP ORF instead of Nef of HIV-1. Significantly, after 24 and 48 hours treatment, the levels of transcription of HIV-1 Gag and GFP genes increased to 3-10 folds, suggesting that HIV Env induces HIV-1 expression especially in latent infected cells at the transcription levels. To understand the underlying mechanism, two protein inhibitors CAN508 and INCA-6 that specifically target NFAT and CDK9/Cyclin T pathways were added before Env treatment. Results showed that the HIV transcription induced by Env was partially inhibited by the two inhibitors. Moreover, western blot showed that the dephosphorylated NFAT protein increased upon the treatment of Env, providing a link between HIV Env and host transcription factors. Overall, this study provides evidences to support that HIV-1 envelope can activate viral transcription in HIV-infected latent cells. We believe that this study can provide us a new insight into the mechanism of reactivation of HIV latency.
Introduction: The adoption of electronic medical records (EMR) is a priority for the Canadian health care system. Improved provider effectiveness and health outcomes require better adoption of advanced EMR features by primary care physicians (PCP). However, the majority of PCPs do not fully adopt these advanced features. The literature widely suggests that end-user support (EUS) is a critical success factor for increasing EMR adoption (i.e., overall use of all EMR features). EUS is any information or activity (e.g., training) that is intended to help physicians solve problems with and better utilize their EMR. Existing research in EUS is limited and is inconsistently described and measured, which impedes efforts to improve EUS quality and EMR adoption. Therefore, a valid and reliable tool to assess EUS is needed. Additional research on the relationship between EUS and EMR adoption is also required.

Research Objectives:
1. To identify external barriers and facilitators to the uptake of EUS among PCPs.
2. To develop a valid and reliable instrument to measure EUS for EMRs in primary care.
3. To measure EUS and EMR adoption for PCPs in British Columbia.
4. To explore the quantitative relationship between EUS and EMR adoption.

Methodology: A sequential (exploratory) mixed methods research design will be used. Objective 1. Individual interviews with a purposive sample of PCPs (n=15-20) will be conducted. A descriptive content analysis will be used. Objective 2. Instrument items will be generated through a systematic review of the EUS literature. The instrument will be refined using a three round Delphi technique, with an expert panel of PCPs (n=15-20). The instrument will be psychometrically tested (internal consistency, test-retest, construct validity, and criterion validity) with a separate sample (n=5-7 PCPs per instrument item). Objective 3. Data will be collected using the EUS instrument (Objective 2) and the EMR Adoption Survey with a random sample of PCPs (n=100 based on CI=10, 95% Confidence Level) representing all health regions in British Columbia. Objective 4. Pearson’s correlation will be used.
PPARdelta Promotes the Progression of Post-Traumatic Osteoarthritis
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Introduction: Osteoarthritis (OA) is a chronic degenerative joint disorder primarily characterized by articular cartilage breakdown. Current findings from our laboratory indicate that activation of the nuclear receptor PPARdelta induces expression of enzymes involved in cartilage breakdown, prompting us to speculate whether inhibition of PPARdelta could be a viable treatment strategy.

Hypothesis: We hypothesize that inhibition of PPARdelta will slow the progression of OA in animal models.

Methods: In order to examine the role of PPARdelta in-vivo, cartilage-specific knockout mice and wild-type littermate controls were subjected to destabilization of medial meniscus surgery (DMM) at 20 weeks of age. 8 weeks post-surgery mice were compared through classical histological and biochemical measures of OA progression including Safranin-O staining with OARSI scoring, immunohistochemistry for cartilage matrix breakdown products, and picrosirius red staining for collagen fiber structure and orientation. To investigate what enzymes were acting to breakdown cartilage matrix, dye binding assays were conducted concurrently with Western Blots for breakdown products of aggrecan on cartilage explants treated with PPARdelta agonist GW501516. Microarrays were conducted on immature murine articular chondrocytes treated with GW501516 to elucidate information on targets of this nuclear receptor.

Results: PPARdelta agonism (by GW501516) results in significantly increased proteoglycan breakdown in an explant culture system. Dye-binding assays of medium and guanidine extracts demonstrate significantly increased quantities of aggrecan breakdown products released from treated explants. Microarray analyses identified targets of PPARdelta, such as those involved in lipid oxidation and transport, and metabolism. OARSI histopathological scoring and immunohistochemistry demonstrated strong protection of PPARdelta KO mice from cartilage matrix breakdown after surgical induction of OA.

Conclusion: This study provides strong evidence for catabolic roles of endogenous PPARdelta in post-traumatic OA and suggests that pharmacological inhibition of PPARdelta is a promising therapeutic strategy. Future studies will examine the potential of pharmacological treatment in a post-traumatic model of OA, as well as provide mechanistic insights into the action of this nuclear receptor in response to injury.
Do Non-Nutritive Sweeteners have Adverse Effects on Metabolic Health in Infants and Children? A Systematic Review Randomized Controlled Trials and Prospective Cohort Studies


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Background: Non-nutritive sweeteners (NNS) have recently gained enormous popularity due to their perceived health benefits in weight loss and management, however their long-term impact on human health is unknown, particularly when exposure occurs during early development. We conducted a systematic review of prospective cohort studies and randomized controlled trials (RCTs) to evaluate the association between NNS exposure in the prenatal period, infancy and childhood (age < 12 years) and metabolic health outcomes.

Methods: A comprehensive peer-reviewed search strategy was used to search the Medline (OVID) database from inception to present. Using Distiller SR software, citations were screened in duplicate to identify RCTs and prospective cohort studies evaluating metabolic outcomes after NNS exposure during gestation or childhood. The primary outcomes were change in weight-for-length (WFL) z-score in infants and change in body mass index (BMI) z-score in children; secondary outcomes included growth velocity, birth weight, incidence of overweight/obesity, change in central and total adiposity, and incidence of adverse metabolic effects. Quality was assessed using the Cochrane Collaboration Risk of Bias (RoB) tool in RCTs and the Newcastle Ottawa Scale in prospective cohort studies.

Results: From 4591 citations, 12 studies met our inclusion criteria; 3 RCTs and 9 prospective cohort studies. Studies were heterogeneous in the type and duration of NNS exposure and outcomes reported. Three of the nine prospective cohort studies identified positive associations between NNS exposure and BMI z-score or BMI, yet five of the nine studies reported no association. Two RCTs that evaluated sucralose exposure identified positive associations in weight gain, fat accumulation, and WFL z-score in children. In two prenatal studies, NNS exposure was not associated with infant birth weight and no long-term metabolic outcomes were reported. No studies reported on incidence of metabolic syndrome, insulin resistance, or Type 2 Diabetes Mellitus.
Conclusions: There is limited and inconsistent evidence for the effects of NNS exposure in children, and no current studies have evaluated NNS exposure in gestation or infancy with subsequent metabolic effects. Further research and extended follow up is required to provide sufficient evidence of the effects of NNS exposure during the prenatal period, infancy, and childhood.
Examining Gaze Behaviours to Moving Faces in Adults with and without Autism Spectrum Disorders
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Background: There is an abundance of social information present in a moving face; therefore dynamic faces are more challenging to process continuously than photographs of faces, particularly in social situations where multiple people are present (Stoesz & Jakobson, 2014). We asked whether individuals with autism spectrum disorders (ASD) show atypical gaze behaviours when viewing social scenes compared to their peers. This might be expected given other evidence of difficulties with social perception in this population (Barton et al., 2007; Speer et al., 2007).

Methods: Sixteen adults with ASD and 16 sex-, age-, and IQ- matched typical controls participated in this study. We assessed participants’ direction of attention to faces when passively viewing photographs and movies of social scenes that varied in complexity. We studied 2 types of gaze behaviours (number of fixations and fixation length) within 3 areas of interest (AOIs: faces, bodies, and backgrounds) in 4 scene conditions (static-single-character, static-multiple-character, dynamic-single-character, and dynamic-multiple-character).

Results: Both groups spent more time fixating on the background and less time fixating on faces in multiple-character compared to single-character scenes. However, adults with ASD made shorter fixations on faces than controls and, when viewing multiple-character scenes, these fixations were of shorter duration than controls’. Although both groups made fewer fixations on faces in dynamic compared to static scenes, overall, the duration of face fixations was impacted by the introduction of motion cues in controls (with fixations on faces in static scenes being shorter than those made to faces in dynamic scenes) but not in those with ASD.

Conclusions: The fact that adults with ASD were not affected by the introduction of motion cues may be related to atypical motion processing in this population (Bertone et al., 2003). Adding characters to scenes influenced fixation patterns differently in the two groups, possibly due to the increased social complexity of the scenes. The observed group differences in gaze behaviour highlight the importance of studying dynamic face processing abilities in order to understand better how deficits in these abilities contribute to atypical social cognition.
Injuries are a serious public health concern and identifying risk factors for injury is a research priority. Previous research consistently supports the link between alcohol and risk of injury and between mental health and alcohol use. There is also some research to indicate an association between mental health and risk of injury. Given the nature of these independent relationships, examining how these variables are inter-related could have significant implications for injury prevention and informing public health policies. There is however, a dearth of research examining how mental health and alcohol interact and contribute to injury risk. The present study examines the independent and shared contributions of mental health and alcohol to injury. Furthermore, gender differences in these relationships are examined. The results indicate both alcohol use and mental health are significantly associated with increased risk of injury. Moreover, a synergistic effect between alcohol and mental health on injury is found among women. The implications for these results in practice and policy are discussed.
Investigating the Knowledge of Young Adults Related to Omega-3 Fats and the Impact of Providing Genetic Information on Dietary Choices

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Introduction: The consumption of omega-3 fats is encouraged due to their recognized health benefits; however, the average Canadian adult does not consume enough in their diet. The factors influencing omega-3 fat consumption are unclear. Nevertheless, we hypothesize that personalizing an individual’s diet according to their genotype may lead to an increase omega-3 intake. To accomplish this goal, we first need to assess the baseline knowledge of young adults regarding omega-3 fats and genetics. Then, we will study whether providing young adults with genetic information related to lipid metabolism will promote increased intake of omega-3 fats.

Methods: Study participants for both projects will be young adults recruited from the University of Guelph. An online Qualtrics survey was created to investigate the knowledge, awareness, attitudes and beliefs that young adults have about omega-3 fats, health, and genetics. Focus groups and cognitive interviews were completed to ensure that the questions and options were clearly interpreted and representative for this population. The second part of this project involves providing participants with genetic information related to lipid metabolism. Participants will complete online surveys to investigate whether knowledge of genetic information influences diet and behaviour changes throughout a 24 week period. Cardiometabolic markers of health will be also measured and participants will be genotyped using DNA from saliva samples.

Results: The results of this study will provide new insights into why young adults are not consuming omega-3 dietary fats, as well as the best ways to communicate nutrition and health information to this demographic. Our preliminary results show that individuals who learn about omega-3 fats from health care professionals and academic sources know significantly more about the positive health benefits compared to those who learn about omega-3 fats using other sources of information. Additionally, intake patterns will be studied over time to determine the impact of receiving personal genetic information.

Conclusion: This nutrigenomics research is amongst the first of its kind, with the hope that data generated by our research will lead to the tailoring of dietary advice in clinical settings.
HIV-Exposed Seronegative Men Who Have Sex with Men Express Antiproteases with Novel Antiviral Activity

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Introduction: HIV-Exposed Seronegative (HESN) individuals have shown altered mucosal immune responses in cervical, salivary and foreskin secretions associated with reduced HIV-susceptibility; however, this has not been investigated in rectal mucosa. This is the first comprehensive proteomic study defining innate mucosal immune differences in the rectal secretions of HESN MSM.

Methods: Rectal lavage from HESN MSM (n=23) and non-exposed, healthy controls (n=14) from the Venhälсан clinic, Sweden, were analysed by label-free mass spectrometry. Identification of proteins, differential expression analysis and pathway analysis were performed. Protein levels were correlated to variables relating to partner HIV exposure. One differentially abundant factor showed innate expression and was screened for anti-HIV activity in PBMC and colorectal-explant culture and in the presence of R5- and X4-tropic HIV lab strains (HIV-Bal and HIV-3B, respectively) using p24 ELISA.

Results: HESN MSM differentially expressed 31 proteins (p<0.05, adjusted for multiple hypothesis testing). Pathway analysis linked these factors to reduced LXR/RXR pro-inflammatory signaling (z=-0.4, p=3.5x10^-5). Functional enrichment analysis demonstrated increased immune activity and epidermal development in HESN men (p=0.01). Three overabundant factors were antiproteases. These proteins did not show a significant correlation (p>0.05) with clinical variables (frequency oral/anal sex, HIV-neutralizing IgA, and VL of HIV+ partner). One overabundant antiprotease (AP1) demonstrated antiviral activity in vitro. Neutralization assays showed that AP1 reduced Bal infection by a maximum of 61% and reduced IIIB infection by 90% (20 μg/ml) in PBMCs with negligible effect on cell viability (cell viability >60%, p<0.05); AP1 inhibited HIV infection in rectal explants at a maximum of 75% (25 μg/ml).

Conclusion: HESN MSM show a state of decreased immune activation at the rectal mucosa, and overexpress an antiprotease with previously undescribed antiviral activity, which may contribute to reduced susceptibility to HIV at the rectal mucosa. This is likely a result of innate differences rather than HIV-exposure. Our findings overlap with previous studies of showing an overabundance of antiviral factors in the cervical secretions of HESN women, supporting further study into their roles in HIV infection. This knowledge is critical for the design of safe, effective HIV-prevention technologies for MSM.
Amiodarone Monitoring in Patients with Implantable Cardioverter Defibrillators

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Introduction: Although amiodarone has been shown to be efficacious in suppressing implantable cardioverter defibrillator (ICD) shocks, its use is associated with many adverse side effects, including lung, liver and thyroid dysfunction. The Canadian Cardiovascular Society recommends routine clinical exam, careful history and measurement of thyroid stimulating enzyme (TSH) and liver enzymes every six months in patients on chronic amiodarone in order to elicit signs of early amiodarone toxicity. It is unclear how routinely this is monitoring is done in clinical practice.

Objectives: We investigated if a population of ICD patient prescribed amiodarone had thyroid and liver enzymes monitored per guideline recommendations.

Methods: We retrospectively analyzed all patients followed in an academic institution with an ICD in situ who are prescribed amiodarone in the previous 6 months. We determined if TSH and liver enzyme levels were monitored.

Results: Two hundred and eighteen patients were included. Mean age was 69 years (+/-10.5) 192 (88%) were male. 61% (132) of patients had a TSH checked in the last 6 months. Of these, 33 results were abnormal and 17 patients were on thyroid replacement therapy. 47% (103) of patients had liver enzymes checked in the previous six months, with 16 abnormal results.

Conclusion: Despite guideline recommendations, thyroid function and liver enzymes were checked in only 61% and 47% of this cohort of patients with ICDs who are prescribed amiodarone. Lack of compliance is likely multifactorial.
Phase Analysis of Gated Blood Pool SPECT for Multiple Stress Testing Assessments of Ventricular Mechanical Dyssynchrony in A Dilated Cardiomyopathy Canine Model

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**Introduction:** Ventricular Mechanical dyssynchrony seems to be present in almost all chronic heart failure (HF) patients, even patients with a narrow QRS complex. While this parameter could change during the stress condition, no comprehensive analysis has been made to see the range of difference in ventricular dyssynchrony between rest and levels of stress. Our objective was to investigate the range of difference in inter- and intraventricular dyssynchrony parameters between rest and levels of dobutamine stress in a non-ischemic dilated cardiomyopathy (DCM) canine model with normal QRS complex using gated-blood pool SPECT (GBPS).

**Methods:** Stress was induced by dobutamine infusion in 10 dogs with DCM. Hemodynamic and ventricular (dys)synchrony data were analyzed by left ventricular (LV) pressure measurements and GBPS. Count-based indices were extracted for assessing intra and interventricular mechanical (dys)synchrony. A comparison was performed between DCM and 8 healthy dogs.

**Results:** LV ejection fraction increased from 22.6±6.0% in baseline versus 48.1±5.8% in 20μg/kg/min; (p<0.0001). Ventricular performance (dP/dtmax) increased from 949.5±238 to 3020.8±568.9 at 20μg/kg/min; (p<0.0001). Contraction homogeneity index (CHI) showed a significant increase in synchronism from baseline to the stress levels of 5, 10, 20μg/kg/min dobutamine (p<0.05). Similar results were found for entropy and phase SD. While, no profound difference was found between baseline and levels of stress for interventricular delay, introduction of stress was coincided with improved synchrony in 90% of dogs with negative delay.

**Conclusions:** Intermediate levels of dobutamine significantly reduced the intraventricular dyssynchrony. There was a remarkable inter-animal variability in interventricular dyssynchrony pattern by different levels of dobutamine stress. However, introduction of stress significantly improved the synchrony of contraction in 90% of DCM subjects with negative delay. Further investigation is needed to assess whether lower mechanical dyssynchrony indices from stress GBPS in DCM patients with normal QRS have the ability to predict response of these patients to cardiac resynchronization therapy.
**Cost-Effectiveness of Prostate Cancer Management Strategies in Canada**

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**Introduction:** In developed countries prostate cancer (PCa) is the most common non-melanoma cancer in men. Over the years novel PCa management strategies have been adopted by health care systems to improve patient outcomes. However, adoption and diffusion of novel strategies have lead to growing economic burden. Contemporary estimate indicate PCa management of men 65 years and older cost $4.0 billion to the United States health care system. The current literature is sparse on lifetime costs and quality-adjusted-life years (QALYs) associated with management strategies across risk strata.

**Objective:** To assess direct health care costs and QALYs associated with PCa management strategies in Quebec, Canada from diagnosis to end-of-life.

