CSHRF +
GAIRDNER
SYMPOSIUM 2022
CELEBRATING
EXCELLENCE
JUNE
13–20
IN HEALTH
RESEARCH

CSHRF 2022

PRESENTATIONS ON
INNOVATIVE RESEARCH

LECTURES BY INTERNATIONALLY RENOWNED
EXPERTS FROM THE GAIRDNER FOUNDATION

RECOGNITION OF OUTSTANDING
STUDENT SCIENTIFIC ACCOMPLISHMENTS

DEAN'S MESSAGE

Brian Postl, MD

Dean, Max Rady College of Medicine, Rady Faculty of Health Sciences

Welcome and congratulations on participating in the 2022 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. After several years in "virtual" mode, it is a pleasure to be welcoming participants in-person!

The engagement 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 Career Development Workshop and numerous networking opportunities. In partnership with the Gairdner Foundation, we are pleased to host a Gairdner Symposium and note the exceptional opportunities for mentorship from these internationally recognized scientists who will be sharing their insights and experience.

We extend warm greetings and a "friendly Manitoba" welcome to participants from across Canada as well as internationally who are taking part in the CIHR National Research Poster Presentation. We trust that the valuable connections you make with your peers, judges and AFMC leadership will provide career-enhancing opportunities as well as a thoroughly enjoyable experience. In testament to the world-class quality of our participants, we are proud to recognize both our academic partnership with the Lindau Nobel Laureate meetings and the participation by our past winners at Lindau. The Lindau lecture within our event will share that experience. Our thanks to the Health Sciences Graduate Student Association 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 explore all that our city has to offer and enjoy your visit with us at the University of Manitoba Bannatyne campus.

2022 AGENDA

CANADIAN STUDENT HEALTH RESEARCH FORUM

JUNE 13–20

2022 GAIRDNER SYMPOSIUM PROGRAMME

Thursday, June 16, 2022

Frederic Gaspard Lecture Theatre

Basic Medical Sciences Building

730 William Avenue

8:00 **Registration & Refreshments (Concourse)**

9:00 **Welcome and Opening Remarks** Dr. Peter Nickerson
Vice-Dean (Research) and Distinguished Professor, Flynn Family Chair in Renal Transplantation, Rady Faculty of Health Sciences, University of Manitoba

9:05 **Chair's Overview** Dr. Geoff Hicks
Director and Professor, Regenerative Medicine Program, Dept. of Biochemistry & Medical Genetics, Max Rady College of Medicine, University of Manitoba

9:10 **Gairdner Overview** Dr. Janet Rossant
President & Scientific Director of the Gairdner Foundation

9:15 **Dr. Mathieu Lupien** (Introduced by Dr. Tamra Olgivie, University of Manitoba)
Senior Scientist, Princess Margaret Cancer Centre, Professor, University of Toronto & Ontario Institute for Cancer Research.
"How to Find Genetic Drivers across the Cancer Genome to Guide Precision Medicine"

10:00 **Coffee Break** (Joe Doupe Concourse)

10:30 **Dr. Sheena Josselyn** (Introduced by Dr. Tabrez Siddiqui, University of Manitoba)
Senior Scientist, the Hospital for Sick Children (SickKids) & Professor, University of Toronto. "Making Memories in Mice"

11:15 **Dr. Janet Rossant** (Introduced by Dr. Barbara Triggs-Raine, University of Manitoba)
Senior Scientist Emeritus, the Hospital for Sick Children; University Professor Emeritus, University of Toronto; & President and Scientific Director of the Gairdner Foundation. "Human Embryos and Stem Cell-Derived Embryo Models: The 14 day rule and beyond?"

12:00 **Lunch** (Brodie Atrium)

14:00 **Dr. Zulfiqar A. Bhutta** (Introduced by Dr. Cheryl Rockman Greenberg)
Founding Director, Centre of Excellence in Women and Child Health & the Institute of Global Health and Development, The Aga Khan University as well as the Inaugural Robert Harding Chair in Global Child Health, and Co-Director of SickKids' Centre for Global Child Health.
"Thrown Under the Bus? Young People's Mental Health in the Pandemic"

14:45 **Coffee Break** (Joe Doupe Concourse)

15:15 **Dr. James Blanchard** (Introduced by Dr. Sharon Bruce, University of Manitoba)
Professor and Executive Director, Institute for Global Public Health, University of Manitoba & Canada Research Chair, Epidemiology and Global Public Health
"Science in Global Public Health: Charting a new course"

16:00 **Round Table Discussion**

16:30 **National Awards Ceremony**

2022 GAIRDNER SYMPOSIUM SPEAKERS

Zulfiqar A. Bhutta

Centre for Global Child Health & Hospital for Sick Children, University of Toronto

James Blanchard

Institute for Global Public Health, University of Manitoba

Sheena Josselyn

Hospital for Sick Children, University of Toronto

Mathieu Lupien

Princess Margaret Cancer Centre & Ontario Institute for Cancer Research, University of Toronto

Janet Rossant

Hospital for Sick Children, University of Toronto & Gairdner Foundation

Zulfiqar A. Bhutta

PhD, FRS

Centre for Global Child Health & Hospital for Sick Children, University of Toronto

Dr. Zulfiqar A. Bhutta is Founding Director of the Centre of Excellence in Women and Child Health and the Institute of Global Health & Development, The Aga Khan University as well as the Inaugural Robert Harding Chair in Global Child Health, and Co-Director of SickKids' Centre for Global Child Health.