**Methods:** A validated Markov Montel Carlo model was use to predict lifetime direct costs and QALYs from the perspective of the Quebec health care system. Health states modeled by risk at diagnosis were: active surveillance (AS), initial treatments (radical prostatectomy or radiation therapy), PCa recurrence, PCa recurrence free, metastatic castrate resistant prostate cancer (mCRPC) and death (cause specific/other causes). Treatment trajectories were based on state transition probabilities derived from the literature. Unit costs were amassed from Regie de l’Assurance Maladie du Quebec (RAMQ), Ministere de la Sante et des Services Sociaux (MSSS), Montreal General Hospital pharmacy list, and published literature.

**Results:** Total cost per patient for the overall cohort increased from $17034 at 5 year to $22072 and $26407 at 10 and 15 year, respectively. Further, results indicated influence of risk group on total cost with high risk accrued maximum cost followed by intermediate, and low. AS conferred most QALYs (13.1 years) and was the least costly strategy ($11267) for low risk. For intermediate and high risk, radical prostatectomy and radiation therapy with androgen deprivation conferred most QALYs and were least costly strategies; $20843, 11.6 years and $86560, 10.1 years, respectively.

**Conclusion:** Public healthcare system in Canada and elsewhere are operating under economic constrains to allocate finite health care resources to maximize health at population level. To improve efficiency of the health care delivery relative cost and QALY conferred by management strategies would be paramount for decision making and patient care.
Characterization of the Phage-Induction Time Course Required For Detection of Stx1 and Stx2 Toxins in Shiga Toxin-Producing Escherichia coli Cultures by Mass Spectrometry

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**Introduction:** Shiga toxin-producing \textit{E. coli} (STEC) can cause severe renal damage. The genes encoding AB5-class Stx holotoxins, \textit{stxA} and \textit{stxB}, are found on integrated lambdoid bacteriophages. Transition to lytic cycle phages, induced by DNA-damage activation of the \textit{SOS} response, increases Stx toxin production. Toxin detection \textit{in vitro} is challenging as Stx1 / Stx2 are undetectable without prior phage induction. Using mitomycin C (MMC), a DNA-damaging agent, our study sought the optimal induction conditions for detection of Stx1 and Stx2 applying mass spectrometry.

**Methods:** We applied shotgun proteomics (a bottom-up technique identifying bulk proteins in complex mixtures) to STEC isolates EC0004 (O157:H7) and EC0001 (O26:H11) using isobaric tag for relative and absolute quantitation (iTRAQ) two-dimensional liquid chromatography tandem mass spectrometry (2D-LC/MS/MS) on a LTQ Orbitrap Velos mass spectrometer. Bacterial cultures were induced with 1 µg/ml MMC for 1-hour, 2-hours or 3-hours. 2D-LC/MS/MS analysis was performed in triplicate on cell pellet and culture supernatant fractions to determine relative Stx1 and Stx2 levels at each time point relative to no MMC treatment.

**Results:** Roughly 2000 and 800 proteins were detected in the STEC cell pellets and culture supernatants, respectively. Stx1 was identified in cell pellets but not culture supernatants; whereas Stx2 was identified in both fractions. Stx1B subunit was detected in EC0004 pellets starting at 1-hour post-MMC induction and steadily increased thereafter; whereas Stx1A, Stx2A, and Stx2B subunits were not detected until 2-hours post-MMC induction. Both Stx1A and Stx1B subunits were detected in EC0001 cell pellets and maintained at relatively constant levels. Stx2A subunit was detected at high levels after 1-hour of induction; whereas Stx2B was not detected.

**Conclusions:** We observed the induction time required for Stx toxin detection varies for different isolates and cellular fractions. Pellets from 2-hour MMC-induced cultures were deduced as optimal for MS detection of Stx toxins. Owing to independence from strong acid protein precipitation and incomplete cell lysis at 2-hours post-MMC, processing of pellet fractions is advantageous and more efficient than for supernatants. Determination of optimal induction time will aid our development of a mass spectrometry-based method to detect Stx toxin in clinical samples.
High-Resolution Genotyping of Closely Related Measles Virus Isolates Sequencing of the MF Untranslated Region

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Background: Measles virus (MeV) is a highly infectious virus and caused an estimated 145,700 deaths globally in 2013. Measles has been eliminated from the Americas in 2002 and all cases resulting from importation from endemic areas. These importations are tracked by surveillance, which requires a high-resolution genotyping method to overcome the current difficulty in distinguishing decreasing diversity of MeV isolates. Traceability of measles cases in outbreaks received heightened media attention as over one hundred measles cases are reported in Canada since the beginning of 2015.

Objective: The proposed study would (1) develop a practical whole genome sequencing method of clinical MeV isolates, and (2) analyze the sequences for hypervariable regions for development of high-resolution genotyping test suitable for routine molecular surveillance of measles cases in Canada.

Methods: For the 2011 outbreak, MeV of clinical specimens is propagated in Vero/hSLAM cells, followed by RNA purification, reverse transcription (RT) and PCR. Amplicons are subject to Sanger and next-generation sequencing. For consequent outbreaks, RNA purification from clinical specimens is followed by RT-PCR amplification of MF Untranslated Region (MF UTR) with universal MeV-primers and Sanger sequencing.

Results: During outbreaks, the WHO target sequences of the N gene and the H gene remain invariant for several MeV genotypes. Whole genome sequencing allows determination of distinct importation events and chains of transmission and the sequence alignment leads to the identification of a hypervariable region of 1366-nt in the MF UTR. Sequencing of the MF UTR successfully resolved the phylogenetic relationship of several hundred cases in four recent outbreaks of genotype B3, D4, D8, and additional strains of D9 and H1. Performed in real time, this agile surrogate enhanced surveillance during four outbreaks determining MeV transmission in geographical regions over a given time course.

Conclusion: Since the MeV genome changes very slowly during outbreaks, unlike other RNA viruses, the WHO-standardized regions on N and H gene are not diverse enough to discriminate between different importations events during outbreaks. The newly discovered high-resolution genotyping target satisfactorily allows effective molecular surveillance during measles elimination filling the gap between epidemiological contact tracing and molecular surveillance.
Gene Variants and the Innate Immune Pathways in Canadian First Nations Populations
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Introduction: Canadian First Nations experience a significantly higher rate of Mycobacterium tuberculosis (TB) infection than non-aboriginal Caucasian Canadians. This is largely attributed to differences in the social determinants of health. However recent research has shown that single nucleotide polymorphisms (SNPs) in key immune regulatory genes play a critical role in host immune response to TB. Canadian First Nations are known to have an altered frequency of SNPs that may have functional consequences in the Th1 and Th2 immune pathways, but SNPs in the Th17 pathway and the P2X7 gene have not been explored in these groups despite their important role against infectious diseases.

Methods: Ethics approval from the Health Research Ethics Board, the Chief and Councils of the First Nation groups’ involved and individual informed consent from study participants has been obtained. This has been done with respect to the First Nations groups and their culture in mind. SNP profiles were identified through literature research. The NCBI database was used for identifying gene motifs, primer locations for PCR and potential Restriction Enzyme cut sites for RFLP analysis. Collecting of samples for First Nations groups and for a Caucasian cohort has been done through previous studies. The sample sizes include 113 Dene, 44 Saulteaux, 46 Cree, and 100 Caucasians. PCR analysis will be performed as per the reference articles. RFLP will be performed via a 30 min digest at 37°C and then analyzed on a 3% agarose gel. Each genotype will be entered into a spread sheet and once the work is completed the genotypes of each subset will be compared with each other to establish individual SNP profiles.

Results: Pending

Conclusion: It is anticipate that First Nations will have different SNP profiles in these genes as compared to a non-First Nations cohort. This study will provide researchers with a new understanding of these differential immune-regulatory molecular mechanisms in Canadian First Nation populations and possibly offer new cellular targets for infectious disease prevention and treatment for chronic and autoimmune diseases among this, and possibly other, populations.
Introduction: Cognitive behaviour therapy is an effective treatment for anxiety that often involves exposure to feared stimuli. Early theories of fear reduction posit that using distraction during exposure will interfere with treatment outcome; therefore, distraction has historically been discouraged during exposure. Recent research suggests that distraction may not in fact be countertherapeutic, which is especially important given high rates of treatment refusal and drop-out: incorporating distraction during exposure may increase treatment acceptability without reducing treatment benefits. Previous studies investigating distraction use in exposure have used a wide variety of distraction tasks, leading to difficulties drawing definitive conclusions. The current study therefore aimed to investigate the impact of differing levels of distraction on exposure outcome. We predicted that moderate levels of distraction would facilitate fear reduction while high levels of distraction would interfere, and that using distraction would lead to greater treatment acceptability and changes in self-efficacy.

Method: Participants (n = 121) were assigned to one of four conditions: no, low, moderate, or high distraction. Behavioural approach (a measure of fear based on willingness to approach a feared stimulus) and self-efficacy were measured before and after a 20-minute exposure and at one-week follow-up. Treatment acceptability was measured immediately following the exposure.

Results: Behavioural approach increased for all conditions pre- to post-exposure, F(1, 120) = 125.27, p < .001, and post-exposure to follow-up, F(1, 117) = 20.01, p < .001; however, there were no significant time by condition interactions. Self-efficacy increased pre- to post-exposure in all conditions, F(1, 120) = 43.11, p < .001, and there was also a significant time by condition interaction, F(3, 120) = 3.40, p = .020, with more significant increases for individuals using moderate distraction; there were no changes over the follow-up period. Treatment acceptability differed significantly across conditions, F(3, 123) = 7.23, p < .001, with the moderate and high distraction conditions rating the treatment as more acceptable.

Conclusions: Distraction, at any level, may not have a negative impact on treatment outcome, and is effective in increasing self-efficacy and treatment acceptability. Distraction use during exposure may therefore be helpful in reducing treatment refusal and drop-out rates.
Multiple T-cell Epitopes of HIV-1 Nef Containing Positively Selected Mutations Associated with Different Disease Outcomes

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Objectives: HIV-1 Nef plays a major role in enhancing the pathogenicity of the virus through various mechanisms such as down-regulation of CD4 and HLA class I surface expression and interfering with cell signaling pathways. Identifying and characterizing CD8+ epitopes in Nef that are under host immune selection can help in selecting targets for an effective vaccine.

Methods: 326 subtype A Nef sequences from treatment naïve patients of a Kenyan sex-worker cohort were generated using 454 pyrosequencing. Positively selected (PS) mutations were determined using a bioinformatics approach, quasi analysis. Peptides were designed with mutation placed in anchor position 2, 5, 8, 9 of epitopes of HLA class I alleles for validation with ELISpot assay using patient PBMCs.

Results: E70D, I109V and I176M were associated with rapid CD4 decline (p=0.010, 0.015, 0.025 respectively). H124N and K190M were associated with slow CD4 decline (p=0.001 and 0.029). The five PS mutations were significantly associated with HLA class I alleles including A*23:01 (E70D, p=0.002; I176M, p=0.003), A*02:01 (I109V, p=0.028; H124N, p=0.021), B*58:01 (I109V, p=0.048), A*3002, B*57:03 and C*02:01 (H124N, p=0.026, 0.0004, and 0.011 respectively) and C*06:02 (K190M, p=0.037). ELISPOT analysis identified 27 novel epitopes containing either the consensus or the PS mutations. Six new epitopes contained E70D, five epitopes contained K190M, and I109V and H124N were each contained by eight new epitopes. No epitopes containing I176M was confirmed by ELISpot assays. It is possible that I176M represents compensatory mutations due to functional requirements under host immune selective pressure.

Conclusion: Identification and characterization of epitopes containing beneficial and detrimental PS mutations can provide important insight for selecting immunogens for an effective HIV vaccine. More detailed investigation of T-cell responses, such as poly-functionality and proliferation to these mutations will be conducted to further characterize these Nef epitopes.
Long Noncoding RNA Regulation of the Cancer Stem Cell Phenotype in Glioblastoma Multiforme

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**Introduction:** Glioblastoma multiforme (GBM) is the most common primary malignant brain tumor in adults, with a two-year survival rate of less than 25%. Patients with GBM usually experience an initial remission after aggressive multimodal therapy comprising surgery, radiation, and chemotherapy, but inevitably succumb to disease recurrence and progression. The recurrence of GBM has been attributed to the presence of glioma stem cells (GSC), which are thought to play a central role in tumor development and progression. The property of “stemness” itself may be responsible for GSC resistance to chemotherapy and radiation, and might underlie their ability to drive tumor recurrence following disease remission. Long noncoding RNAs (lncRNAs) have been suggested to play a role in maintaining pluripotency, self-renewal, and differentiation in embryonic stem cells. We hypothesized that lncRNAs functionally contribute to GBM development and tumor propagation by maintaining the cancer stem cell phenotype in glioblastoma.

**Methods and Results:** Initially, an *in silico* “nearest-neighbor” approach was employed to identify 112 lncRNA candidates that were close to the transcription factors that have been implicated in regulating self-renewal and pluripotency of embryonic stem cells, or have been used to reprogram somatic cells into induced pluripotent stem cells (iPSCs), as well as factors that have been used to reprogram GSCs. Based on further *in silico* analyses and *in vitro* studies, we have identified two novel long noncoding RNAs, lincSox2 and lincPOUSF1, that show differential expression in stem vs. differentiated normal human fetal neural stem cells (NSCs) and GSCs. The expression of lincSox2 decreased after NSCs differentiated into astrocytes. In contrast, lincSox2 was enriched after GSCs underwent differentiation, suggesting its possible role in regulation of the cancer stem cell phenotype. In a similar manner, lincPOUSF1 expression increased in differentiated GSCs, but was decreased in differentiated NSCs. This implicates the role of these two lncRNA candidates in glioma biology. Further knockdown experiments followed by *in vivo* studies will provide insight into functional relevance of these candidates in maintaining “stemness” in GSCs.
Conclusions: Based on *in silico* and *in vitro* studies, we have identified two novel long noncoding RNAs that show differential expression in stem vs. differentiated NSCs and GSCs. LncRNAs may functionally contribute to glioma biology by regulating the cancer stem cell phenotype. Further characterization is needed to fully understand the role of lncRNAs in glioblastoma multiforme.
Background: Selective reporting is a problem in systematic reviews (1,2), detection of which is only possible when review protocols can be compared to completed reports. Outside of established review organizations (e.g. the Cochrane Collaboration), protocol documentation is rare (3). Initiatives have emerged to improve reporting of reviews (4,5). This project aims to understand factors influencing the reporting of protocols and develop a strategy to overcome identified barriers.

Methods: Barrier and facilitator interviews, structured around the theoretical domains framework (TDF) (6), will be conducted with systematic review authors, funders, and editors of journals that publish reviews. For each group, interviews will continue until data saturation is reached, starting with an initial analysis sample of 10 participants (7).

Planned Analysis: Interviews will be transcribed and will be independently coded by 2 assessors into the 12 domains of the TDF. Next, statements describing specific beliefs based on the textual data will be developed within each domain by 2 assessors (together). Problematic behavioural domains will be identified as those containing specific beliefs that reflect barriers to SR protocol reporting.

Expected Outcomes: This project should yield findings and a strategy that is applicable to develop implementation interventions to improve reporting across the biomedical literature.
Vitamin A Transporter RBP4 Concentrations are Specifically Altered In Obese and in Type 2 Diabetes Patients Blood
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Hepatic RBP4 (retinol binding protein) is the circulating vitamin A (ROL) carrier. The transthyretine (TTR) enhances ROL-RBP4 complex molecular weight, to prevent early kidney filtration, and allow ROL to tissues. The RBP4 is also an adipokine, secreted by fat tissues. Exogenous administration of RBP4 induces insulin resistance (IR) in animals. Circulating RBP4 were found elevated in type 2 diabetes (T2DM) but their raise in obesity alone or in obesity and diabetes, together with their relation with TTR in these diseases are subjects of some controversy.

Hypothesis: Serum concentrations of RBP4 and TTR may directly correlate with metabolic syndrome features and serve as pathophysiology indicators in obesity and diabetes.

Objective: Investigate the potential correlations of serum retinoid transporters RBP4 and TTR levels with traditional established markers in obesity, metabolic syndrome (MS) and T2DM.

Methods: Four groups of subjects (n=48) participated: Group A, healthy controls; Group B, obese diabetics with A1c > 7%; Group C, obese diabetics with A1c < 7%; Group D, obese non-diabetics. RBP4 and TTR were quantified by immunedetections Western Blot and ELISA respectively. Biometry: (waist circumference-WC, weight, height, body mass index-BMI); Blood biochemistry: (insulin resistance-HOMA, glycated hemoglobin-A1C, triglycerides-TG, cholesterol-HDL; LDL, albumin/creatinine ratio-A/CR, uric acid, C-reactive proteins); Hematology and blood pressure.

Results: RBP4 were 2.58 ± 0.21 nmol/ml in healthy subjects, slightly increased in obese non-diabetics (3.81 ± 0.75), and markedly increased in obese diabetics, both well controlled (5.59 ± 0.02) and poorly controlled (5.92 ± 0.03). RBP4 correlated directly with BMI, WC, IR-HOMA, TG and A/CR-ratio and inversely with HDL. No significant differences were found for TTR.

Conclusions: Serum levels of RBP4 but not the TTR are increased in obesity and diabetes and correlates with traditional biochemical and biometrical indicators of metabolic syndrome and diabetes. That should influence the retinoid metabolism and homeostasis in these metabolic conditions. The RBP4, RBP4/TTR and other retinoid related molecules may show potential as pathophysiology indicators in these diseases.
Resistance-Nodulation-Division (RND) Efflux Pumps Expression in Multidrug Resistant Isolates of *Pseudomonas aeruginosa* Obtained from Cystic Fibrosis Patients

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**Introduction:** *Pseudomonas aeruginosa* is a Gram-negative opportunistic pathogen known for causing pneumonia, urinary tract infections, surgical site infections, and bloodstream infections in immune compromised individuals. It is the major cause of progressive lung deterioration in cystic fibrosis (CF) patients. Elevated intrinsic resistance to many clinically important antibiotics, mainly through the activity of Resistance-Nodulation-Division (RND) efflux pumps, makes the treatment of infections very challenging. These efflux pumps are tripartite complex with a membrane fusion protein (MFP), and an outer membrane factor (OMF) protein to form a channel across the cell envelope of Gram-negative bacteria. The purpose of this study was to compare the expression of four RND complexes, namely MexAB-OprM, MexCD-OprJ, MexEF-OprN, and MexXY, in 15 CF clinical isolates of *P. aeruginosa* using real-time reverse transcription PCR.