Dr. Bhutta holds adjunct professorships at several Schools of Public Health including Johns Hopkins University, Harvard, and the London School of Hygiene & Tropical Medicine. Dr. Bhutta leads large research groups based in Toronto, Karachi and Nairobi focused on, scaling up evidence-based community interventions, and implementing reproductive, maternal, newborn, child and adolescent health interventions in humanitarian settings. Dr. Bhutta is a Fellow of the Royal Society, the 2021 IHME Roux Prize recipient for significant research contributions to women and child health and was awarded the John Dirks Canada Gairdner 2022 Global Health Award, one of the most prestigious global health awards.

GLOBAL CHILD AND ADOLESCENT HEALTH: CURRENT CHALLENGES AND OPPORTUNITIES FOR ACTION

The challenges to global child and adolescent health are more important than ever. Dr. Bhutta will outline and discuss current trends and progress in global child and adolescent survival and health and explain key determinants of child health and issues in the Millennium Development Goals to Sustainable Development Goals transition. Dr. Bhutta has tried to address some of the equity and evidence gaps around the "First Thousand Days" of life through implementation research and effectiveness studies and explains the key opportunities for accelerating progress in child and adolescent health today in the context of relevant Sustainable Development Goals.

James Blanchard

MD, MPH, PhD

Institute for Global Public Health, University of Manitoba

Dr. Blanchard is an epidemiologist and public health specialist focusing on global health. He received an MD from the University of Manitoba and an MPH and PhD in Epidemiology from the Johns Hopkins University. He is a Professor and the Executive Director of the University of Manitoba's Institute for Global Public Health and holds a Canada Research Chair in Epidemiology and Global Public Health.

Dr. Blanchard's research focuses on how the characteristics of individuals, communities and large populations contribute to the local and global distribution of communicable and non-communicable diseases. Over the past twenty-five years he has also provided leadership to the design and implementation of large public health programs related to sexually transmitted infections, HIV/AIDS and maternal, neonatal and child health in Canada, India, other Asian countries and Africa. He is actively engaged with policy makers and public health leaders to translate scientific knowledge and approaches to improve the effectiveness and efficiency of public health programs, with an emphasis on improving the health of disadvantaged populations.

SCIENCE IN GLOBAL PUBLIC HEALTH: CHARTING A NEW COURSE

As the global health community continues to strive to achieve Sustainable Development Goals (SDGs) in health, there is a strong emphasis on improving equity in health outcomes and ensuring that health systems and programs "leave no one behind". Progress has been made in many areas, and science has contributed substantially by providing evidence and new biomedical breakthroughs to improve health outcomes. However, as the global COVID-19 pandemic has highlighted, there remain substantial gaps in the application of public health science to improve outcomes, particularly for those who are socially and economically disadvantaged and living in contexts with weak public health systems. As scientists from Canada and the global research community seek to meet these challenges, there is a need to move to transdisciplinary research models, whereby complex public health challenges are addressed by collaborative groups of researchers from diverse disciplines working closely with affected communities, public health programs, and global health institutions. To achieve this, academic institutions and funding organizations will need to make fundamental changes to how they function. This presentation will focus on these challenges, with a focus on charting a new course for fulfilling the academic mission in global public health.

Sheena Josselyn

PhD

Hospital for Sick Children, University of Toronto

Sheena Josselyn is a Senior Scientist at The Hospital for Sick Children (SickKids) and a Professor in the departments of Psychology and Physiology at the University of Toronto in Canada. She holds a Canada Research Chair in Brain Mechanisms underlying Memory, and is a Fellow of the Royal Society of Canada.

Dr. Josselyn holds undergraduate degrees in Psychology and Life Sciences and a Masters degree in Clinical Psychology from Queen's University in Kingston (Canada). Sheena received a PhD in Neuroscience/Psychology from the University of Toronto with Dr. Franco Vaccarino as her supervisor. She conducted post-doctoral work with Dr. Mike Davis (Yale University) and Dr. Alcino Silva (UCLA). Dr. Josselyn received numerous awards, including the Innovations in Psychopharmacology Award from the Canadian College of Neuropsychopharmacology (CCNP) and the Effron Award from the American College of Neuropsychopharmacology (ACNP).

Dr. Josselyn is interested in understanding how the brain encodes, stores and uses information. Her primary model organism is mice. However, several human disorders (ranging from autism spectrum disorder to Alzheimer's disease) may stem from disrupted information processing. Therefore, this basic knowledge in mice is not only critical for understanding normal brain function, but also vital for the development of new treatment strategies for these disorders.