**Methods:** Relative expression for different RND efflux pumps in 15 CF isolates were measures using real-time reverse transcription PCR along with their minimum inhibitory concentration (MIC) against clinically relevant antibiotics. All the RND expressions were normalized against the wild type *P. aeruginosa* PA01 strain.

**Results:** The relative expression of MexAB-OprM, MexCD-OprJ, MexEF-OprN, and MexXY in these CF isolates were 40% (6), 13% (2), 54% (8), and 87% (13) of the total isolates, respectively. Among the four RND pumps, MexXY was highly expressed by the majority of CF isolates followed by MexEF-OprN, while MexCD-OprJ being the least expressed. Among the CF isolates, 47% (7) and 13% (2) of the isolates showed simultaneous overexpression for three and two efflux pumps, respectively.

**Conclusions:** Collectively, these results demonstrates that the majority of the clinical isolates overexpress MexXY and can even overexpress more than one RND efflux pumps simultaneously to cover broad range antibiotic resistance. Identifying the minimum inhibitory concentration (MIC) of CF isolates for clinically relevant antibiotics and correlating them with the expression of different RND efflux pumps will help in identifying the possible cause of high antibiotic resistance in these isolates.
A Novel Model of Brain Metastasis from Lung Cancer: Targeting the Brain Metastasis Initiating Cell

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**Introduction:** Metastases are the most common type of cerebral tumour in adult, occurring at a rate 10 times greater than that of primary brain cancers. Despite the prevalence and lethality of brain metastases, very little research exists that completely elucidates the metastatic phenomenon in its entirety, nor is there a clinically relevant model that fully reflects metastasis in patients. The inherent abilities of a primary tumour cell capable of initiating a brain metastasis (BM) resembles that of a brain tumour initiating cell (BTIC). We hypothesize that there exists a subset of cells capable of metastasizing to the brain, termed BMICs, which are exclusively identified by genes that regulate self-renewal and invasion.

**Methods:** We established in house early passage cell lines from patient samples of BMs of lung origin, enriched for cells capable of completing the lung-to-brain metastatic cascade, termed BMIC lines. These cells were then injected into immunocompromised mice via three routes (intracranial, intrathoracic, intracardiac), brains harvested and cultured to isolate and characterize BMICs. We also performed a shRNA dropout screen with 150 genes implicated in BM formation followed by in silico analysis to determine genes that regulate the metastatic process.

**Results:** We have generated a novel human-mouse xenotransplantation model of brain metastasis that allows for serial in vivo enrichment and propagation of the functional TIC population that initiates BMs. We have identified characteristic differences in BMICs as they undergo the metastatic cycle. From the shRNA screen we identified approximately 60 hit genes that are required for survival/sphere formation. We are currently performing \textit{in vitro} and \textit{in vivo} validation of these hits.

**Conclusion:** We have developed a novel model of brain metastasis from lung cancer. This work has the potential to identify genes essential to development of brain metastases from the lungs a possibly a predictive metastatic gene signature. This will potentially offer therapeutic targets would lead to the inhibition of lung metastasis to the brain, allowing the arrest of BMs and keep the primary cancer localized and ultimately increase patient survival.
Epidermal Growth Factor Receptor (EGFR) signaling is essential for animal development. Mutations that activate EGFR signaling are commonly found in human cancers and most cancers originate from epithelial cells, where EGFR is required on the basolateral membrane. However, the mechanisms underlying EGFR regulation in polarized epithelial cells are not fully understood. In the nematode Caenorhabditis elegans EGFR signaling from the basolateral membrane of the vulva precursor cells is essential for vulva development, providing a unique in vivo model to study EGFR signaling and localization using genetic and cell biological approaches.

EGFR endocytosis and lysosomal degradation play an important role in regulation of the strength of signaling. In mammalian cells Rab7 promotes EGFR degradation; however its effect on signaling had not been demonstrated. We found that C. elegans RAB-7 antagonises EGFR signaling and its loss leads to EGFR accumulation in the cytoplasmic foci, suggesting a potential role for Rab7 as a tumor suppressor.

Identification of rab-7, a gene essential for viability, as a strong negative regulator of EGFR signalling, suggested that other essential regulators of EGFR signalling and trafficking might exist. I conducted a genetic screen designed to uncover essential regulators of EGFR signalling and identified mutations in the agef-1 and dhc-1 which code for an Arf GTPase Guanine nucleotide Exchange Factor and the heavy chain of the Dynein minus-end microtubule motor protein, respectively.

I found that AGEF-1 functions with two Arf GTPases and the AP-1 clathrin adaptor complex to negatively regulate EGFR signaling by antagonizing the basolateral localization of the receptor. A human homolog of AGEF-1 is frequently mutated in numerous cancer cell lines supporting a tumor suppressive function in humans.

Loss of dhc-1 leads to EGFR accumulation in plasma membrane proximal foci suggesting that Dynein functions at an early step of EGFR endosomal trafficking. I recently identified a kinesin plus-end directed microtubule motor ZEN-4, a homolog of KIF23, as functioning antagonistically to DHC-1 in EGFR signaling. Understanding how these genes regulate EGFR signaling and trafficking in C. elegans will inform our understanding of EGFR signaling in human epithelial cells.
Physician Assistants Making a Difference: A Retrospective Study on Discharge Times in Community Orthopedics
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Introduction: Physician Assistants (PA's) have been key components to health care teams around the world for quite some time now. In Canada, PA's have been utilized very effectively in the military and are now transitioning to become integral parts to civilian health care across the country with Manitoba leading the way. Doctors are seeing the many potential benefits of PA's but it is not always clear how best to utilize their unique skill sets and talents. Looking at how discharge times are affected is one way to analyze their effectiveness in practice. The purpose of this paper was to look at a community orthopedic surgery service and investigate how hiring a PA affected discharge times post-op.

Methods: 120 systematic chart reviews were completed at the health records office at the community health care center. Dates ranged from 6 months prior and 1 year after the PA was hired. To keep the sample size simple, one surgeon was chosen who specialized in total hip arthroplasties and total knee arthroplasties, with no bias being given to age or sex of the patient.

Results: The first data set looked at 6 months prior to the PA starting. 45 cases were reviewed and the total length of stay for all cases was averaged and found to be 8.2 days post-op. The next data set looked at 75 cases up to 1 year after the PA was hired. The average length of stay again was averaged and found to be 5.2 days post-op. This shows that with the addition of a PA to the orthopedic team, they were able to decrease length of stay by, on average, 3 days.

Conclusion: This data set proves that PA's are an effective member of the health care team and when used successfully, have dramatic positive benefits to the patients and the health care system as a whole. Not only do they increase effectiveness and efficiency of their supervising physicians, but also with reducing discharge times they are able to reduce costs to the medical system associated with longer hospital stays and associated medical resources.
Effects of haptic forces on locomotion and posture in post stroke and elderly adults
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Introduction: There is growing evidence that walking adaptations and post-adaptations can be achieved in post-stroke populations by perturbing walking surfaces (e.g. split belt treadmills). It is also believed that haptic inputs can have an attenuating effect on both static and dynamic posture in the same population. Such inputs can come from a cane, fixed surface or even from a rehabilitation dog. In a post stroke population, would it be possible to render similar adaptation and post-adaptation walking effects using haptic robotics?

Objective: We recruited elderly post-stroke and age-matched controls to investigate adaptation and post-adaptation effects of haptic tension forces, compared to no force, during steady-state walking as evidenced in spatiotemporal gait parameters.

Methods: We have developed an innovative system based on virtual reality coupled with robotics for balance and gait rehabilitation post stroke. The system can systematically control tension forces delivered to the hand, in the direction of locomotion via a leash, while walking on a self-paced treadmill in a virtual environment (VE).

Results: Post stroke participants (n=6) and healthy age-matched controls (n=6) increased walking velocity by as much as 22% in the stroke group and 18.5% in the control group. These changes were accompanied by similar changes in stride distance, which increased as much as 15% when walking with the haptic force in the stroke group. Lastly, double and single limb support times showed some tendency to decrease in proportion to the stride time during the force epoch relative to the pre-force baseline. Further investigation is needed to determine whether there are changes in single and double limb support durations of the paretic limb compared to the non-paretic limb.

Conclusion: In both elderly post stroke and aged-match controls, adaptation and post-adaptation effects were found in spatiotemporal gait measures due to haptic forces on the hand. Future work will include an investigation of postural changes in paretic and non-paretic sides in reaction to leash perturbations.
Migratory Properties of Cytokine-Activated Natural Killer Cells in Natural Killer Cell-Dendritic Cell Crosstalk
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Introduction: Recent work on Natural Killer (NK)-Dendritic cell (DC) crosstalk has focused on how NK-DC interaction can lead to NK activation, DC activation and apoptosis. However, the way in which NK-DC interaction helps in NK recruitment has not been studied in detail. Good understanding of how NK-cell migratory properties are regulated in physiological and pathological microenvironments will provide further insights into the development of NK cell based therapeutic approaches. Using a conventional Trans-well assay and a microfluidic platform in vitro, we reported recently the migratory properties of IL-2 activated NK cells in response to conditioned media from immature or LPS-stimulated mature DC. We hypothesized further that NK migration is differentially regulated by cytokines and/or conditioned media (CM) from mature DC activated by different Toll like Receptor Ligand (TLRL).

Methods and Result: We compared migratory properties of the IL-15 and IL-2-activated NK in the CM from either immature or mature bone marrow derived DC preparations that had been activated by different TLRL (LPS, Pam 3C, polyI:C, CpG) at its optimal concentration. We observed that IL-2 and IL-15 activated NK elicited weak chemotactic responses towards CM from immature DC. CM from all the tested TLRL-stimulated DC promoted chemotaxis of the IL-2 and IL-15 activated NK cells. However, CM from the CpG-activated DC was less chemotactic compared to that of the Pam 3C-, LPS- or polyI:C-activated DC. We further examined such NK-DC crosstalk in a 4T1 mouse model of breast cancer. We showed that CM from 4T1 cells negatively regulated IL-2 activated NK-cell chemotaxis indirectly by impairing the LPS-induced DC maturation. Of interest, CM from 4T1 cells directly impaired also chemotaxis of the IL-2 activated NK cells directly, but not the IL-15 activated NK cells.

Conclusion: TLRL matured DC promote NK chemotaxis, and IL-15 activated NK cells were more chemotactic than IL-2 activated NK cells in all these analyses. IL-15 activated NK cell may be a good candidate cell type in adoptive cell therapy of breast cancer.
Rationale: Regulation of the immune system is a complex process involving many different pathways and signals. LAG-3 is an immune inhibitory marker which regulates T cell effector function when engaged. Conversely, engagement of MHC class II by LAG-3 results in activation of APCs and recruitment of additional immune cells. Expression of immune inhibitory markers during chronic infections, such as HIV, leads to immune exhaustion. Although many exhaustion markers are up-regulated during HIV infection, LAG-3 expression is consistently low on T cells for unknown reasons. The cell subsets contributing to and factors regulating LAG-3 expression are not well defined in humans. Murine studies have demonstrated the presence of an intracellular store of LAG-3 protein that is rapidly expressed. Whether human LAG-3 is regulated in the same manner is unknown.

Methods: Human PBMCs, isolated from 6 healthy donors, were stimulated for up to 24 hours with CD3/CD28 beads to observe when LAG-3 expression peaks in response to engagement of the TCR. LAG-3 expression was measured by flow cytometry. T cells were also sorted into CD4+ and CD8+ subsets and stimulated with CD3/CD28 beads to assess their relative contribution to LAG-3 expression.

Results: Engagement of the TCR using CD3/CD28 beads resulted in significantly increased LAG-3 expression after 4 hours (p = 0.0313). LAG-3 is then down-regulated until the 16 hour time point when expression peaks (p = 0.0313). This trend was consistent between both CD4+ and CD8+ T cell subsets. Pure CD8+ cells are capable of generating significantly more LAG-3 than CD4+ cells (p = 0.0156). Additionally, CD8+ cells express significantly less activation marker CD69 than CD4+ cells (p = 0.0156).

Significance: The low expression of LAG-3 in HIV infection is unique, and the mechanisms involved not understood. This work will provide insight into factors regulating early LAG-3 expression in the context of viral infection and help to clarify its contribution to early versus late immune responses.
The Effects of Sepsis on Hypothalamic Osmosensory Neurons Mediating Thirst

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Introduction: The ability of osmosensory neurons within the hypothalamus to sense changes in blood osmolality is essential for maintaining systemic hydromineral balance. Normally the osmosensitive neurons of the organum vasculosum of the lamina terminalis (OVLT) are stimulated by an increase in blood osmolality, which then stimulate neural pathways to induce thirst sensation. Patient studies show that in sepsis, a deadly disease defined by the systemic inflammatory response to a severe infection, thirst is depressed and that this impairment may be due to a deficit in the osmoregulatory pathway. We tested the hypothesis that sepsis impairs osmotically-induced thirst and that this is due to changes in OVLT neuron properties.

Methods: We used an acute model of the cecal ligation and puncture (CLP) surgery to induce sepsis in male rats. Rats were given access to either water or 2% NaCl, and the amount of fluid drunk was measured at regular time intervals for up to 24 hours. Extracellular single unit recordings from intact hypothalamic explants were obtained to measure spontaneous neuronal firing and OVLT response to hyperosmotic stimulus. NeuN-stained neurons were quantified using confocal microscopy. The electrophysiological properties of OVLT neurons were determined by whole-cell patch clamping techniques using both current- and voltage-clamp configurations.

Results: Septic rats drank significantly less under systemic hypertonic conditions (CLP 4.2 ± 0.58mL; sham 16.4 ± 1.8mL; p < 0.001). Electrophysiological recordings show that septic OVLT neurons display an impaired increase in firing rate in response to hypertonicity (CLP 0.1 ± 0.05Hz; sham 0.52 ± 0.18Hz; p < 0.01). Furthermore, they are less spontaneously active due to their hyperpolarized state (CLP −47.9 ± 2.4mV; sham −41.0 ± 2.2mV; p = 0.02) as opposed to a decrease in neuron density (CLP 15 ± 2 nuclei/200mm3; sham 13 ± 1 nuclei/200mm3; p = 0.33).

Conclusion: These results imply that the CLP rat model mimics the human septic condition, and provide a platform to further our understanding of how central osmoregulation and osmotic thirst are perturbed at a cellular level in sepsis.

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Proposal: Difference-makers in the experience of human affective distress: Perspectives on causation and recovery gained from phenomenological inquiry into lived experience.

Introduction: Depressive disorders have increased in prevalence in recent decades, as has the use of antidepressant medications to treat human affective distress. Social sciences research shows robust and reliable associations between depressive symptoms and social, psychological, and environmental factors including employment status, income, life stressors, and gender. The paradigm currently in use in psychiatry, however, reflects a biologically based, individualist, dualist, reductionist discourse which fails to address social and environmental context, and therefore defaults to an individual-level analysis of a complex phenomenon which could be better understood as the result of interactions between ecological, psychological, and biological factors. Insight into the lived experiences of individuals with stories of recovery from affective distress may point the way forward in integrating relevant social and environmental context into assessment, treatment, and prevention of depressive disorders.

Purpose: The purpose of this research is to: (1) explore the causal factors or ‘difference-makers’ identified by participants as contributing to their experience of distress; (2) identify the ‘difference-makers’ participants perceived as helpful in their recovery; and (3) hear the individuals’ views on their degree of fit or lack of fit with the dominant psychiatric discourse of biomedical individualism and pharmacological treatment.

Methods: In-depth interviewing to obtain a phenomenological understanding of the lived experience of affective distress in context. Data sources will include field notes and audio-recorded, semi-structured, qualitative interviews with research participants. Purposive sampling, as well as ‘snowball sampling,’ will be implemented in order to achieve maximal variability on socioeconomic and demographic characteristics such as income, employment status, educational level, gender, and ethnicity.

Significance: While research in academic psychiatry focuses on individual-level factors in the debates over causation and diagnostic categorization, epidemiology and the social sciences have largely assumed the
validity of depressive disorder as a biological given, without recognition of its historical-cultural context. There are calls from within both traditions for more comprehensive models capable of accommodating complex cross-level mechanisms of causation, and integrating multiple valid explanatory perspectives.
Objective: Effective treatment of brain disorders requires a focus on improving drug permeability across the blood-brain barrier (BBB). Herein, we examined the pharmacokinetic properties of negatively charged iron oxide nanoparticles (IONPs) and capability of using lysophosphatidic acid (LPA) to disrupt the tight junction to allow IONPs to enter the brain.

Methods: Using a mouse model, quantitative determination of IONP in blood and various tissues (liver, spleen, lung, kidney and brain) was performed following IONP administration with or without LPA to transiently disrupt the BBB. Localization of IONPs in tissue was confirmed by transmission electron microscopy (TEM). Potential brain toxicity was evaluated by histological analysis at 2 and 9 days following IONPs treatment.

Results: The half-life of IONPs was 5.9 minute. Liver and spleen were the major organs of IONP deposition. Renal elimination of IONPs was observed. There was limited distribution of IONPs in lung and brain under normal conditions. LPA treatment enhanced brain and spleen accumulation of IONPs up to 3% and 70% of injected dose respectively. Tight junctions in lung and kidney remained intact and no IONPs were observed suggesting LPA-mediated disruption of tight junctions was localized to the brain. Histological examination of brain slices of LPA and IONPs treated mice revealed no significant toxicity with regard to infiltration of peripheral immune cells or activation of microglia and astrocytes.

Conclusion: LPA facilitated enhanced brain penetration of IONPs. The delivery efficiency in the brain parenchyma following LPA treatment is higher than most of receptor mediated transcytosis delivery of NPs reported in the literature. Transit BBB disruption and enhanced IONP delivery to the brain did not lead to inflammation or toxicity. Our findings suggest enhanced brain delivery via transient disruption of the BBB may be a safe and effective method.
Role of the pRb Family Proteins in Axon Growth and Guidance
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Introduction: Neuron development proceeds through a series of stages beginning with division of neural precursor cells and birth of the neuron, and culminating with neuronal maturation. To maintain organization of this complex chain, later developmental stages, like axon guidance, must be coordinated with the culmination of earlier events such as cell cycle exit. Indeed, there is increasing evidence that proteins which regulate cell cycle progression also influence later events in neuron maturation such as differentiation and migration.

Methods: Developmental and molecular consequences of pocket protein loss on axon guidance were analyzed using pRb and p107 double knockout (DKO) mice. Immunohistochemistry and in situ hybridization were employed to examine formation of axon tracts and the midline support structures of the commissural plate at E15.5, E17.5 and P0. Axon tracing was performed through in utero electroporation of a GFP plasmid. In vitro cortical explant cultures were used to examine neurite outgrowth.

Results: Following loss of pRb and p107 in the telencephalon, callosal axons show signs of general disorganization and numerous guidance errors, most notably their extopic re-entry into the ipsilateral cortex and failure to cross the midline between hemispheres. In addition, the corticoseptal boundary is shifted ventro-caudally in DKO brains and there are multiple defects in the midline glial structures, which have known axon guidance functions. Deregulation of several key axon guidance molecules was observed in DKO brains, which could explain these developmental defects. Furthermore, loss of pRb and p107 function was shown to result in an inherent neurite growth defect independent of the environment of the intact brain, which suggests a cell autonomous role of these protein in neurite extension.

Conclusions: We show here that pRb and p107, which are well known to cell cycle exit, also regulate axon growth and guidance during formation of the corpus callosum in the developing brain. This data indicates that the pRb family proteins are crucial for the growth and guidance of callosal axons and provide even further evidence to the growing body of data showing that cell cycle proteins regulate neuron maturation beyond control of proliferation.

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De Novo HTLV-1 Infection Depletes Monocytes via SAMDH1-STING, But Enhances CD4+ T-Cell Persistence through Tax-Mediated FOXO3a Inhibition

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Very little is known concerning the initial stages of Human T-cell leukemia virus type 1 (HTLV-1) infection, despite it being the first human retrovirus discovered and it being present in many regions of the world, including Canada. Although most of the infected individuals remain asymptomatic, chronic infection may lead to a number of pathologies, including adult T-cell leukemia (ATL), as well as HTLV-1-associated myelopathy and tropical spastic paraparesis (HAM/TSP). HTLV-1 has a preferential tropism for CD4+ T-cells but infects many other cells types such as monocytes, which play a key role in innate immunity.

Here we demonstrate that de novo HTLV-1 infection of primary human monocytes leads to cell death mediated by SAMHD1, a restriction factor that prevents retroviral DNA synthesis. Reverse transcription intermediates were produced in the presence of SAMHD1 and complexed with the DNA sensor STING. This resulted in an IRF3-mediated antiviral and apoptotic response. Conversely, de novo HTLV-1 infection of activated primary human CD4+ T-cells results in enhanced cellular persistence due to Tax, an early accessory protein of HTLV-1. Tax expression led to higher levels of phospho-AKT, which inactivated the FOXO3a pathway. This led to enhanced survival of infected cells that were capable of transmitting HTLV-1 infection.

Overall, this study provides new insights into the molecular events following de novo HTLV-1 infection and coincides with observed clinical features. Our results may also lead to important discoveries regarding HTLV-1 pathogenesis, as the depletion of monocytes may influence the development of ATL or HAM/TSP. Furthermore, the importance of the AKT-FOXO3a pathway in the early stages of HTLV-1 persistence may render it vulnerable to new therapeutics.
Introduction: Chronic pain is a debilitating, co-morbid, complex illness with numerous effects to physical and psychological functioning. Coronary artery bypass grafting (CABG) is a surgical intervention to improve quality of life and survival. CABG patients may experience chronic pain from the sternal incision and subsequent manipulation and displacement of chest wall structures damaging thoracic cavity nerves. Nerve injury and prolonged, uncontrolled acute pain can alter pain pathways in the central nervous system resulting in neuropathic pain characterized by sensory abnormalities. Although there is an abundance of literature examining chronic pain after cardiac surgery, few studies have specifically measured neuropathic pain in the cardiac surgery population. This prospective longitudinal cohort study will determine the incidence of neuropathic pain as well as examine the patterns and possible predictors of chronic and neuropathic pain development after CABG.

Methods: Elective CABG patients (n=300) will complete baseline questionnaires to assess for acute pain (SF-MPQ), neuropathic pain (S-LANSS), physical functioning and mental health / well being (SF-36), and pain catastrophizing (PCS) prior to surgery. Following CABG surgery, participants will complete the SF-MPQ, S-LANSS, SF-36 questionnaires, and a sleep questionnaire (MOS sleep), 7-days after discharge, 3-months and 6-months after surgery.

Analysis: The incidence of neuropathic pain will be determined by pain scores from the SF-MPQ and S-LANSS questionnaires at the three postoperative time points. Growth curve modeling will be used to examine individual and group patterns. Pre-operative PCS scores, postoperative SF-36 scores, MOS sleep scores, patient demographic data and surgical factors will be added to the analysis to further explain and predict the patterns of chronic and neuropathic pain development in the first six months after CABG.

Discussion: The results of this study may provide information to improve identification, assessment, and treatment of chronic and neuropathic pain after CABG surgery. More evidence about the patterns and predictors of chronic and neuropathic pain development may enable healthcare providers to better facilitate the cardiac surgery recovery process for patients.
Stretch-Induced ATP Release in Rat Alveolar Type II Cells

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Introduction: Extracellular nucleotides, such as ATP, UTP and their metabolites, initiate the purinergic signaling cascade via binding to cell surface purinergic receptors. Purinergic signaling regulates many functions in the lungs, including mucociliary clearance and surfactant secretion. Our previous work demonstrated that mechanical stresses, particularly stretch, not only increase intracellular calcium ([Ca²⁺]i), but also induce ATP release, whose mechanisms are still poorly understood. We will therefore investigate the physiological mechanism of stretch-induced ATP release and Ca²⁺ signaling in rat pulmonary cells.

Methods: Rat alveolar type II (ATII) cells are seeded onto flexible collagen-coated silicone stretch chambers. During experiments, the 2-3-day-old primary cell cultures are bathed in luciferin-luciferase (LL) containing DMEM medium and subjected to a 1-second stretch of 10% to 25%. Released ATP is visualized in real-time by imaging ATP-dependent LL bioluminescence with high-sensitivity EMCCD camera. [Ca²⁺]i measurements are performed with ratiometric Fura-2 fluorescence imaging.

Results: Following a stretch, the peak concentration of ATP released from ATII cells varies from 1-5 μM, which is sufficient for autocrine/paracrine activation of purinergic receptors in the entire cell culture. The number of responding cells was proportional to the extent of stretch. Stretch-induced [Ca²⁺]i was also elevated, where the number of responses was proportional to the magnitude of stretch. Moreover, 24-hour pre-treatment with an inflammatory mediator lipopolysaccharide (LPS) evoked the increase of [Ca²⁺]i in ATII cells. Their ATP release responses were stronger than those of untreated control cells.

Discussion & Conclusion: Our results indicate the importance of stretch-induced ATP release in mechano-purinergic signaling in the alveolus. LPS-induced inflammation may play a role in [Ca²⁺]i signaling and indirectly in Ca²⁺-regulated ATP release. Our study should contribute to better understanding of the role of purinergic signaling in surfactant and fluid homeostasis in the lungs, which may provide potential novel therapeutic approaches for respiratory diseases and clinical complications, such as acute respiratory distress syndrome (ARDS) and ventilator-associated lung injury (VALI).
Disease Activity Patterns in Rheumatoid Arthritis in the First 3-Years of Follow-up in Usual Care: Markov Modeling Shows Rapid Improvement in States in the 1st Year Followed by Stable States in the Last 2 Years

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**Background:** Disease progression in longitudinal studies of rheumatoid arthritis (RA) is assessed by examining measures of disease over fixed time intervals; results can be presented as the group mean or for common subgroups which do not address individual within-patient changes over time. In contrast, a multi-state model classifies each patient into one of several pre-defined disease states at patient visit and examines moves between disease states over time. Aim: (1) provide descriptive analysis of patients’ disease defined by the DAS-28 throughout the first three years of treatment; (2) determine the time spent in each disease state; and (3) estimate the probabilities of changing between disease states.

**Methods:** From an ongoing prospective study of RA in Ontario, Canada, patients were selected if: (1) incident RA; (2) active disease (>= 1 swollen joint); and (3) at least 2 follow-up visits to their rheumatologist. The DAS-28 disease score was collected at each visit and patients were classified in one of four DAS-28 categories, from remission to high disease activity. Traditional assumptions of Markov models are equal intervals between visits, which do not reflect the clinical reality of irregular visits. We fitted a novel 4-state to describe patient progression through disease states expressing time as a continuous variable.

**Results:** There were 3014 visits in 586 patients included in the study. At baseline, 43% were in DAS-28 high disease activity, but patients moved out of this health state rapidly on average 0.17 years, 95% CI (0.19, 0.23). At baseline 9% of patients were in DAS-28 remission, increasing to 30% at 6 months and 45% at 1 year. Once a patient achieved remission, the mean duration before moving to another disease state was 0.81 years, 95% CI (0.67, 0.97). By 1.5 years after initiation of treatment, patients in each disease state remained constant, indicating no net movement between health states.

**Conclusions:** Our analysis indicates the critical first year of treatment before a steady disease state with no net movement will be reached. Major changes in the first year of treatment do not occur as quickly as specified by treat-to-target guidelines indicating possible gaps in care.
Semaphorin 3E Inhibits House Dust Mite Induced Angiogenesis in Mouse Model of Allergic Asthma

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Rationale: Asthma is a chronic inflammatory disease characterized by airway hyperresponsiveness and airway remodeling. One of the prominent features of airway remodeling is increased angiogenesis, which is defined as a complex process in which new vessels form from pre-existing ones. Angiogenesis is a vital process in the homeostatic condition; and its increase in pathological conditions stems from the imbalance between pro-angiogenic and anti-angiogenic molecules. Surprisingly, the factors regulating this process in allergic asthma are poorly defined. Semaphorin 3E (Sema3E) is an axon-guidance secreted protein in neuronal system that has emerged as an essential mediator involved in cell migration and proliferation that are known to play a key role in angiogenesis. Our objective is to investigate the effect of Sema3E treatment and deletion on angiogenesis events within the airways of murine model of allergic asthma.

Methods: Sema3E knock out (KO) and wild type (WT) mice were subjected to House Dust Mite (HDM) acute exposure. The effect of treatment and deletion of Sema3E on Von Willebrand factor (vWF) and CD31 expression were studied using immunohistochemistry, immunofluorescence staining and confocal laser scanning microscopy on lung tissue. The expression of pro-angiogenic factors in bronchoalveolar lavage fluid and total lung homogenate was assessed by ELISA and Real-Time PCR.

Results: Sema3E treatment reduces the number of blood vessels positive for vWF and CD31 on lung sections of HDM exposed mice compared to untreated groups. Conversely, lung sections of HDM exposed sema3E KO mice displayed enhanced number of blood vessels positive for vWF compared to HDM exposed WT mice. Sema3E treatment inhibited HDM-induced secretion of the pro-angiogenic factor KC in the airway fluid; however, this did not change gene expression level of vWF, VEGF and bFGF, and CD31.

Conclusions: This study provides the first evidence that Sema3E down-regulates angiogenesis in allergic asthmatic airways.
Establishing a High-Content, Image-Based Screen to Identify Chromosome Instability Genes

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Introduction: Chromosome instability (CIN) is defined as an increase in the rate at which whole chromosomes or large chromosomal fragments are gained or lost. It is a characteristic of virtually all cancer types that is frequently observed in highly aggressive, drug resistant tumors. Despite this, the majority of human CIN genes have yet to be elucidated, emphasizing the need for novel assays and studies aimed at identifying the defective genes that drive CIN.

Methods: In this study we developed two image-based assays to detect CIN-associated phenotypes following RNAi-based silencing of candidate genes. The first assay utilizes nuclear area changes following silencing as an indicator of the chromosome content changes that underlie CIN. The second approach monitors micronucleus (MN) formation, where increases in the number of micronuclei are indicative of DNA damage and CIN. These approaches were validated in two unrelated cell lines (HT1080 and hTERT), by silencing the known CIN gene SMC1A, and comparing the results of each assay to the appropriate negative controls, (Untreated and siGAPDH). Once validated, this combinatorial approach was employed in a high-content screen of 164 human candidate CIN genes identified through standard cross-species approaches.

Results: In either cell line, diminished expression of the known CIN gene SMC1A resulted in a highly statistically significant increase in mean nuclear area and a >2-fold increase in MN formation compared to controls, validating the ability of each assay to detect changes associated with CIN. Next, these assays were employed to screen 164 candidates and collectively identified 124 putative human CIN genes. Preliminary data collected through Western blotting, mitotic spreads and flow cytometry, has provided evidence to support the validation of a subset of these putative CIN genes including SKP1, as bona fide human CIN genes.

Conclusion: The results of this study indicate that this approach is capable of detecting phenotypic changes associated with CIN, and can be utilized to uncover novel human CIN genes. Identifying CIN genes will provide critical insights into CIN and tumorigenesis, as well as identify potential targets that could be exploited for the development of superior therapeutic strategies.
Introduction: Like many carcinomas, epithelial ovarian cancer (EOC) exhibits extensive interpatient and intratumoral heterogeneity, which can hinder effective treatment of new molecularly-targeted therapeutics. In addition, the most prevalent high-grade serous subtype of EOC exhibits profound genomic instability fuelling tumour heterogeneity and acquisition of chemotherapeutic resistance. EOC treatment is further complicated by the formation of EOC spheroids which mediate metastasis and contribute to chemotherapeutic resistance.

Methods: We compared the oncolytic efficacies of three different oncolytic viruses—Myxoma, vaccinia, and Maraba (MRBV)—using a three-dimensional spheroid culture model of EOC metastasis. We expanded our results with MRBV by infecting novel EOC patient ascites-derived cell lines to investigate the impacts of temporal and spatial tumour cell heterogeneity on virus oncolysis. Subclones were generated by the expansion of single cells isolated from a single sample to investigate the impact of cellular heterogeneity on MRBV oncolysis. To define and validate mechanisms driving MRBV mediated killing, we performed viral titering, binding experiments, protein expression analysis, and siRNA knockdown of potential tropic factors.

Results: MRBV effectively infects, replicates, and kills EOC cells in both adherent and three-dimensional spheroid culture. However, we observed that spheroids can acquire resistance to MRBV infection and oncolysis in some EOC cell lines when compared with adherent cells. Furthermore, we found that both temporal and cellular tumour heterogeneity can impact MRBV oncolysis. Subcloned cells from one isolate identified two subsets of cells: one highly susceptible to MRBV, and the other with 1000-fold greater resistance. We determined that these differences were not due to reactivation of type I interferon in resistant EOC cells. Binding and entry experiments revealed MRBV restriction at virus entry in EOC spheroids and some resistant subclones. Decreased entry was also correlated with a decrease in the expression of low-density lipoprotein receptor (LDLR). siLDLR knockdown of EOC cell lines and subclones validated our correlative observations and restricted MRBV entry and oncolysis.
**Conclusion:** In summary, our data clearly demonstrates the potential impact of ovarian tumour cell heterogeneity on differential efficacy of oncolytic agents, and reveals that reduced LDLR expression is at least one mechanism which can confer resistance to MRBV-mediated oncolysis.
Background: Monozygotic (MZ) twins are ideal subjects for epigenetic studies because they are genetically identical and share intrauterine and postnatal environments. Currently, epigenetic studies use MZ twins as one homogenous group. However, there are two distinct MZ twin types: dichorionic (DC) MZ twins, a result of zygote splitting within 1-3 days, and monochorionic (MC) MZ twins, a result of the splitting occurring within 4-8 days. Two previous studies reported that DCMZ twins had more similar DNA methylation levels than MCMZ twins. However, studies with better genomic coverage are needed. We hypothesize that DCMZ twins are more similar epigenetically than MCMZ twins, due to either an earlier zygote splitting event, a higher degree of phenotypic similarity, or both.

Methods: We recruited 220 newborn twins and examined the relationship between chorionicity and birth outcomes. In addition, genome-wide DNA methylation profiles were generated for 48 twins, including eight twin pairs for each of the three twin types: DCMZ, MCMZ, and dizygotic (DZ). Intraclass correlation coefficients (ICC) and linear mixed models were used to investigate the global and site-specific relationships between DNA methylation levels and chorionicity.

Results: We found that compared to the DCMZ twins, the MCMZ twins tended to have shorter gestational age (p=0.0003), smaller birth weight (p=0.03), and larger birth length discordance (p=0.02). In general, the DCMZ twin pairs have more similar DNA methylation profiles than the MCMZ and DZ twin pairs (ICC=0.21 vs. 0.13 and 0.14, respectively), after adjusting for gestational age, birth weight percentile, and sex. However, the pattern did not hold at proximal promoter CpG island sites, where the DCMZ and DZ twin pairs were more correlated compared to the MCMZ twin pairs (ICC=0.14 and 0.13 vs. 0.07). Additionally, we identified 5,170 CpG sites (nominal p < 0.01) that had different DNA methylation levels between DCMZ and MCMZ twins, only one site was significant after multiple testing correction.