MAKING MEMORIES IN MICE

Understanding how the brain uses information is a fundamental goal of neuroscience. Several human disorders (ranging from autism spectrum disorder to PTSD to Alzheimer's disease) may stem from disrupted information processing. Therefore, this basic knowledge is not only critical for understanding normal brain function, but also vital for the development of new treatment strategies for these disorders. Memory may be defined as the retention over time of internal representations gained through experience, and the capacity to reconstruct these representations at later times. Long-lasting physical brain changes ('engrams') are thought to encode these internal representations. The concept of a physical memory trace likely originated in ancient Greece, although it wasn't until 1904 that Richard Semon first coined the term 'engram'. Despite its long history, finding a specific engram has been challenging, likely because an engram is encoded at multiple levels (epigenetic, synaptic, cell assembly). My lab is interested in understanding how specific neurons are recruited or allocated to an engram, and how neuronal membership in an engram may change over time or with new experience. Here I will describe both older and new unpublished data in our efforts to understand memories in mice.

Mathieu Lupien

PhD

Princess Margaret Cancer Centre & Ontario Institute for Cancer Research, University of Toronto

Dr. Mathieu Lupien is a Senior Scientist at the Princess Margaret Cancer Centre, a Professor at the University of Toronto and holds a cross-appointment with the Ontario Institute for Cancer Research. He serves on the Senior Advisory Group and the Research Council on Oncology to the Princess Margaret Cancer Centre.

Dr. Lupien's research in chromatin and epigenetics has pioneered the study of the non-coding genome to identify the genetic determinants of oncogenesis and accelerate the development of chromatin and epigenetic-based precision medicine designed for cancer patients. Among other honours, Dr. Lupien is a recipient of the Mona Gauthier Award, the Canadian Cancer Society Bernard and Francine Dorval Award for Excellence, is a two times recipient of the Till and McCulloch Discovery of the Year award, a three-time recipient of the Investigator Award from the Ontario Institute for Cancer Research and co-founder of the CoBE ecosystem.

Dr. Lupien earned his PhD in experimental medicine at McGill University under the leadership of Dr. Sylvie Mader and carried out postdoctoral training in medical oncology as an Era of Hope Fellow at the Dana-Farber Cancer Institute/Harvard Medical School under the mentorship of Dr. Myles Brown followed by a PLDA at Harvard Business School. Dr. Lupien joined the Princess Margaret Cancer Centre and the University of Toronto in 2012.

HOW TO FIND GENETIC DRIVERS ACROSS THE CANCER GENOME TO GUIDE PRECISION MEDICINE

Conquering cancer lies in fulfilling the promise of precision treatment medicine. The latter relies on identifying treatment options designed against the unique disease phenotype of a patient's cancer. The characterization of cancer phenotypes to guide clinical decisions in hard-to-treat tumours relies primarily on genetic profiling to find genetic variants such as mutations and inherited single-nucleotide polymorphisms that define cancer drivers. However, this approach has yielded actionable targets for less than 30% of tumours. This low success stems from a primary focus on identifying cancer driver within gene, despite coding sequences accounting for less than 2% of the human genome. Here we present novel approaches to find novel cancer drivers by mining the non-coding genome. Furthermore, we demonstrate that cancer not only relies on genetic variants but also on chromatin variants that change the "active" versus "inactive" nature of small DNA sequences. Collectively, we present the framework to accelerate precision treatment medicine by adopting genome-wide methods to find cancer drivers across the coding and non-coding genome taking into account genetic as well as chromatin variants.

Janet Rossant

CC, PhD, FRS, FRSC

Hospital for Sick Children, University of Toronto & Gairdner Foundation

Janet Rossant, CC, PhD, FRS, FRSC is Senior Scientist Emeritus at the Hospital for Sick Children in Toronto, University Professor Emeritus at the University of Toronto, and President and Scientific Director of the Gairdner Foundation.

Dr. Rossant is an internationally recognized developmental and stem cell biologist, exploring the biology of the early embryo and its stem cells and their applications to understanding and treating human disease. She has also been actively involved in ethics and public policy discussions around stem cell research and genetic modifications. She led the Research Institute at the Hospital for Sick Children from 2005 to 2015. She has received many honors and recognition for her work, including seven honorary degrees, and election to the Royal Societies of London and Canada and the US National Academy of Sciences. In 2018 she received the N. American L'Oreal-UNESCO Women in Science Award and in 2021 the ISSCR Achievement Award.

HUMAN EMBRYOS AND STEM CELL-DERIVED EMBRYO MODELS: THE 14 DAY RULE AND BEYOND?

New science brings new ethical and regulatory challenges for research on human embryos and stem cells. Improved cultures of human blastocysts are reaching stages close to gastrulation, challenging the long existing rule banning culture of human embryos beyond 14 days. Is there a justification for an extension? Or can new stem cell-derived embryo models like blastoids and gastruloids replace the need for direct embryo research? Do these models raise their own challenges in terms of emergent properties? These questions, plus issues around interspecies chimeras, germline gene editing and in vitro gametogenesis, are just some of the areas where scientists and society need to work together to promote good science and maintain a clear ethical research framework.