Conclusion: We demonstrate that DCMZ twins are more similar epigenetically than MCMZ twins, even after adjusting for birth outcomes. This study highlights the importance of including chorionicity information in epigenetic MZ twin studies. However, studies with larger sample sizes are needed to validate these results.
Defining the Role of Elmo2 in Myoblast Fusion during Muscle Development and Regeneration
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Introduction: Myoblast fusion is a crucial step for skeletal muscle development and regeneration, and it has been mainly studied in Drosophila. Until now, little is known about myoblast fusion in mammals, but studies in mice have shown a conserved role for proteins involved in actin rearrangement, such as Dock180 and Rac1. The Dock180 is a GEF that needs to formed a complex with the scaffold protein Elmo to induces Rac1 signalling. Elmo functions are still not well understood, but our lab has recently identified an auto-inhibition regulation, where its activation leads to the recruitment of Dock180/Elmo complex to the cell membrane, for Rac1 signalling. Since Dock180 and Rac1 functions during myoblast fusion are conserved, we suggest that ELMO is also essential to coordinate Rac1 activity during muscle development and regeneration, in mammals.

Methods/Results: 1) Elmo2KO mice have been generated. Exploiting the presence of a LacZ reporter gene, we detected Elmo2 expression in maturing somites. Immunohistofluorescence on E14.5 embryos confirmed its expression in muscle and also showed its broad expression in embryonic tissues. Analysis of E14.5 Elmo2KO embryos, using α-MHC (the latest differentiation marker) showed impaired myoblast fusion. 2) To investigate the biological relevance of the auto-inhibition regulation of Elmo2 in vivo, Elmo2EID knockin mice (with a mutation that induce the constitutively opened form of Elmo) have been generated. The Elmo2EID mice are viable, but fine analysis of muscles revealed the presence of larger fibers, suggesting an important role for this regulation in myoblast fusion. Preliminary results of cardiotoxin-induced muscle injury suggest a more efficient regeneration of the muscle in Elmo2EID/EID mice. Finally, Elmo2EID mice were breed with Elmo1KO mice to remove compensation by Elmo1. These double mutant mice demonstrated embryonic lethality and impaired myoblast fusion.

Conclusion: Little is known about the molecular mechanisms promoting myoblast fusion in mammals. Progress in understanding this biological step could have important implications in the treatment of myopathies by enhancing engraftment of muscle stem cells.
**Evaluating the Impact of Hydroxychloroquine-Loaded Polyurethane Intravaginal Rings on Lactobacilli**

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**Introduction:** Intravaginal ring (IVR) drug delivery systems have been extensively evaluated for the delivery of hormones for contraception and in the development of microbicides. In this study, we investigated the impact of polyurethane IVRs loaded with the immunomodulatory drug hydroxychloroquine (HCQ) on the major flora found within the FGT specifically Lactobacillus Jensenii, Lactobacillus Cryspatus and Lactobacillus Hansen and Mocquoct.

**Methods:** IVR segments were fabricated using medical grade polyurethane HP-60D-35 by hot-melt injection molding. The lumen of the IVR segments were filled with HCQ mixed with hydroxypropyl methylcellulose at a 1:1 weight ratio. The impact of free HCQ alone on lactobacilli was evaluated using the microplate dilution method. The toxicity of drug-free IVR segments was evaluated on bacteria using an elution assay method. The impact of HCQ-loaded IVR segments on lactobacilli growth was evaluated by incubating the IVR segments in MRS broth for various time points, followed by the addition of bacteria ($10^5$ CFU/mL) into the media. *In vitro* cytotoxicity of the IVR segments was assessed using the vaginal epithelial cell line VK2/E6E7 and the ectocervical cell line Ect1/E6E7 using the MTS assay.

**Results:** Sustained and controlled release of HCQ was achieved from the IVR segment for up to 2 weeks with an average release of 40 μg/mL/day. HCQ concentrations of up to 1.8 mg/mL appeared to have no significant impact on lactobacilli growth. Furthermore, drug-free IVR segments had no significant impact on the growth of lactobacilli or the viability of vaginal and cervical epithelial cells when compared to controls.

**Conclusions:** We describe for the first time, an IVR drug delivery system that can provide controlled release of HCQ for 14 days and is non-cytotoxic towards lactobacilli and vaginal/cervical epithelial cells.
DNM3 Genetic Variability Modifies Age-At-Onset in LRRK2 p.G2019S Parkinsonism

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Methods: Genome-wide short-tandem repeat (STR) markers were scored in 21 multi-incident LRRK2 p.G2019S families with parkinsonism. Non-parametric linkage analysis with MERLIN assessed age-at-onset as the trait. Complementary genome-wide genotyping of single nucleotide polymorphisms (SNPs) was performed for DNA from 263 North African and 98 Northern European patients with LRRK2 p.G2019S. PLINK, SnipSnip and BEAGLE algorithms were used for haplotype association analysis of age-at-onset in regions of suggestive linkage (LOD-1 intervals). Subsequent genotyping, candidate gene and protein expression analysis used Taqman probes, real-time PCR and Western blotting in human striatal brain tissues and mice neuronal cultures.

Results: Age-at-onset linkage analysis in LRRK2 p.G2019S families with parkinsonism highlighted five genomic regions (LOD>2.0), with suggestive linkage on chromosomes 1q24.3 (LOD=3.3) and 12q12 (LOD=3.4). Haplotype association analysis underneath suggestive linkage reveals significant association for DNM3 rs2421947 (p<10⁻⁵). DNM3 mRNA and protein expression were found to correlate with rs2421947 (p=0.006). Over-expression of V5-tagged dynamin-3 constructs in LRRK2 knock-out mice neuronal cultures show increased synuclein levels, whereas knock-down of dynamin-3 show decreased levels of synuclein (p=0.02).

Conclusions: The study illustrates the potential of genetically homogeneous populations to identify disease modifiers of Mendelian disorders. The candidate gene, DNM3, is expressed abundantly in the brain and promotes synaptic vesicle fission during endocytosis. In recent literature, Stafa and colleagues (2014) have reported biological associations between the dynamin GTPase superfamily and LRRK2. Thus, dynamin 3 is a compelling candidate to consider as a novel modifier of LRRK2 parkinsonism.
Introduction: Protease-activated receptors (PARs) and their activating enzymes have been suggested to play a role in inflammatory bowel disease (IBD) pathogenesis. While previous studies have shown that PAR2 is highly expressed on intestinal epithelial cells and its activating enzymes are increased in IBD, the specific roles of PAR2 in disease initiation and progression remain unclear. It has been previously shown that PAR2 activation can increase cyclooxygenase (COX)-2 expression in intestinal epithelial cells, and can promote cellular migration and proliferation. We tested the hypothesis that PAR2-induced COX-2 could promote epithelial wound healing.

Methods: Using colonic epithelial Caco2 cells, PAR2 was activated using the selective activating peptide 2f-LIGRLO (0.5μM-10μM). An inactive, reverse-sequence peptide served as the control. COX-2 protein levels were assessed by western blot, and PGE2 metabolites measured by ELISA. For wound healing experiments, circular wounds were made in cell monolayers with a pipette tip and monitored over time with live-cell imaging. Individual cells were tracked during wound healing to assess migration, while proliferation was determined by EdU incorporation.

Results: Activation of PAR2 in Caco2 cells significantly increased COX-2 protein levels (peak 4.2 fold increase at 4 hr) and PGE2 metabolites (peak 9.6 fold increase at 6 hr) compared to control. Contrary to our hypothesis, PAR2 activation significantly inhibited the rate of wound closure over 48 hr (79.3±2.5% wound closure) compared to control (94.3±0.5%), which was independent of COX-2 activity. Although PAR2 activation was able to increase proliferation in confluent cells, it had no effect on cells at the leading edge of the wound. However, PAR2 activation was able to significantly inhibit cell migration.

Conclusions: We uncovered a novel effect of PAR2 activation that was independent of COX-2. Although PAR2 activation had no effect on Caco2 proliferation at the leading edge of the wound, it significantly slowed the rate of wound healing by inhibiting cell migration. These data could have implications on the rate of epithelial barrier recovery following periods of intestinal inflammation during IBD.
Ly49-Dependent Cancer Immunosurveillance and Tumour Immunoediting by NK Cells
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Introduction: Natural killer (NK) cells are an integral part of the innate immune response, and are characterized by their ability to detect and kill tumour and virally-infected cells. Based on the missing-self hypothesis, NK cells surveil for abnormal cells which lack expression of class I major histocompatibility complex (MHC-I) molecules. The Ly49 receptor family recognizes MHC-I and plays a crucial role in the education of NK cells leading to their functionality. This work addresses the importance of Ly49 in cancer immunosurveillance.

Methods & Results: Genetically-manipulated mice with knocked-down expression of the Ly49 receptor (Ly49KD) were used in various cancer models. The appearance and growth of flank tumours in response to subcutaneous challenge with MHC-I-deficient tumour cell lines, RMA-S and B16F10, was accelerated in Ly49KD mice. As well, Ly49KD mice possessed a greater number of experimental pulmonary metastatic nodules. In addition, Ly49KD mice also exhibit defective control of primary tumour surveillance of methylcholanthrene-induced sarcoma and myc oncogene-driven B cell lymphoma. Tumour analysis for MHC-I expression levels showed tumour immunoediting, in which expression levels of the MHC-I molecules, H-2Kb and H-2Db, are decreased in Ly49KD mice. Introduction of a transgene for the inhibitory self-receptor Ly49I, thus restoring NK cell licensing, enhanced the ability of Ly49KD mice to suppress cancer onset and spread. Interestingly, activating receptor-mediated surveillance is intact in Ly49KD mice, as exhibited by stimulation of the NKG2D receptor with tumour cells or splenocytes ectopically expressing the ligand Rae1.

Conclusion: Together, these data provide evidence for the integral role of Ly49 in NK cell-mediated control of carcinogenesis and metastases. Impaired expression of the receptors results in uncontrolled tumour growth due to loss of MHC-I-dependent ‘missing-self’ cancer immunosurveillance.
Introduction: Metabolic transporters used by pathogens have gone under researched due, in part, to an established dogma that transporters solely provide nutrients to bacteria during an infection. Recent research has shown that various bacterial transporters are responsible for evasion of the host immune system. One of these transporters DalS, was found to sequester d-alanine from D-Amino acid Oxidase (DAO) preventing the production of reactive oxygen species and promoting survival of Salmonella within neutrophils. Follow up work has uncovered a second metabolic transporter, CycA, which is critical for systemic survival of Salmonella during an infection but is dispensable in vitro even if nutrients are limiting. We propose that CycA, like DalS, sequesters nutrients from the host to promote infection.

Methods: Infections of both primary immune cells and in a murine model allows for the characterization of a bacterial mutant's fitness. We generated a chromosomal deletion mutant of cycA and compared it against a fully virulent wild type strain in both neutrophils and our murine model of infection. In addition we monitored cycA mRNA levels under various conditions through qPCR.

Results: We found that CycA is critical for survival when exposed to neutrophils and in in vivo competitive infections. Data also suggests that cycA expression may be suppressed by the virulence regulators PhoP, OmpR, and SsrB.

Conclusion: Although we know CycA is important for Salmonella virulence and is potentially expressed in extracellular environments the exact mechanisms of how CycA contributes to pathogenesis remains elusive.
Perceived Need, Help-Seeking and Barriers to Treatment among Adults who Experienced Child Abuse in Canada
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Introduction: Previous research has shown that a wide range of childhood adversities are associated with mental disorders and perceiving a need for treatment. However, no current studies have examined the relationship between child abuse and barriers to mental health treatment in Canada. Objectives: The objectives of this research were to: 1) determine the relationship between child abuse history and perceived need for mental health treatment 2) determine the relationship between child abuse history and help-seeking for mental health problems and 3) determine the relationship between child abuse history and barriers to mental health treatment.

Methods: Data were obtained from the 2012 Canadian Community Health Survey- Mental Health, collected in the 10 provinces. Abuse types included physical abuse, sexual abuse and intimate partner violence.

Results: Experiencing any type of child abuse was associated with increased odds of perceiving a need for mental health treatment after adjusting for demographic covariates, any diagnosed or self-reported mental disorder and other types of child abuse. Experiencing any type of child abuse was also associated with increased odds of help-seeking for a mental health problem after adjusting for demographic covariates and any diagnosed or self-reported mental disorder. Dose response relationships were found for increasing number of abuse types experienced corresponding to increased odds of help-seeking and perceiving a need for mental health treatment. Those with a child abuse history were more likely to experience barriers to mental health treatment after adjusting for demographics and any diagnosed or self-reported mental disorder.

Conclusions: This study indicates that experiencing child abuse is associated with increased perceived need and help-seeking for mental health treatment, independent of the presence of mental disorders. Additionally, those with a child abuse history were more likely to experience barriers to mental health treatment independent of the presence of mental disorders. This study is limited by the fact that child abuse could be associated with mental disorders not assessed in this survey and there is no measure of child neglect, a prevalent form of child maltreatment. Clinicians should be aware of child abuse histories when providing mental health treatment to ensure appropriate care.
Self-Rated Health: A Potentially Modifiable Prognostic Factor for Disability Progression in Multiple Sclerosis

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**Introduction:** Disability progression remains a difficult consequence of multiple sclerosis (MS). While characteristics such as male gender, later age and motor symptoms at onset, are helpful in identifying those most at risk for disability progression, they are not modifiable. Patients’ assessment of their own health, referred to as self-rated health (SRH), may be one potentially modifiable prognostic factor. The purpose of this study is to determine if baseline SRH is an independent prognostic risk factor for disability progression in persons with MS.

**Methods:** This study is part of a larger study evaluating the impact of disease modifying therapies on the disability level and quality of life of persons with MS over time. Baseline SRH was measured by asking respondents: “In general, would you say your health is: Excellent - Very Good - Good - Fair - Poor?” The responses were dichotomized into excellent/very good/good vs. fair/poor. The outcome was change in disability level from baseline to year 3. Disability level was assessed using the Expanded Disability Status Scale (EDSS), an MS-specific measure of disability. Data were analysed using general linear models.

**Results:** The majority of the respondents (N=204) were female (70.7%), with an average age of 38.3, disease duration of 8.1 years and EDSS of 2.4. Less than one-quarter of the sample rated their health as fair or poor (21%). Potential confounding by the following factors was assessed for, gender, age, depression, fatigue, and none changed the crude estimate by more than 10%. The estimate of the association between SRH and disability at three years was -0.55 (95%CI:-1.08,-0.02).

**Conclusions:** SRH is an independent prognostic factor for disability progression. The ease of interpretation - “poor health” (fair/poor) is prognostic of disease progression - is a useful way for clinicians to begin their patient visits. By obtaining the patients’ perspective of how healthy they perceive themselves to be, clinicians have a starting point for directed conversations and potential interventions to make positive modifications.
Longitudinal Analyses Of CD3ζ-Chain Expression in the Correlation of the Disease Status in a Cohort of Head and Neck Cancer Patients

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Purpose: Despite advances in multimodality treatment, the 5-year survival rate of Head and neck squamous cancer (HNSCC) patients has not improved significantly over the last 4 decades. CD3ζ has emerged as a clinically important immunological molecule in HNSCC. Its relevance as a prognostic biomarker of HNSCC, however, has not been formally addressed in a longitudinal study.

Experimental design: A cohort of 46 HNSCC patients and 53 healthy controls were recruited in this study. Peripheral blood mononuclear cells (PBMCs) were collected at the time of diagnosis and at different time-points (up to a 2-year period) post-treatments. Expression of CD3ζ in the T cells of the PBMCs samples were analyzed in flow cytometry. Following in vitro stimulation of PBMCs with CEF peptide and Staphylococcus Enterotoxin B (SEB) superantigen, IFN-γ producing T cells were determined using flow cytometric methodology.

Results: We established a standardized method to conduct longitudinal analyses of intracellular CD3ζ expressions in the PBMC samples. We considered a <10% baseline increase in the normalized MFI of CD3ζ expression as a predictor of disease status in evaluating the follow-up samples of the HNSCC patients. Correlation analysis showed that 27/29 HNSCC patients who showed an increase in the CD3ζ expression relative to their baselines were disease free (negative predictive value. 93.1%). 10/17 HNSCC patients who showed a reduced/no change in the CD3ζ expression died or had recurrent disease (positive predictive value, 58.8 %). Overall accuracy of the assay was 80.43%. The sensitivity and specificity were respectively 83.3% and 79.41%. In our baseline samples, T cells from the HNSCC patients produced a significantly weaker IFN-γ response (MFI P<0.0001, and % P<0.05), in comparison to the healthy controls, when they were stimulated by the recall viral CEF peptide antigens. No significant differences in the IFN-γ responses were observed when T cells from either the HNSCC patients or healthy controls were stimulated by a potent SEB.

Conclusion: Our longitudinal analyses supported that a >10% increase in the CD3ζ expression against baseline could be a good prognostic biomarker in HNSCC patients.
Effects of Hempseed Oil on N-3 Fatty Acids Enriched Tissue Function and THC Metabolites in Vivo

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Purpose: Hempseed oil (HSO) is known to have optimal balanced ratio (3:1) of two essential polyunsaturated fatty acids, n-6 linoleic and n-3 alpha-linolenic acids. Due to a 60-year ban of hemp cultivation because of its highly lipophilic psychoactive component, Δ-9-tetrahydrocannabinol (THC), its health benefits are not entirely known. This investigation aims to explore the health benefits in n-3 enriched neural tissues (retina and brain) and n-6 enriched (in rodent) testis by providing dietary HSO with short and long-term use.