2022 AWARDS

Caroline A. Cope Major Award – Oncology

Children's Hospital Foundation Major Award – Child Health

Dean of Graduate Studies – Poster Presentations

Dean of Medicine – Poster Presentations

E.L. Drewry Memorial Major Award – Health Research

Emergent BioSolutions Inc. Major Award – Infectious Disease

Emil & Lynette Hain Scholarship – Oncology

Gairdner Awards of Excellence – Poster Presentations

Graduate Students Association – Poster Presentations

Health Sciences Centre Foundation Inc. Major Award – Infection & Immunity/Neurobiology

Lindau Award

Manitoba Medical Service Foundation Inc. – Poster Presentations

Manitoba Medical Service Foundation Inc. Major Award – Health Research

Right Honourable Don Mazankowski Major Award – Oncology

St. Boniface Hospital Foundation Inc. Major Award – Cardiovascular Biology

The Winnipeg Foundation – Travel Awards

2022 AWARD COMMITTEES

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Jeffrey Wigle (Chair)
Christine Doucette
Larry Hryshko
Xi Yang
Bradley Doble
Etienne Leygue
Sari Hannila
Katinka Stecina
Lyle McKinnon
Stephanie Booth

Dr. Forough Khadem Memorial
Tamra Werbowetski-Ogilvie (Chair)
Soheila Karimi
Robert Beattie
Brad Doble

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Jiuyong Xie
Versha Banerji
Susan Logue

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Tara Beattie, AFMC
Jeffrey Wigle

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Eftekhar Eftekharpour
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Paul McLaren
Tooru Mizuno
Thomas Murooka
Thomas Netticadan
Chris Pascoe
Afshin Raouf
Ruey Su
Peter Thompson
Geoff Tranmer
Kristine Cowley
Saeid Ghavami
James Gilchrist
Sandy Kiazyk
Don Miller

National Poster Judges
Jeffrey Wigle (Chair)
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Paul McLaren
Donald Miller
Tara Beattie
Tooru Mizuno
Christopher Pascoe
Robert Shiu
Sanjiv Dhingra
Catherine Card
Depeng Jiang
Tanveer Sharif
Richard LeDuc
Galen Wright
Janice Dodd
Razvan Romanescu
Ayesha Saleem
Barbara Porto
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Women's Health Research Foundation

2022 ABSTRACTS

Tracking Tumor-TME Interactions Using *in vivo* Models of Glioblastoma

Aly O. Abdelkareem[1,2,3], Samuel Brown[1,2,3], Donna Senger[1,2,3], Jennifer A. Chan[1,2,3], A. Sorana Morrissy[1,2,3]

[1]Department of Biology and Biochemistry; [2]Cumming School of Medicine; [3]University of Calgary, Calgary, AB

Introduction: Glioblastoma multiforme (GBM) is the most common adult brain tumour, and despite aggressive treatment, it recurs fatally. GBM tumours include diverse populations of malignant and non-neoplastic cells with distinct molecular capabilities and with differential levels of sensitivity to treatment. Understanding the cell dynamics that occur during the development of GBM resistance to therapy could reveal key aspects of this process, including how resistance is acquired in time and how the diverse cell types of the tumour microenvironment (TME) contribute to this phenotype. In addition to the role of the TME, GBM exhibits significant tumour heterogeneity with diverse genetic clones coexisting in the same tumor, as well as cells with similar genetic backgrounds capable of adopting distinct transcriptional states and subtypes. The complex and dynamic interactions between tumor and TME remain to be fully studied. This work focuses on the *in vivo* spatial organization in GBM during disease progression.

Methods: We generated spatial transcriptomic data from a set of adult GBM samples grown as patient-derived xenograft (PDX) models, profiled at different time points of the disease. Three PDX lines from one GBM patient (derived from tumor core, vascularized area, and infiltrating front) were used to recapitulate the genetic and phenotypic heterogeneity observed in the human disease. Two replicates from each of 8 PDX mice were collected from early, mid, and late time points of tumour growth and data was generated using the 10X Genomics Visium platform. We developed a robust computational pipeline capable of distinguishing admixture of human (tumour) and mouse cells (TME), using state-of-the-art tools. Human and mouse cell types and states were identified using pooled and separate single-cell references of human GBM states, and mouse brain cells from both normal and tumour conditions.

Results: we observe spatially distinct patterns of both (a) tumour infiltration patterns specific to the each PDX line that includes non-random distribution of GBM transcriptional states and genetic clones, and (b) spatially distinct infiltration of TME components including microglial and macrophage populations.

Conclusion: our approach addresses the challenge of understanding the tumor-TME relationship by application of spatial profiling in PDX models and provides a computational pipeline complex multi-species analysis in the spatial transcriptomic field.

Targeted Lipid Nanoparticles for Transplacental Delivery of MicroRNA to Correct Fetal Lung Anomalies

Amr Abostait, Wai Hei Tse, Jackie Wang, Daywin Patel, Mike Jackson, Richard Keijzer, Hagar Labouta

College of Pharmacy, University of Manitoba; Children's Hospital Research Institute of Manitoba, Winnipeg, MB

Introduction: Congenital diaphragmatic hernia (CDH) is a fetal lung abnormality that accounts for 1-2% of infant deaths. MicroRNA-200b (miR-200b) was found to reverse epithelial-mesenchymal transition and rescue abnormal lung development, thus decreasing the incidence of CDH in the off-springs. This work uses lipid nanoparticles (LNPs) and utilizes the natural maternal-fetal transfer of passive immunity, to deliver miR-200b to fetal lung and avoid off-target maternal and fetal accumulation.

Methods: LNPs composed of different types of lipids were synthesized by a microfluidic platform. The microfluidic parameters were optimized to give formulations with different size ranges and low polydispersity index (PDI). LNPs were surface functionalized with IgG antibodies using simple click chemistry. A dynamic placenta-on-a-chip model was developed using BeWo human placental epithelial cells and HUVEC (human umbilical vein endothelial cells) to evaluate the LNPs versus control static conditions.