Methods: Male Sprague Dawley rats (n=40) were fed for either 3 or 9 weeks with semi-purified diets containing either 17% HSO (n=10) or 17% corn oil (control) (n=10). After each feeding period, retinal function was assessed with an electroretinogram (ERG), the brain volume with magnetic resonance imaging (MRI), and testis function with a light microscope using sperm concentration and morphology. Fatty acids were also measured in these tissues.

Results: Animals fed the HSO diet had significantly (p < 0.05) higher inner neural retina ERG a-wave (photoreceptor cells) and b-wave (inner retina bipolar cells) amplitudes in both rod and cone cells than the control group with increased n-3 long chain polyunsaturated fatty acids in the retina. The increased ERG parameters were achieved in both 3 and 9 weeks of feeding. Brain MRI parameters and sperm morphology showed no differences between the groups that were fed HSO or corn oil.

Conclusion: This study indicates that HSO may be a healthy dietary option for visual function by providing the essential fatty acids required for retinal health. The long-term effects of HSO consumption on THC metabolism are currently under investigation.
Effect of Antibiotic Administration Sequence on Blood Bacterial Counts in a Rat Model of E. coli Peritonitis Septic Shock


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Introduction: Severe sepsis and septic shock with sepsis-associated multiple organ failure has a high associated mortality (30-50%), despite newer antibiotics, improved diagnosis and deeper knowledge of sepsis pathophysiology. Microbial load is the key determinant of septic shock; thus the faster it is reduced to a sub-critical threshold, the less organ dysfunction and the higher survival. To improve pathogen clearance, we can optimize antimicrobial therapy by using antibiotics in combination. In-vitro studies have suggested that the sequence of antibiotic administration may have a significant impact on bacterial kill rates of several organisms using different antibiotic combinations. Few animal studies have been performed. In addition, previous studies have been inconsistent suggesting varying responses, depending on antibiotic class, organism and timing between doses.

Hypothesis: Administration of a β-lactam antibiotic simultaneously with or immediately prior to a fluoroquinolone or aminoglycoside would yield higher blood bacterial kill rates than the reverse order in a bacteremic rat model of E. coli peritonitis septic shock.

Methods: We created gelatine capsules filled with 20,000 E. coli (O18:K1:H7), and cellulose, encapsulated in a 3% fibrinogen clot. We surgically implanted the capsules in the abdomen of young adult Sprague-Dawley rats (250 g). After 16 hours, animals entered septic shock (hypotension and increased heart rate). At this time, animals were again anesthetized and underwent carotid and jugular cannulation. Antibiotics were administered alone (cefotaxime, ciprofloxacin or gentamicin) and in combination (simultaneously, cefotaxime followed in 30 minutes by the second agent and reverse order). Animals were monitored for 6h and blood was collected for quantification bacterial count at different time points. Animals were euthanized after the experiment.

Results: Administration of cefotaxime with or followed by ciprofloxacin or gentamicin yields larger bacterial clearance than the reverse order by 0.75-1 log CFU reduction, at 6 hours after antibiotic administration. The reverse order has similar bacterial clearance as ciprofloxacin or gentamicin alone.
Conclusion: The order in which the antibiotic combination is given has an impact on bacterial clearance. Variations in blood bacterial clearance could potentially impact clinical responses and outcome in life-threatening bacterial infections including septic shock.
A Novel Proteogenomic Approach to Identify Leukemia-Specific Antigens for Cancer Immunotherapy


Allogeneic hematopoietic cell transplantation (AHCT) led to the discovery of the allogeneic graft-versus-leukemia effect. This process is mediated by T cells and it currently represents the most convincing evidence that immune cells can cure cancer in humans. Two types of antigens can be found at the surface of leukemia cells: 1) Minor histocompatibility antigens (MiHAs) arise from inter-individual variations in the genome that translate to differential antigen expression at the cell surface. It is known that AHCT is mediated mostly by T cells targeted to MiHAs, 2) Antigens found at the surface of leukemia cells that are either overexpressed compared to their normal counterpart (leukemia-associated antigens; LAAs) or derived from leukemia-specific somatic mutations (leukemia-specific antigens; LSAs).

Two major problems still arise from AHCT, a rudimentary form of immunotherapy. First, a successful transplantation requires the identification of compatible donors. Second, about 60% of patients manifest graft-versus-host disease because the expression of MiHAs is not restricted to cancer cells. This can be circumvented by the injection of autologous CD8 T cells targeted to leukemia antigens. Our studies and that from other groups suggest that only non-self epitopes, such as MiHAs but not LAAs, can elicit protective anti-leukemic responses. A key corollary is that, since they represent non-self epitopes, LSAs might be as effective as MiHAs for leukemia immunotherapy.

Unfortunately, LSAs are difficult to identify due to their very unique nature. Because they are truly leukemia specific, we believe that LSAs are ideal targets for antigen-specific immunotherapy and have the potential to induce strong antitumor responses. Thus, we have developed a novel high-throughput proteogenomic approach to identify murine LSAs and validate their immunotherapeutic potential in vivo. With our innovative approach, we will be able to get a global view of the mutational landscape of the murine EL4 cell line. Most importantly, our work will lay the foundations that will enable us to identify LSAs in humans.
Miracles for Babies with Abnormal Lungs: The Story of miR-10a and Lung Development

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**Introduction:** Worldwide, 150 babies are born every day with congenital diaphragmatic hernia (CDH). These babies have a hole in their diaphragm, which allows the abdominal organs to crowd the developing lungs. One third of these babies will die from respiratory failure because their lungs are too small and very stiff (hypoplastic). MicroRNAs are essential epigenetic factors for lung development. We have identified two microRNAs as regulators of lung development in CDH: miR-200b and miR-10a. The purpose of our investigation is to define the role of miR-10a in lung development.

**Methods:** Using a nitrofen rat model to induce abnormal lung development and CDH, we employed RT-qPCR and in situ hybridization to study miR-10a expression during development. In addition, control- and nitrofen-treated fetal rat lungs were extracted for explant cultures and treated with miR-10a inhibitors and mimics. We are investigating miR-10a’s interaction with the retinoic acid signalling pathway using RT-qPCR and will confirm miR-10a’s participation in the retinoic acid signalling pathway in vitro using a retinoic acid response element (RARE) luciferase assay.

**Results:** MiR-10a was highly expressed in the lung bud epithelium, but lower in nitrofen-induced hypoplastic lungs. Using nitrofen-treated rat lungs as explant cultures, we could reverse the hypoplastic phenotype by treating early (E13) lungs with a miR-10a mimic. We are currently using RT-qPCR on total RNA extracted from the lung explants to confirm that miR-10a affects lung development through the retinoic acid signalling pathway. These results will be validated using a retinoic acid response element (RARE) luciferase in human bronchial epithelial and smooth muscle cells.

**Discussion:** MiR-10a contributes to lung organogenesis via the retinoic acid pathway. In CDH, reduced expression of miR-10a in development contributes to pulmonary hypoplasia. By treating developing lungs with miR-10a mimics, we can reverse the hypoplastic phenotype. Our ultimate goal is to treat CDH pups with miR-10a during pregnancy and improve their lung development. If we can do this, we may be able to give CDH babies the breathe of hope they need for a fighting chance at life.
A Proposed Pilot Randomized Controlled Trial of an Online Communication Tool for Collaborative Care in Patients with Advanced Cancer

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\textbf{Introduction:} Management of advanced cancer requires complex therapeutic strategies and follow-up from a variety of health care providers (HCPs). There is no standardized approach to communication between different HCPs, including allied health professionals, the caregiver and the patient. As a result, patients and their family are left shouldering the burden of coordinating care. We have developed a novel online tool for clinical collaboration, called Loop, to facilitate team-based communication with the hopes of improving care coordination and continuity of care. Loop assembles a patient’s care team in order to arrive at plans of care together in a virtual setting.

\textbf{Methods:} This is a pilot pragmatic stratified cluster randomized controlled trial. Physicians from oncology or palliative care will be stratified by specialty. Randomization to the intervention or control group will be done at the level of the physician within each stratum with patients as the unit of analysis. Patients and their care team (health care providers and caregivers) will receive either the intervention or usual care, as allocated to the physician to which they are registered. Patients with Stage III or Stage IV cancer will be eligible to participate.

\textbf{Results:} The primary outcome of interest is feasibility of conducting a full scale RCT. Patient, health care provider and caregiver recruitment and adherence to the study procedure will be measured. Preliminary measurements of efficacy between study arms will be obtained including on continuity of care, quality of care, health care utilization costs, and physician participation. Statistics on participant tool use will be captured with the tool’s audit functionality. Also, interviews will be conducted to gather qualitative data on user experience.

\textbf{Conclusion:} This pilot study will enable us to optimize the design of a full scale RCT, including changes to the delivery of the intervention, adjustments to recruitment strategies, assessment of outcome responsiveness, and participation.
Gestational Diabetes Mellitus Induces Chronic Neuroinflammation, Synaptic Degradation and Behavioral Changes in Offspring

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**Introduction:** Gestational diabetes mellitus (GDM) is the most common complication of pregnancy and population health studies have linked it to impaired cognitive performance in the offspring. GDM and diets containing excess fats and sugars promote inflammatory responses. Prolonged inflammation can impair the neuronal circuitry development in the fetus, resulting in lifelong effects on cognitive functions. We hypothesized that GDM causes adverse inflammatory responses in the fetus. This inflammatory environment could disturb the fine-tuning of developing neuronal networks impairing the neurocognitive abilities of the offspring.

**Methods:** We induced GDM by exposing rat dams to “junk food” diets high in sucrose and fatty acids 2 weeks prior and throughout their pregnancy. Fetal (18.5E) and 15-week old offspring (young adult) from GDM and lean dams were examined. The neurocognitive abilities of 15-week old offspring were evaluated with standard behavioural tests and the brains from both age groups were analyzed by immunohistochemistry. Complementing *in vitro* experiments involved analyzing microglial responses to elevated levels of glucose and/or fatty acids.

**Results:** The offspring from GDM dams showed atypical exploratory behaviour in the open field test, while their learning abilities remained unaffected. Analysis of brain tissues from fetal offspring of GDM dams showed increased astroglial GFAP expression, increased morphological activation of microglia, and expression of synaptic vesicle protein was reduced. 15-week old offspring showed similar results, in addition to reduced numbers of microglia. Postnatal junk food diet further promoted inflammatory responses and reduced synaptic integrity. Microglial cultures exposed to high glucose and/or fatty acids (palmitate) transformed into activated, amoeboid morphology, and significantly increased nitric oxide production.

**Conclusion:** The *in vivo* data strongly supports our hypothesis that GDM induces chronic inflammatory responses in the brain of the offspring. The robust neuroinflammation accompanied with reduced microglial number in the young adult brains suggest microglial senescence caused by chronic activation. Microglia
Culture experiments confirmed that excess glucose and/or fatty acids induce pro-inflammatory responses. Detrimental pro-inflammatory responses combined with impaired microglial neurotropic functions could explain synaptic degradation and behavioural changes. It remains to be seen whether fetal exposure to GDM predisposes offspring for memory and learning impairments later in life.
Decreased Expression of the Putative Cardiac Survival Factor FGF-16 Gene by Doxorubicin is Csx/Nkx2.5 Related

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Fibroblast growth factor 16 (FGF-16) plays an important role in various aspects of embryonic development, including of brown adipose tissue, the ear and the heart. However, FGF-16 is preferentially expressed in the heart myocardium after birth, and this “cardiac” and “time” specific expression suggests an important role for FGF-16 in the adult mammalian heart. FGF-16 is released by cardiomyocytes, and evidence from “knockout” mouse studies indicates that FGF-16 can limit the negative effects of heart remodeling (hypertrophy and fibrosis) after injury in vivo. In addition, mutations in the human FGF-16 gene appear to increase the risk for cardiovascular diseases. Moreover, we showed previously that exogenous addition of FGF-16 increased resistance to the loss of left ventricle contractility in an isolated mouse heart model of acute doxorubicin (DOX) induced injury. There is no report, however, on whether FGF-16 can influence viability in cardiomyocytes, and if so whether a negative effect on FGF-16 production or endogenous availability might contribute to any cellular damage and by extension function. DOX is a widely used and effective chemotherapy drug, but cardiotoxicity is a side effect and can lead to heart failure thereby limiting its effectiveness. Thus, strategies are needed to protect the heart while still allowing the benefits of the chemotherapy. Our data suggest that FGF-16 has a positive effect on postnatal cardiomyocyte survival from DOX-induced plasma membrane damage. This benefit is, at least partially due to the increased efflux of DOX by FGF-16. Interestingly, endogenous FGF-16 transcripts were significantly and rapidly decreased with 1μM DOX by 2 hours. “Knockdown” of endogenous FGF-16 using siRNA decreased cardiomyocyte viability. Thus, this reduced viability resulting from the decrease of FGF-16 levels may contribute to the cardiotoxic effects associated with DOX treatment. The lack of any effect on FGF-16 RNA stability together with a similar DOX-induced decrease in hybrid reporter gene expression driven by 747 base pairs of FGF-16 promoter sequences are consistent with control exerted at the level of transcription. Assessment of sequences in the proximal promoter region of murine and human FGF-16 sequences identified a highly conserved putative binding site for the transcription factor Csx/Nkx2.5 in a previously characterized TATA box. Csx/Nkx2.5 siRNA knockdown significantly decreased endogenous FGF-16 RNA
levels compared to a control siRNA. In addition, overexpression of Csx/Nkx2.5 was able to increase FGF-16 RNA levels, and increase the resistance of DOX-induced decrease in FGF-16 gene expression. Binding of Csx/Nkx2.5 to FGF-16 promoter was confirmed and shown to decrease rapidly in response to DOX \textit{in situ}. These observations support a role for FGF-16 in the maintenance and/or survival of postnatal cardiomyocytes, and suggest that a decrease in FGF-16 production via a negative effect on Csx/Nkx2.5 contributes to the cardiotoxic effects of DOX treatment.
Does Phosphorylation of Claudin 1 Play a Role in its Mislocalization in Human Invasive Breast Cancer?

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Introduction: Claudin 1, a major tight junction protein, is frequently down regulated in invasive human breast cancer (HBC), indicative of a putative tumor suppressor. The deregulated expression of junctional proteins is believed to be a key step for metastatic development and cancer progression. Intriguingly, our laboratory has made the novel observation that claudin 1 is highly expressed and often mislocalized to the cytoplasm in HBC. Moreover, it has been shown that protein kinase activity is important in controlling both claudin 1 expression and its localization in several cancers, but this has not been addressed in breast cancer. We hypothesize that the mislocalization of claudin 1 in human breast cancer is regulated by phosphorylation. The objective of this study is to determine whether deletions/modifications of phosphorylation sites on claudin 1 alter its subcellular localization in HBC.

Methods: Immunofluorescence and subcellular fractionation techniques were used to determine endogenous claudin 1 localization in a panel of HBC cell lines. C-terminus truncated claudin 1 constructs were generated by PCR and cloned into pEGFP-C1 vector. Additionally, claudin 1 mutants were generated to mimic constitutive phosphorylation or to render putative phosphorylation sites non-phosphorylatable. Immunofluorescence and confocal microscopy were used to assess the localization of the mutated claudin 1 protein following transfection into HBC cells.

Results: Mutation of the putative phosphorylation sites to mimic a non-phosphorylated state did not cause a shift of claudin 1 from the cell membrane. However, both claudin 1 constructs lacking the entire C-terminus and mutations that mimic constitutive phosphorylation resulted in a decrease in claudin 1 membrane localization.

Conclusion: We show in vitro, that C-terminus of claudin 1 is required for its membrane localization and phosphorylation of claudin 1 causes mislocalization to the cytoplasm in HBC cells. Such mislocalization has been shown to enhance the metastatic potential in some cancers, and thus, may also promote metastasis in breast cancer.
Introduction: In order to achieve abnormal gene expression in cancer, epigenetic silencing is often orchestrated with genetic aberrations. Certain genes are mutated or deleted to obtain the loss-of-function phenotype, whereas others are epigenetically silenced to stifle expression; how this is determined is currently unknown. Bidirectional promoters are prevalently represented in the human genome. We demonstrate a novel mechanism in pediatric brain cancer medulloblastoma, whereby the preferential hypermethylation of a bidirectional promoter results in the silencing of a tumour suppressor gene pair.

Methods/Results: Using the UCSC database of all known genes in the human genome, a putative list of potential bidirectional promoters was generated. Previously reported bidirectional promoters were validated using this method. One of the gene partners potentially regulated by a bidirectional promoter is HIC1, a tumour suppressor gene involved in the p53 apoptotic pathway, and miR-cluster 212/132, miRNAs involved in neuronal differentiation. Expression profiling of both HIC1 and miR-212/132 in human medulloblastoma revealed a significant correlation between their respective expressions. When looking at the expression in a subgroup-specific manner, it is evident that the expression of HIC1 and miR-212/132 is highest in normal cerebellum and lowest in groups 3 and 4 tumours, which are subgroups with the poorest prognosis. Inducible overexpression of Hic1 and miR-212/132 decreases medulloblastoma proliferation in vitro and extend survival in vivo. Knockout mice harbouring floxed alleles of either Hic1 or miR-212/132 cluster were crossed to Nestin-cre in a Ptc+/- background and resulted in increased tumour incidence.

Conclusions: We have identified a novel bidirectional promoter that is epigenetically regulated and silenced in the context of medulloblastoma pathogenesis. This analysis led to the identification of a novel tumor suppressor microRNA cluster in medulloblastoma; miR-212/132. Re-expression of Hic1 and miR-212/132 in medulloblastoma patient-derived cell lines resulted in significant reduction in tumour proliferation in vitro and in vivo. This novel mechanism of epigenetic regulation of bidirectional promoters may be relevant to a wide array of cancers and can be applied to identify novel cancer genes.
Plasma Fibronectin in Arterial and Venous Thrombosis: Evidence for the Fibrin Dependent and Independent Mechanisms

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Introduction: Hemostasis is the physiological process to stop bleeding, while thrombosis is the pathological vessel occlusive process leading to heart attack, stroke, and deep vein thrombosis, the leading cause of mortality and morbidity worldwide. Plasma fibronectin (pFn), the plasma form of fibronectin (Fn), exists in circulation in high abundance. However, the exact role of pFn in hemostasis and thrombosis is unclear.

Methods and Results: pFn has long been suspected to play a role in hemostasis but direct evidence has been lacking. Using our unique Fg−/−/pFn−/−, VWF−/−/pFn−/−, and Fg−/−/VWF−/−/pFn−/− mouse models, we demonstrated that pFn is vital for the control of bleeding in fibrinogen deficient mice and in wild-type mice treated with anticoagulants. We further found that, at the site of vessel injury, pFn rapidly deposits and initiates hemostasis even before platelet accumulation (the first wave of hemostasis). This pFn deposition is independent of fibrinogen, von Willebrand factor, β3 integrin, and platelets. Our perfusion chamber assay indicates that subendothelial collagen can mediate the rapid pFn deposition. Using confocal and scanning electron microscopy, we reveal that pFn actively integrates into fibrin, which increases fibrin fiber diameter and enhances the mechanical strength of clots as determined by thromboelastography. Using an inferior vena cava thrombosis model, we found that pFn depletion attenuated venous thrombosis, suggesting the involvement of pFn in deep vein thrombosis.

Interestingly, pFn promotes platelet aggregation when linked with fibrin but inhibits this process when fibrin is absent. Therefore, pFn may gradually switch from supporting hemostasis to inhibiting thrombosis and vessel occlusion following the fibrin gradient that decreases farther from the injured endothelium, thus preventing excessive thrombosis and rescuing downstream blood supply.

Conclusion: Our data established that pFn is a supportive factor in hemostasis, which is vital under coagulation deficient (both genetic and therapeutic) conditions. By interacting with fibrin and platelet β3 integrin, pFn plays a unique self-limiting regulatory role in thrombosis, suggesting pFn transfusion may be a potential therapy for bleeding disorders, particularly in association with anticoagulant therapy.
Access to High Quality Colonoscopy and the Colorectal Cancer Diagnostic Interval
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Background: There is concern that patients are waiting too long to be diagnosed with colorectal cancer (CRC) after presenting to the healthcare system. A prolonged time from first presentation to diagnosis, also known as the diagnostic interval, may be harmful to patients and indicate problems with the healthcare system. Variations in the length of the CRC diagnostic interval and care received within the interval reflect issues with patients’ access to care. We know that wait times for colonoscopy, a key test used to diagnose CRC, are longer than recommended by guidelines. However, most research examining risk factors for a delayed CRC diagnostic interval has focused on patient characteristics rather than characteristics of the health care system. The purpose of this study is to describe access to high quality colonoscopy resources in Ontario and examine the association between access and the length and characteristics of the CRC diagnostic interval.

Methods: This is a population-based, cross-sectional study of access to high quality colonoscopy resources and the CRC diagnostic interval in Ontario between 2009 and 2013. This study will use administrative health data housed at the Institute for Clinical Evaluative Sciences. Access to high quality colonoscopy resources will be defined based on characteristics of colonoscopy providers, including physician density, specialty, and colonoscopy outcomes, as well as the distance that patients must travel for colonoscopy. The diagnostic interval will be defined as the date of the first colorectal-related healthcare encounter to the date of diagnosis. Analyses will describe access to high quality colonoscopy resources across Ontario. Cluster analysis will identify groups of CRC patients with similar diagnostic intervals based on the care received within the interval. Multivariate analyses will evaluate the associations between access to high quality colonoscopy resources and the length of the diagnostic interval and care received within the interval.

Results: Pending.

Conclusions: This study will identify areas in Ontario that lack resources required for a timely diagnosis of CRC. These areas could be targeted in interventions to improve service provision, which could ultimately improve patient outcomes by ensuring that patients are diagnosed in a timely and effective manner.
Identification of Regulatory Mechanisms Governing GATA4 Functional Diversity in the Heart
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**Introduction:** Cardiovascular disease is the leading cause of death in North America and encompasses a wide variety of developmental and post-natal defects. Despite advances in our understanding of cardiac formation and function, the underlying mechanisms and how they are altered leading to disease remain incompletely understood. The zinc-finger transcription factor GATA4 is a key component of multiple cardiac processes including proliferation, differentiation, survival and hypertrophy. Using a mass spectrometry-based approach, this project aims to identify novel GATA4 post-translational modifications and protein partners central to cardiac function. As well, we aim to determine how these pathways are altered leading to disease.

**Methods:** To study novel GATA4 regulators, a stable GATA4-overexpressing cell line was developed via the transduction of a 3xFlag-GATA4 construct into the TC13 endocardial precursor cell line. 3xFlag-GATA4 was immunoprecipitated from nuclear extracts and analyzed by HPLC-ESI-MS/MS. Confirmation and assessment of the functional relevance of novel post-translational modification sites and protein partners was completed in both rat primary cardiomyocyte cultures and *in vivo*.

**Results:** Via our mass spectrometry-based approach, three novel phosphorylation sites were identified, implicating phosphorylation by kinases previously unknown to modify GATA4 and further elucidating mechanisms underlying GATA4 nuclear-cytoplasmic shuttling. Our approach has also identified several interacting partners key to GATA4 functionality, including the pro-angiogenic and pro-proliferative immediate-early gene Nur77 and the chaperone protein Heat Shock Protein 70 (Hsp70). We establish that interaction with Hsp70 rescues GATA4 from cleavage by caspase-1, a protease we show to be central to cardiomyocyte death caused by the chemotherapeutic agent Doxorubicin. Activation and nuclear translocation of caspase-1 leads to decreased GATA4 protein levels, increased cardiomyocyte cell death and development of fibrotic tissue *in vivo*. However, direct binding of Hsp70 onto the caspase-1 recognition motif blocks protein cleavage and rescues cellular GATA4 protein levels and transcriptional activity.
Conclusions: Through the identification of novel post-translational modification sites and protein partners, this work has shed further light on the diverse and context-specific GATA4-mediated mechanisms key to proper cardiac function. This information also lends further insight into how these pathways are altered causing disease.
**Quality Of Life in Prostate Cancer: Active Surveillance Versus Radical Prostatectomy**
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**Introduction:** This purpose of this review is to summarize current literature surrounding quality of life research in men with prostate cancer who choose either active surveillance (AS) or radical prostatectomy (RP), targeting anxiety, urinary and sexual symptoms.

**Methods:** A PUBMED search on anxiety, urinary, and sexual symptoms in active surveillance and prostatectomy in prostate cancer was performed. Literature in English from 2005 to 2015 was included, and augmented with relevant articles found amongst reference lists. Original research articles were selected for review.

**Results:** Men in AS vs RP groups do not significantly differ in terms of anxiety, depression or distress surrounding their diagnosis. A higher proportion of men have difficulty achieving erections firm enough for intercourse after RP. Higher rates of urinary incontinence also occur in RP. No data was found comparing urinary obstructive symptoms in RP and AS, though obstructive symptoms are known to be higher in watchful waiting.

**Conclusion:** Active surveillance is advantageous in having lower rates of erectile dysfunction and urinary incontinence, and is not associated with higher rates of anxiety. There are few studies comparing health related quality of life in active surveillance to radical prostatectomy, and more literature is required to fully understand the outcomes of each treatment choice.
Background: Aboriginal health is a national health priority as they experience some of the worst disease burdens and outcomes in Canada. Despite governments’ funding, policies, and health promotion, this concerning health inequality remains largely unresolved. The health Insurance Registry and other administrative databases in most Canadian jurisdictions lack an Aboriginal identifier. As a result, the continual and reliable tracking of individuals’ and communities’ health needs, disease patterns, and health-related behaviours for Aboriginals is currently absent. This inability to track Aboriginal status becomes a major barrier for researcher to uncover community-specific root causes responsible for the persistent health issues experienced by Canadian Aboriginals.

Objectives: In attempt to fill this gap of Aboriginal status information, we aim to: 1) use Canadian Census data to train the supervised-learning algorithm for classifying Canadian residents into Aboriginal and non-Aboriginal status based on people’s full names alone and in conjunction with sex and residential locations, 2) validate the performance internally using the test set from the Canadian Census data, 3) validate the performance externally using the Canadian Community Health Survey (CCHS), and 4) use our algorithm to classify individuals with missing ethnicity in the CCHS survey and conduct ethnic-specific analysis on health behaviours (i.e. smoking).

Methods: Our research will be the first study to use machine-learning classification to identify Aboriginal status using commonly-collected information (i.e. names). We will create two-levels of Aboriginal classification: 1) Aboriginal and non-Aboriginal, and if identified as Aboriginal then into 2) First Nations, Métis, and Inuit. The Naive Bayes training algorithm will be used. The Canadian Census 1901 data will be split into 60% training and 40% testing set. For internal validation, the predictive performance will be demonstrated through sensitivity, specificity, and accuracy. For external validation, the 2005 Canadian Community Health Survey will be used with the same predictive performance measures.

Once satisfactory level (70%) of overall accuracy is achieved, we will use the algorithm to predict the ethnicity of the missing ethnicity fields within the CCHS data. Subsequently, descriptive statistics on smoking, alcohol, and vegetable consumption will be computed, and compared qualitatively with published CCHS reports.
The Impact of Exercise Training and Leucine Supplementation in Frail/Prefrail Elderly Women with an Exploration into Mechanistic Explanations

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Aging is associated with frailty in which sarcopenia is a prominent component. Muscle protein synthesis (MPS) measured via fractional synthesis rates (FSR) from muscle biopsies is generally shown to be altered with aging. The amino acid (AA) leucine is a potent stimulus of anabolism, independent of insulin. While leucine can acutely correct the defective anabolic response in the elderly, chronic supplementation of leucine has yielded conflicting results. Combined resistance training (RT) with protein supplements has additive benefits over placebo in healthy older adults (Kim et al., 2012). However, neither FSR nor molecular mechanisms governing increases in protein accretion have been measured.

Our objective is to test for the effect of added leucine to combined RT and adequate protein intake on muscle mass and physical performance, and to provide mechanistic understanding of its effect. We hypothesize that the addition of leucine to an adequate daily protein intake with RT will be superior to the combination of protein and RT alone in stimulating FSR, improving muscle mass and physical performance in frail/prefrail elderly women.

**Study Design:** Double-blinded, placebo controlled trial.

**Intervention:** 12-week RT program with a protein optimized diet and additional leucine supplementation (2.5 g 3x/d leucine or isonitrogenous equivalent of the placebo alanine).

**Participants:** Pre-frail and frail (n=24) elderly women 70 yrs or older, as defined according to the criteria of Fried et al., 2001.

**Primary Outcome:** Changes in body composition pre- and post-intervention.

**Secondary Outcomes:** The difference in the change in FSR from the post-absorptive to post-prandial state, physical functioning tests, phosphorylation of signaling proteins in MPS mTORC pathway, fiber typing, and mitochondrial function tests pre- and post-intervention.

**Significance:** To our knowledge, no data exists in frail elderly women regarding how might be benefited through RT in combination with leucine supplementation. This is the population who would benefit the most from such an intervention in order to prevent disability and permit maintenance of autonomy. If we find abnormalities in mTOR pathways that are benefitted by the supplementation regime, we would have insight into mechanistic explanations on which to base pharmacological interventions in future studies.
Role of PI3K Enzymes in Chronic Lymphocytic Leukemia and Interaction with Lymphoid Tissue Microenvironment

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Chronic Lymphocytic Leukemia (CLL) is a lymphoproliferative disorder of mature B cells. A common feature of the disease is abnormal accumulation of malignant B cells in bone marrow and peripheral lymphoid tissues. Mesenchymal stromal cells present in the lymphoid tissue and bone marrow microenvironments can enhance the survival, proliferation and drug resistance of CLL cells through direct contact, thus selective disruption of this cell-cell interaction has been proposed as an effective therapy. Recently approved breakthrough therapies Ibrutinib and Idelalisib are kinase inhibitors targeting signaling enzymes in the phosphoinositide 3-kinase (PI3K) pathway. Recently, work done in our lab indicate that blocking the PI3K pathway impairs malignant B cell adhesion to stromal cells and migration in response to chemotactic factors produced by stromal cells. We hypothesize that targeting the PI3K pathway will effectively impair malignant B cell adhesion and migration functions required to access and colonize protective niches in lymphoid tissues. The mechanisms by which the PI3K pathway regulates CLL cell migration and interactions with stromal cells are still unclear. Here we propose several experiments to define the roles of individual PI3K enzymes on different aspects of this process, namely overall cell motility, chemotactic gradient sensing, cell-cell interaction and chemokine receptor trafficking. This study will apply novel technologies including live cell imaging and microfluidic devices to understand a critical aspect of malignant B cell biology. Preliminary data showed PI3K inhibitors can inhibit migration of CLL cells by SDF-1α in Transwell. Idelalisib (PI3K δ isoform specific inhibitor) can inhibit SDF-1α induced chemokinesis. CLL cells migration on the surface of stromal cells can be directly observed in co-culture system. Migratory capacities of the cells as well as cell-cell interactions were further analyzed in a quantitative way. These results will provide fundamental insights into the roles of PI3Ks in cell biology and help understand the underlying mechanisms by which different PI3K inhibitors act upon B cell leukemias. Furthermore, these results could provide evidence for specific PI3K inhibitor treatments of CLL patient in the future.
Comparison of Whole Genome Multi-Locus Sequence Typing (wgMLST) and Single Nucleotide Variant Phylogenomics for Foodborne Disease Outbreak Response in Canada

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**Introduction:** For the past fifteen years, the gold-standard for molecular subtyping of food-borne bacterial pathogens has been pulsed-field gel electrophoresis (PFGE). While this method has been suitably discriminatory for identifying and investigating most food-borne illness outbreaks, it only provides a snapshot of an organism’s total genetic material. Whole genome sequencing (WGS) has the potential to provide better case categorization in delineating outbreak strains from unrelated isolates. Critical elements to the successful application of WGS are the bioinformatics methods applied to process, assess and compare results. In Canada, the single nucleotide variant phylogenomic (SNVPhyl) pipeline developed at the National Microbiology Laboratory (NML) has been used for foodborne disease outbreak response, and whole genome multi-locus sequence typing (wgMLST) is also being explored.

**Methods:** Technical and qualitative comparisons of the wgMLST and SNVPhyl pipelines were performed and assessed against requirements for national reference laboratory standards. Both methods were used to retrospectively assess a recent outbreak of \textit{Salmonella} Thompson and were compared to the current gold standard (PFGE).

**Results:** SNVPhyl and wgMLST vary significantly in conceptual approaches to comparing genomes; SNVPhyl assesses high-quality single nucleotide polymorphisms in the core genome; wgMLST assesses allelic differences among thousands of loci in the whole genome. SNVPhyl provides resolution that can be “zoomed in” or “zoomed out” depending on the nature of the outbreak investigated and relies on a suitable reference genome. The wgMLST method is being customized into BioNumerics, the software currently used by the PulseNet Canada national network, and does not require a reference genome. Both methods provided increased resolution to a S. Thompson outbreak beyond the information provided by PFGE alone.

**Conclusion:** Both SNVPhyl and wgMLST have the ability to inform outbreak investigations with laboratory evidence that surpasses their traditional molecular counterparts. While the SNVphyl pipeline is well-established at the NML, wgMLST is still in development within the infrastructure/framework of PulseNet Canada. Further validation of wgMLST for outbreak detection and response is recommended and underway.

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**Introduction:** Preterm premature rupture of fetal membranes (PPROM) is a complication seen in obstetrics that occurs in 30% of premature birth and 3% of all pregnancies. Rupture of membrane is most commonly diagnosed clinically with physical examination; however, approximately 20% of women present very subtly, thus, making it difficult for a clear diagnosis. An accurate diagnosis is crucial in this case as it may influence subsequent management and care plan. Amnisure is a highly sensitive tool kit used reliably in diagnosing preterm rupture of membranes, but the cost of tool kit is significant enough that we do not offer to everyone. The current standard is ferning and/or nitrazine which performs at very low cost but does not carry the same sensitivity and specificity as Amnisure. This study is survey-designed, to collect information for analysis in order to estimate an actual cost in introducing Amnisure in a selective fashion and for every patient with query PROM, to inform decision making for use in triage unit at Women’s Hospital.

**Methods:** For an approximately one month period, data will be gathered for all women presenting to triage unit at The Women’s Hospital in Winnipeg with possible PROM. Data gathered will include gestational age, strength of history of rupture of membranes, whether there is obvious fluid in the vagina, record of ferning test, if a referral to fetal assessment unit was done, whether the patient went on to induction, and an assessment by medical staff if an AMNISURE kit would have been necessary.

**Result:** Survey currently ongoing, results to follow

**Conclusion:** To follow
RNAi-Based Nanomicrobicide for the Efficient Intravaginal Gene Knockdown of CCR5 and Nef in CD4+ Immune Cells

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Introduction: This study is to develop a RNA interference (RNAi)-based nanomicrobicide for the delivery of small interfering RNAs (siRNAs) knocking down host gene CCR5 and viral gene nef simultaneously as a pre-exposure prophylaxis to prevent intravaginal transmission of HIV-1. This technology platform comprises siRNA-encapsulated nanoparticles (siRNA-NPs) loaded into a vaginal gel.

Methods: siRNAs targeting CCR5 and nef were condensed by polyethyleneimine and then co-encapsulated into NPs by double-emulsion evaporation method using biodegradable polymer, poly(lactic-co-glycolic acid)-polyethylene glycol. Resulting NPs were formulated into a 0.5% hydroxyethyl cellulose vaginal gel.