Results: The optimized microfluidic parameters gave reproducible monodispersed LNPs in the size range of 60-200 nm. LNPs were stable over three weeks at 4 °C. The dynamic recirculation resulted in the increased formation of dense microvilli over the surface of the cells compared to the static conditions. LNPs were not toxic to placental models under different flow conditions. IgG coating increased nanoparticle transport by 2.7-2.8 folds under static conditions compared to non-functionalized nanoparticles, with dependency on LNP composition, size and IgG coating density.

Conclusion: The dynamic recirculating model of the placenta-on-a-chip mimics the micro-physiology of the placenta. The natural maternal-fetal transfer of passive immunity could be utilized as a targeting mechanism to facilitate the transplacental delivery of LNPs. Next, LNPs will be tested for gene silencing in CDH rat model.

Impact of COVID-19 Pandemic on Preterm Birth and Stillbirth Rates in Manitoba, Canada: A quasi-experimental study

Laila Aboulatta[1], Kaarina Kowalec[1,2], Christine Leong[1,3], Joseph Delaney[1,4], Jamie Falk[1], Silvia Alessi-Severini[1,5], Sherif Eltonsy[1,6]

[1]College of Pharmacy, Rady Faculty of Health Sciences, University of Manitoba; [2]Department of Medical Epidemiology and Biostatistics, Karolinska Institutet, Stockholm, Sweden; [3]Department of Psychiatry, College of Medicine, Rady Faculty of Health Sciences, University of Manitoba; [4]Department of Epidemiology, University of Washington, Washington, SE; [5]Manitoba Centre for Health Policy; [6]The Children's Hospital Research Institute of Manitoba, Winnipeg, MB

Introduction: Conflicting evidence exists on the impact of the COVID-19 pandemic restrictions on preterm birth (PTB) and stillbirth rates. We aimed to evaluate changes in PTB and stillbirth rates before and during the pandemic period.

Methods: Using the linked administrative health databases from Manitoba, Canada, we conducted a quasi-experimental study among all pregnant women during the pre-pandemic period (1 October 2016 to 29 February 2020) and the pandemic period (1 March 2020 to 31 March 2021). We used interrupted time series analysis using autoregressive integrated moving average models - with step and ramp intervention functions - to investigate the immediate change in level and lagged effect change in slope in the quarterly rates of PTB (<37 weeks) and stillbirth. We also calculated the forecasted trends based on pre-pandemic period data.

Results: We examined 70,931 pregnancies in Manitoba during the study period (1 October 2016-31 March 2021). During the first quarter of 2021, the absolute differences in the observed and forecasted PTB and stillbirth percentages were 2.05% and 0.04%, respectively. Immediately after the implementation of COVID-19 restrictions, there was no significant change in the levels of both PTB (p=0.0942) and stillbirth (p=0.9584) rates. However, over the pandemic period, the PTB change in slope significantly decreased with parameter estimate (β_3)=-0.629; SE=0.226 and p=0.005. The change in slope in the stillbirth rate was not significantly different during the COVID-19 period (β_3=-0.0122; SE= 0.0801; p=0.878).

Conclusion: The pandemic led to an unprecedented and substantial reduction in the incidence of PTB in Manitoba, Canada. While the onset of COVID-19 pandemic restrictions was not associated with significant effects on PTB and stillbirth rates, we observed a significant lagged effect on PTB rates. Further studies are needed to detect future trend changes during subsequent pandemic waves and assess potential underlying mechanisms.

Determination of the Adherence Patterns to the Use of Anticoagulants Among Atrial Fibrillation Patients in Alberta Using the Interdisciplinatry Chronic Disease Collaboration Data Repository

Joel Adekanye, Amity Quinn, Derek Chew, Stephen Wilton, Tolulope Sajobi

Libin Cardiovascular Institute of Alberta, University of Calgary, Calgary, AB

Introduction: Atrial fibrillation is a disease of public health importance. Despite the wealth of knowledge and expertise available in cardiovascular science, the incidence and prevalence of atrial fibrillation continues to increase. Atrial fibrillation patients develop complications such as stroke and myocardial infarction. Atrial fibrillation results in a four-fold increased risk for developing stroke. It accounts for about 50,000 stroke cases annually in Canada. One of the goals of management of atrial fibrillation patients is the prevention of stroke. To achieve this, oral anticoagulants (OAC) are prescribed. Oral anticoagulants significantly reduce the risk of developing stroke by 65% among atrial fibrillation patients. Despite the proven efficacy of oral anticoagulants in the prevention of stroke among atrial fibrillation patients, some people end up not adhering to these prescriptions. When patients do not adhere to prescriptions, they may have worse treatment outcomes, increased hospital visits and stay which means increased healthcare costs. This study aims to examine the adherence to the use of oral anticoagulants among atrial fibrillation patients in Alberta.
Methods: This study will be a retrospective cohort study between April 1, 2008 and March 30, 2020 using de-identified data from the Interdisciplinary Chronic Disease Data Repository. To be included in the study, a new diagnosis of atrial fibrillation must be made in an adult who is at least 18 years old. The patient must be followed up for at least a year after diagnosis and commenced on an oral anticoagulant during this period. Outcomes of interest are stroke and bleeding. To determine adherence, the proportion of days covered (PDC) will be used. The PDC will be calculated for each 90 day window. in the case of over-refill, the date of refill for the second prescription will be adjusted to when the medication supply for the current refill is estimated to be completed. Next, we will do a subgroup analysis using pampilon deprivation index and comorbidities such as hypertension, diabetes and renal function to understand how these factors influence adherence to the use of prescribed oral anticoagulants among atrial fibrillation patients.