Results: NPs showed a particle size of 256.6±8.3nm with a zeta-potential of -9.78±1.03mV at pH 5.0 and a particle size of 246.3±10.2nm with a zeta-potential of -24.95±5.55mV at pH 7.4. Encapsulation efficiency was 86.76±0.14%. NPs showed a pH-dependent release profile, with sustained release of siRNA under pH 7.4 (40% over 13 days) and minimal release of siRNA under pH 5.0 (less than 5% over 2 days). siRNA-NPs were rapidly taken up by CD4+ Sup-T1 cells (40% siRNA+ cells in 2hr and 100% siRNA+ cells in 24hr). siRNA-NPs were formulated into a vaginal gel with 17% of siRNA-NPs released within 24hr. In a vaginal mucosal co-culture cell model (upper chamber comprising a vaginal epithelial cell layer and a lower chamber comprprising CD4+ T cells stably expressing viral gene nef), vaginal gel loaded with siRNA-NPs could efficiently knock down CCR5 (>50% gene knockdown) and nef (>70% gene knockdown) in CD4+ T cells over 3 days after a 24hr treatment.

Conclusions: We have developed a novel RNAi-based nanomicrobicide that can efficiently knock down CCR5 and nef simultaneously in CD4+ immune cells. NPs have desirable particle size and zeta potential for intravaginal delivery and a pH-dependent release profile to preserve siRNA under acidic environments. NPs are formulated into a gel dosage-form to provide ease in administration and retention within the vagina.
Investigating the Neuronal Function of MeCP2 Isoforms and the Relevance to the Pathobiology of MeCP2-Associated Neurological Disorders

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Introduction: Methyl CpG Binding Protein 2 (MeCP2) is a transcriptional regulator that is highly expressed in the brain, with an exceptionally high abundance in neurons. Mutations in the MECP2 gene are the primary cause of Rett Syndrome (RTT), a neurodevelopmental disorder and a leading cause of mental retardation in females. Two MeCP2 isoforms MeCP2E1 and MeCP2E2 have been linked to the RTT pathogenesis. Currently, RTT has no cure. However, studies in RTT mice models have demonstrated the potential of selected MeCP2 target genes to reverse RTT phenotypes, indicating the possibility of therapeutic approaches for RTT patients. Advances in this approach are hindered by limited knowledge of the functions of the two MeCP2 isoforms.

Objectives: Recent studies have demonstrated that aberrant neuronal protein synthesis is a prominent feature of RTT. However the precise mechanisms by which MeCP2 regulates neuronal protein synthesis has not been fully elucidated. In this study, we aim to investigate the role of MeCP2 isoforms in regulation of neuronal protein synthesis.

Methods: To study the functions of the two MeCP2 isoforms, we have generated MeCP2 isoform-specific antibodies. The expression and regulation of MeCP2 isoforms were investigated within neurons and various regions of the mouse brain. Based on previous studies, we have chosen a subset of MeCP2 target genes, which are potentially involved in neuronal protein synthesis. In vitro models of MeCP2 isoform-specific loss- and gain-of-function studies were used to elucidate the regulatory role of MeCP2 in controlling these targets.

Results: We have identified a specific subset of MeCP2 target genes that are involved in neuronal protein synthesis. Currently, we are aiming to ascertain if these genes are direct or indirect targets of MeCP2.

Conclusion: Our novel findings on differential function of MeCP2 isoforms in protein synthesis, provides further insight on the role of MeCP2 in neuronal development. Our results also provide insights on selecting MeCP2 target genes for future RTT therapeutic interventions.
**Introduction:** The bacterial infection process often relies on protein-based virulence factors. *Listeria monocytogenes*, the bacterium that causes Listeriosis, has a virulence factor of interest called “Auto”. Auto is the only enzyme that is essential for entry into host cells. *L. monocytogenes* opportunistically infects the central nervous system causing meningitis and brain abscesses in the young, elderly, immunocompromised, etc. The bacterium is ubiquitous in the environment and infects through outbreaks of contaminated food. The process by which listeria enters the host cell requires its peptidoglycan cell-wall structure to be edited by Auto, to allow other virulence factors to gain access to the host cell. The family to which Auto belongs is known as Glycoside Hydrolase 73 family (GH73). Previous studies have shown one important aspect that regulates Auto activity is the presence of an autoinhibition loop that binds into active sites of these enzymes, which must be proteolytically cleaved to activate them. Here we describe the crystal structure of an Auto homologue FlgJ from *Salmonella typhimurium* from which the inhibition loop was removed to yield a free active site, enabling its use to understand the catalytic mechanism of GH73 enzymes and rational GH73 inhibitor development.

**Methods:** The wild-type GH domain of the FlgJ protein and variants thereof were overexpressed and purified from *E. coli*. The protein was then crystallized and the structure determined in-house via a single wavelength anomalous diffraction phasing experiment using the anomalous signal from iodide ions that co-crystallized with the protein.

**Results:** Upon solving the structure of the FlgJ GH domain, it was found that the active site was occluded by a C-terminal alpha helix, which blocked the active site similarly to the autoinhibition loop of Auto. Using the structure as a guide, we deleted the helix to generate a variant of FlgJ with an unclouded active site that was crystallized and its structure determined.

**Conclusion:** Current work is being done to place substrate molecules into the active site to biochemically describe the mechanism of GH73 enzymes. With the active site characterized, inhibitor development can begin to specifically target Auto and other GH73 enzymes of pathogenic bacteria.
Molecular Mechanisms Of The Ski:Scleraxis Negative Feedback Axis In Cardiac Myofibroblasts

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Introduction: In the healthy heart there is a balance among pro-fibrotic protein activation and expression (R-Smads, Scleraxis) and that of anti-fibrotic proteins (I-Smad, Ski). Scleraxis (Scx) is a basic helix-loop-helix transcription factor that is up-regulated in the fibrosing heart. Scleraxis has been shown to bind to the promoter region of the collagen 1α2 gene and induce gene transcription. Ski is a key regulatory protein in TGF-β1/Smad signalling that incorporates into the DNA binding complexes of Smad proteins and negatively regulates gene transcription. The premise of the current investigation is that Ski and Scx directly regulate the transcription of one another (in a reciprocal transcriptional regulation) and that this relationship is disrupted following myocardial injury leading to a pro-fibrotic stimulus.

Methods: Primary adult rat cardiac myofibroblasts were isolated via retrograde Langendorff perfusion. First passage (P1) cells were infected with adenovirus containing HA-Ski, HA-Scx, or LacZ at the time of plating. Twenty-four hours later cells were harvested for Western blot, mRNA, and electrophoretic gel shift assays (EMSA). For functional studies, NIH-3T3 cells were transfected with equal quantities of plasmid DNA for 24h prior to harvesting for luciferase assays.

Results: Overexpression of Ski in P1 myofibroblasts resulted in a 60% and 80% reduction in Scx mRNA and protein levels (p<0.05) respectively. Conversely overexpression of Scx modestly but significantly (p<0.05) reduced Ski protein levels as determined by Western blot. Functional luciferase assays demonstrated that Scx was significantly induced by TGFβ1 treatment in a concentration (0.5-10ng/mL) dependent manner. However Smad2/3 luciferase and EMSA analysis were negative for activation of Scx suggesting a non-canonical TGFβ1 pathway may be responsible for activation of Scx.

Conclusion: We conclude that the Ski and Scx play important roles in regulating the expression of one another and may represent a novel mechanism by which myofibroblasts regulate the production of fibrillar collagens following cardiac injury.
Vaccine Approaches Targeting Colonization by *Streptococcus pyogenes*

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**Introduction:** *Streptococcus pyogenes* is a superantigen-secreting pathogen that can cause a myriad of illnesses from pharyngitis to necrotizing fasciitis and rheumatic heart disease, resulting in over 500,000 deaths annually. Worldwide, it is a top-ten pathogen in terms of human mortality from an infectious agent, and despite decades of effort, a safe, effective vaccine does not exist.

**Methods:** Humanized mice (conventional C57Bl/6 mice expressing human leukocyte antigens) were used in all experiments. Mice were vaccinated with wild-type or mutant toxoid superantigens three times over 28 days (day 0, 14 and 28). Two weeks after vaccination, blood was taken to assess antibody titres (via enzyme-linked immunosorbent assay [ELISA]). To examine the protective effect of vaccination, twenty-four hours post-bleed, mice were intranasally infected with *S. pyogenes* for 48 hours and sacrificed and bacteria infecting the nasopharynx were enumerated. To determine the effects that superantigen vaccination had on specific T cell subsets, mice were vaccinated as above, however, instead of infection, splenocytes were isolated and either exposed to varying concentration of superantigens (responsiveness assay; interleukin-2 ELISA readout) or stained for flow cytometry (to examine specific T cell subset ratios). To specifically measure antibody-mediated protection, mice were passively immunized with anti-SAg or control rabbit serum twice (-24 and -2 hours) before infection (completed as stated above).

**Results:** Mice vaccinated with wild-type superantigens streptococcal pyrogenic exotoxin (Spe) A and staphylococcal enterotoxin B (both targeting $\nu$β8 T cells), and mutant SpeA$_{Y100A}$, were protected from infection by *S. pyogenes*. Sham vaccinated mice were not protected, whereas mice vaccinated with SpeA$_{\text{Hexa}}$ showed a bimodal phenotype. Only mice vaccinated with SpeA mutants showed significant antibody production. Mice vaccinated with wild-type superantigens and SpeA$_{Y100A}$ showed impairment in specific T cell subset responsiveness compared to control vaccination. Mice passively immunized with anti-SpeA, but not control serum, were protected from infection.

**Conclusion:** These findings suggest a requirement for T cells in *S. pyogenes* nasopharyngeal infection and supports superantigens as a mechanism for *S. pyogenes* immune system manipulation to promote nasopharyngeal persistence. These data support the consideration of toxoid superantigens as a vaccine candidate to target *S. pyogenes*.
Misfolded SOD1 Increases Intracellular A-Beta Aggregation
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Introduction: Protein aggregation is a common feature of neurodegenerative diseases. A-beta aggregation is the main component of amyloid plaque and is also believed to play a key role in the development of Alzheimer’s Disease (AD). The mechanism by which A-beta aggregates remains unclear. Nevertheless, the fact that ageing is the most prominent risk factor of amyloid formation suggests a role of oxidative stress in A-beta aggregation. SOD1 as an antioxidant helps to relieve cells from oxidative stress but itself is also a major target of oxidative damage. SOD1 becomes misfolded and gains toxic function after oxidation or mutation. Evidences from different groups suggested a close relationship between misfolded SOD1 and A-beta aggregation. Thus, misfolded SOD1, either induced by oxidation or mutation, potentially plays a role in amyloid formation.

Methods: APP/G37R double transgenic mice were generated by crossing single transgenic G37R mice expressing mutant SOD1 and single transgenic APPsw mice expressing excessive A-beta. APP/G37R mice and APP/G37R double transfected N2A cells were used to study the role of misfolded SOD1 in A-beta aggregation. Thioflavin T binding assay, Double-immunofluorescence staining, A-beta ELISA as well as Congo red staining were utilized to measure the difference of A-beta aggregation between groups.

Results: Although there is no significant difference of amyloid plaque formation between the double and the single transgenic mice by 12 month, the intracellular A-beta expressing and aggregation was observed increased when SOD1 was misfolded. Mutant SOD1 and A-beta were co-localized intracellularly, suggesting a direct interaction between the two proteins.

Conclusion: our results suggest misfolded SOD1 improve A-beta expression and intracellular aggregation in vitro and in vivo. This indicates misfolded SOD1 could potentially be an initial reason for AD.
Development of A Sensitive and Safe Ebola-GP-Mediated Virus Entry System and Investigation of the Anti-Ebolavirus Effect of CHRP

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Background: Ebolavirus (EBOV) cause severe hemorrhagic fevers in humans and non-human primates, with case fatality rates as high as 90%. Without an approved treatment, Ebola outbreak management has been limited to palliative care and barrier methods to prevent transmission. Ebola glycoprotein (GP) forms the trimeric spikes that locate on the surface of the virus, it plays a central role in cellular binding, fusion, and viral entry. EBOV entry is an essential step of the viral life cycle and an attractive target for therapy because inhibition of this step can block the propagation of virus at an early stage.

Results: We have generated a HIV-based vector containing Gaussia luciferase (Glu) gene (Lent-Glu). EBOV-GP pseudotyped vector particles (EB-GP VLP) were produced by co-transfected Lent-Glu, a Gag-Pol packaging plasmid with EBOV-GP expresser. We demonstrated that EB-GP VLP can efficiently infected different cell lines including 293T cells and the virus entry levels can be measured by detecting the levels of Gluc activity in the cell culture supernatants, which providing a simple and fast system for screening anti-EBOV compounds in vitro. Using this system, we identified CHRP, a purified extract from a Chinese herb, has potent inhibition of EB-GP VLP mediated infection. Results showed that about 80% of the infection can be blocked by CHRP at non-cytotoxic concentration, and this effective inhibition results from CHRP blocking the binding site attachment of EBOV-GP. Furthermore, the potent synergy effect of the combination of an EBOV-GP-specific MAb (2G4) with CHRP was observed. The results suggest that in the presence of CHRP, 2G4 can more efficiently inhibited EBOV entry.

Conclusions: In this study, we have established and characterized an Ebola glycoprotein (gp)-pseudotyped HIV-based vector system that utilizes Ebolavirus entry machinery for screening anti-EBOV agent(s) and investigating its antiviral mechanism of action in a lower biosafety level laboratory environment. Furthermore, we have identified an extract from a TCM (Traditional Chinese Medicine) that can efficiently inhibit Ebola virus infection by blocking gp-mediated entry.
Antitubulin drugs, such as paclitaxel (Taxol), vinblastine (Velban), are commonly used for various clinical cancer treatments, including breast cancer, lung cancer, ovarian cancer, etc. However, either the intrinsic or acquired resistances of patients to these drugs always result in the failure of the treatment and high mortality of cancers. Therefore, identifying molecules that involved in anti-tubulin drug resistance and studying the regulation of these molecules may be effective to solve the resistance issues. Recently, increasing studies have suggested that one of the downstream components in the Hippo pathway, TAZ (Transcriptional coactivator with PDZ-binding motif), is overexpressed in basal-like breast cancer cells as well as other types of cancer cells. Moreover, increasing TAZ also contributes to the resistance of breast cancer cells to Taxol through up-regulating its downstream targets, Cyr61 and CTGF, suggesting an essential role of TAZ in anti-tubulin drugs treatments. However, how TAZ is regulated in response to Taxol and other anti-tubulin drugs is largely unknown. In this study, CDK1 was identified as the kinase regulating TAZ phosphorylation and proteasome-based degradation in response to anti-tubulin drugs by targeting six novel sites on TAZ. Moreover, as soon as 6 hours’ drug treatment, three out of these six sites of TAZ were phosphorylated and led to TAZ band-shift. However, only the mutation of all the 6 sites into Alanine can fully abolish anti-tubulin drug induced TAZ degradation, suggesting the rest 3 sites were also phosphorylated with longer drug treatments. In addition, normal mammary cells (MCF10A) with the mimic phosphorylation mutation of TAZ (TAZ6D) overexpressed were more sensitive to anti-tubulin drugs compared with the cells overexpressing either wild type TAZ or block-phosphorylation mutation (TAZ6A) of TAZ, suggesting the phosphorylation of TAZ by CDK1 demonstrates the sensitivity of cancer cells to anti-tubulin drugs. Therefore, the activation status of CDK1 as well as TAZ level and its phosphorylation status may serve as possible biomarkers for checking anti-tubulin drug sensitivity of tumor cells. Moreover, approaches by modulating CDK1 activity or identifying the E3 ubiquitin ligase targeting CDK1-induced TAZ degradation may be capable for solving the TAZ related resistance of cancer cells to anti-tubulin drugs.
Estrogen Receptor a (ERα) is Recruited to the Centrosome in Breast Cancer Cells.
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**Introduction:** Estrogen receptor-α (ERα) is known as a nuclear transcriptional factor, which is overexpressed in two thirds of primary breast cancers. Measuring ERα status is of clinical importance in breast cancer diagnostics and determination of treatment response to hormone therapy. Aurora A, a centrosomal kinase, was shown to phosphorylate ERα at S167 and S305 previously. Polo-like kinase 1 (PLK1) which locates at centrosome and midbody can regulate ERα transcriptional activity during interphase. However, the localization of ERα at centrosome and its regulation by kinases in breast tumor cells are largely unknown.

**Methods and results:** Immunofluorescence assay (IF), biochemical analysis of isolated centrosomes and proximity ligation assay (PLA) showed, for the first time, that ERα is a novel centrosomal protein in the breast tumor cell line MCF7. ERα interacts with Aurora A and PLK1 in ER+ MCF7 and T47D cells by co-immunoprecipitation (Co-IP) assay, and the interactions are increased when the cells are arrested at prometaphase by nocodazole. Moreover, PLA showed that while ERα interacts with PLK1 at centrosome in interphase, centrosome interaction of ERα/Aurora A mainly occurs during mitosis in MCF7 cells. IF data also suggest that phosphorylated ERα pS118 colocalizes with Aurora A at centrosomes and with PLK1 at centrosome and midbody in mitotic MCF7 and T47D cells. Recombinant PLK1 was shown to phosphorylate ERα, and promote ERα polyubiquitination and proteasomal degradation in in vitro kinase assay and in vitro ubiquitination assay. Furthermore, overexpression and nuclear accumulation of Aurora A and PLK1 were observed in some ER+ breast tumors compared with normal mammary epithelial cells. In human breast tumor tissues, using PLA in situ centrosome interaction was only detected for ERα/PLK1 in interphase cells, but not ERα/Aurora A in mitotic cells.

**Conclusion:** These data suggested that ERα might be directly involved in centrosome function in breast cancer cells, and its dysregulation might play a role in tumor initiation and progression. Our research findings could have further implications for finding more accurate biomarkers of prognosis, and providing an alternative treatment strategy against ER+ breast cancers.
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