Acute Intermittent Hypoxia Augments Cardiac Function in Rodents with Spinal Cord Injury: A Preliminary Investigation

Mehdi Ahmadian[1,2,3], Liisa Wainman[2,3,4], Erin Erskine[2,3,4], Glen E. Foster[5], Christopher R. West[2,3,4]

[1]School of Kinesiology, Faculty of Education, UBC, Vancouver, BC; [2] International Collaboration on Repair Discoveries, UBC, Vancouver, BC; [3] Centre for Chronic Disease Prevention and Management, UBC, Kelowna, BC; [4] Department of Cellular and Physiological Sciences, Faculty of Medicine, UBC, Vancouver, BC; [5]Centre for Heart, Lung, & Vascular Health, School of Health and Exercise Sciences, University of British Columbia, Kelowna, BC

Introduction: High-thoracic spinal cord injury (SCI) impairs heart function due to the loss of supraspinal sympathetic control. Acute intermittent hypoxia (AIH; exposure to short, repeated bouts of reduced inspired oxygen) is a novel therapeutic that has shown promising outcomes in eliciting neural plasticity and improving respiratory and motor function in rodents and humans with SCI, yet the cardiovascular effects of this therapy have not been investigated. We explored whether AIH can augment cardiac function by inducing plasticity in the sympathetic nervous system (SNS) in a rodent model of high-thoracic SCI at 2 weeks following injury.
Methods: Eleven male Wistar rats (250-350g, 10-11wks) were anesthetized and ventilated. Left ventricular pressure-volume catheterization was performed to measure cardiac pressure-generating capacity (maximum pressure, P_{max}, and dP/dt_{max}) at baseline, 30 min, 60 min, and 90 min post-AIH. The AIH protocol consisted of 10 bouts of hypoxia (10% inspired O_2 balanced with N_2) lasting for 60 s, followed by 2 min of hyperoxia (100% inspired O_2).
Results: Relative to pre-exposure, cardiac pressure-generating capacity significantly increased at 60 min (9% for P_{max}, 95±12 vs. 104±9 mmHg, P=0.002; 16% for dP/dt_{max}, 6217±884 vs. 7092±1156 mmHg/s, P=0.01) and 90 min (12% for P_{max}, 95±12 vs. 106±9 mmHg, P=0.002; 18% for dP/dt_{max}, 6217±884 vs. 7263±1254 mmHg/s, P=0.01) post-exposure.
Conclusion: These preliminary findings suggest that AIH can augment cardiac function in a rodent model of high-thoracic SCI. Introducing such an intervention may provide a straightforward, non-pharmacological therapy in people with SCI suffering from reduced cardiovascular function associated with the loss and/or weakened SNS activity.

The Incorporation of Novel Analyses to Identify Genes and Pathways Involved in Age-Related Hearing Impairment

Samah Ahmed[1], **Britt Drogemoller**[1-3]

[1]Department of Biochemistry and Medical Genetics, University of Manitoba; [2]The Children's Hospital Foundation of Manitoba; [3]Research Institute in Oncology and Hematology, Winnipeg, MB

Introduction: Age-Related Hearing Impairment (ARHI) affects one-third of the population over 65 years. ARHI has a large impact on people's quality of life and the national economy. Both genetic predisposition and environmental factors contribute to the pathology of ARHI. Although Genome-Wide Association Studies (GWAS) have uncovered genetic variants underlying ARHI, there are large gaps in our understanding of all the genetic factors involved in this diverse group of hearing phenotypes. This may be due to challenges associated with accurately phenotyping large cohorts of patients with ARHI. In this study, we will use machine learning to classify hearing loss based on audiogram measurements.

Methods: We have obtained genomic and audiogram data from 30,097 healthy individuals (age: 45 to 86 years) participating in the Canadian Longitudinal Study on Aging (CLSA). We have calculated the geometric mean of audiogram measurements in both ears and selected the better ear for further analyses. We will build a machine learning model to classify audiograms for CLSA individuals into (i) older-normal hearing; (ii) metabolic hearing; (iii) sensory hearing; and (iv) hidden hearing loss. These data will be used to perform a GWAS to identify genetic variants that are associated with different types of hearing loss. Finally, functional enrichment analyses will be conducted to identify genetic pathways underlying hearing variability.

Results: Initial investigations of the CLSA cohort revealed that we have complete audiogram measurements for 27,208 individuals. Audiometric measures revealed that hearing declines with age in all frequencies, but this effect is more pronounced at higher frequencies. Females show worse hearing at lower frequencies (0.5 and 1 kHz) while males show worse hearing at high frequencies (4-8 kHz). Among individuals with normal audiograms, 2.25% reported hearing difficulty and 26% reported hearing difficulty in background noise, indicating hidden hearing loss.

Conclusion: Initial analyses have shown that there are differences in hearing between males and females. To account for these differences, we will perform GWAS in males and females separately. This will be the first large-scale GWAS of ARHI that does not rely on self-reported phenotypes. These novel analyses will provide increased power to uncover new genes associated with ARHI.

Visualizing the Migratory Behaviors of Latent HIV-Infected CD4+ T-cells *in vitro* and *in vivo*

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Introduction: Antiretroviral therapy (ART) has helped HIV become a manageable chronic infection by repressing viremia to undetectable levels in People living with HIV (PLWH). However, this treatment is not curative, as treatment interruption causes viremia to rebound to pre-treatment levels due to viral latency, a state of reversible non-productive infection of mainly CD4+ T cells established early during acute infection. Thus, viral latency poses a significant barrier to achieving HIV cure and requires a better understanding of latency maintenance to develop effective eradication measures. Latently-infected CD4+ T cells are long-lived and are largely found in various lymphoid organs, but their migratory behaviours and recirculation properties *in vivo* are unknown.

Methods: To address the unknown question, I generated a fluorescent protein-based HIV reporter system that allows us to better understand HIV latency generation and maintenance, with an advantage of reliably tracking latently-infected cells over time. The new full-length, R5-tropic dual-fluorescent HIV reporter encodes two fluorescent markers: Nef-ZsGreen fusion protein under the control of the HIV-LTR and dTomato protein driven by constitutively expressed EF1a-HTLV1 promoter. Infection of primary CD4+ T-cells with this reporter clearly differentiated productively-infected (dTomato+/-ZsGreen1+) and latently-infected (dTomato+ZsGreen1-) cells by flow cytometry and microscopy.

Results: Our live-cell imaging studies in 3D collagen showed that latent CD4+ T-cells displayed robust migration similar to that of uninfected CD4+ T-cells, suggesting that the production of HIV proteins is suppressed and that latently infected CD4+ T-cells retain normal migratory behaviours. Using humanized BLT mice, we are investigating whether latent CD4+ T-cells retain their migratory ability within and between lymph nodes *in vivo* and their ability to access survival signals required for their long-term survival.

Conclusion/Significance: This study will be the first to visually characterize latently-infected CD4+ T-cells *in vivo*. Studying the localization, distribution and migration of these cells will reveal novel insights into latent T cell behaviours and their potential interaction with other cells that help maintain their survival in tissues. My study will identify possible "tissue niches" where latent T cells reside in order to better understand ways to disrupt latent T cell expansion and survival in ART-treated individuals.

Hepatocyte-Targeted Delivery of Imiquimod Reduces Hepatitis B Virus Surface Antigen: a Potentially Improved Therapeutic for Chronic Hepatitis B

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Introduction: Hepatitis B virus (HBV) can rapidly replicate in the hepatocytes after transmission, leading to chronic hepatitis, liver cirrhosis and eventually hepatocellular carcinoma. Interferon a (IFN-a) is included in the standard treatment for chronic hepatitis B (CHB). However, this therapy causes serious side effects. Delivering IFN-a selectively to the liver may enhance its efficacy and safety. Imiquimod (IMQ), a Toll-Like Receptor (TLR) 7 agonist, stimulates the release of IFN-a that exhibits potent antiviral activity. However, the poor solubility and tissue selectivity of IMQ limits its clinical use. Here, we demonstrated the use of lipid-based nanoparticles (LNPs) to deliver IMQ and increase the production of IFN-a in the liver.
Methods: We encapsulated IMQ in two liver-targeted LNP formulations: phospholipid-free small unilamellar vesicles (PFSUVs) and DSPG-liposomes targeting the hepatocytes and the Kupffer cells, respectively. *In vitro* drug release/retention, *in vivo* pharmacokinetics, intrahepatic distribution, IFN-a production, and suppression of serum HBV surface antigen (HBsAg) were evaluated and compared for these two formulations.
Results: PFSUVs provided >95% encapsulation efficiency for IMQ at a drug-to-lipid ratio (D/L) of 1/20 (w/w) and displayed stable drug retention in the presence of serum. DSPG-IMQ showed 79% encapsulation of IMQ at 1/20 (D/L) and exhibited ~30% burst release when incubated with serum. Within the liver, PFSUVs showed high selectivity for the hepatocytes while DSPG liposomes targeted the Kupffer cells. Finally, in an experimental HBV mouse model, PFSUVs significantly reduced serum levels of HBsAg by 12-, 6.3- and 2.2-fold compared to the control, IFN-a, and DSPG-IMQ groups, respectively.
Conclusion: The results suggest that the hepatocyte-targeted PFSUVs loaded with IMQ exhibit significant potential for enhancing therapy of CHB.

Utilization of master's-prepared Omani nurses working in clinical settings in the governmental health care system: results of a national survey

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Aim: To identify characteristics, utilization, and factors impacting the utilization of master's-prepared Omani nurses working in clinical settings in the governmental health care system in Oman.
Design: A quantitative study using a descriptive cross-sectional survey.
Methods: A link to an online self-administrated survey was sent to 111 master's-prepared Omani nurses working in clinical settings at the Ministry of Health affiliated hospitals and the Sultan Qaboos University Hospital from November 2020 to February 2021.
Results: 61 completed the survey for a response rate of 54.9%. Respondents were mainly utilized in administrative and educational roles. Only 10% of respondents were involved in direct clinical care. Respondents believed they spent more time than ideal in organizational leadership and less than ideal in research. Role ambiguity, lack of clear job descriptions, and administrator support were the commonly reported barriers. Almost half of the respondents believed that they were not currently utilized to the maximum of their educational preparation.
Conclusions: Utilization of Master's prepared Omani nurses could be enhanced and maximized, especially, in direct clinical roles, by increasing role clarity and providing administrative support from stakeholders.
Impacts: To our knowledge, this is a first study explored the main characteristics, utilization and factors impacting the utilization of Master's prepared Omani nurses. To meet the national strategic plan of maximizing the utilization of the nursing workforce, patient, organization, and health care system needs that can be met by master's-prepared Omani nurses should be identified. National strategies are needed to assess how current and future master's-prepared Omani nurses could be better utilized to meet those needs. Strategies should focus on promoting the clinical roles of master's-prepared Omani nurses by increasing role clarity, establishing well-defined job descriptions and increasing support from nursing administrators and policymakers. Different stakeholders should be engaged in establishing and implementing those strategies.

The Role of Scleraxis in Inducing Vascular Stiffness

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Vascular dysfunction underlies numerous significant diseases including diabetes, atherosclerosis and hypertension. Vascular dysfunction can be a result of altered smooth muscle contraction/relaxation, and impaired endothelial cell function within the vessel wall. Vascular fibrosis involves an increase in the thickness of vessel wall. This contributes to either an increase in extracellular matrix (ECM) synthesis or induced vascular smooth muscle proliferation within the vessel wall, or both, causing stiffer vessels with impaired tone and reduced lumen diameter. Our lab identified the transcription factor scleraxis as a novel master regulator of fibrotic signaling in the myocardium, showing scleraxis is sufficient to induce fibroblast to myofibroblast phenotype conversion, a critical step in the development of fibrosis, and directly up-regulates ECM genes in cardiac fibroblasts. Angiotensin II (AngII) was reported to induce vascular fibrosis via activation of the transcription factor Smad3 in aortic vascular smooth muscle cells. Our lab has shown that Smad3 physically interacts with scleraxis, and critically requires scleraxis to drive TGFβ/Smad fibrotic signaling in cardiac fibroblasts. Our preliminary data has revealed that scleraxis is detectable in the arterial wall, and scleraxis expression is elevated in high pressure versus low pressure regions of vessels. Loss of scleraxis in the aortas of scleraxis knock-out mice reveals a discontinuation and disarrangement in the structure of vascular wall. We thus hypothesize that scleraxis is sufficient and necessary to induce vascular fibrosis. Pressure myography data reveals reduced compliance of 3rd order mesenteric arteries of smooth muscle-specific scleraxis overexpression mice. Also, our data shows that the compliance is significantly increased in AngII-induced scleraxis overexpression mice with a relative increase in telemetry blood pressure measurements. Histological sections suggest a reduction of the translamellar ECM accumulations in AngII-induced scleraxis overexpression aortas. To summarize, scleraxis may contribute to vascular stiffness by inducing vascular smooth muscle proliferation.

BCL2L13 Regulation of Non-Small Cell Lung Cancer Metastasis via Mitophagy and Anoikis

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Introduction: Lung cancer is one of the most important causes of cancer-related death worldwide and Non-Small Cell Lung Cancer (NSCLC) comprises ~80% of lung cancer cases. Mitophagy, a selective autophagy, refers to continuous mitochondrial housekeeping, ensuring recycling of damaged mitochondria. Our group has recently showed that general autophagy is possibly associated with NSCLC metastasis through regulation of epithelial to mesenchymal transition regulation. Also, one of the requirements of metastasis is acquiring a mesenchymal phenotype and resistance to detached induced apoptosis (anoikis) in tumor cells. Therefore, we continued our investigation on regulation of NSCLC metastasis focusing on mitophagy and anoikis. Several mediators are involved in mitophagy induction including BCL2L13, a member of the Bcl2 family. It has been shown that BCL2L13 is associated with tumor metastasis. In the present study, we have investigated the regulatory role of BCL2L13 in NSCLC metastasis via mitophagy and anoikis

Methods: Human A549 and mouse LLC lung cancer cells were treated with transforming growth factor-beta1 (TGFβ1) to analyze mitophagy induction and subcellular localization of BCL2L13 using Immunoblotting, Immunocytochemistry (ICC) and Transmission Electron Microscopy (TEM). Next, BCL2L13 gene was inactivated in human A549 and mouse LLC lung cancer cells using specific shRNA against BCL2L13. BCL2L13 role in cellular phenotype and resistance to anoikis was evaluated by immunofluorescence microscopy, immunoblotting and flow cytometry.

Results: We showed that TGFβ1 induces mitophagy ([LC3β-II lipidation, p62 and TOMM20 degradation using immunoblotting for mitochondrial fraction]; [colocalization of LC3 puncta and MitoTracker, colocalization of TOMM20 and LAMP1 using ICC]; [mitochondrial structures in autophagosomes using TEM]) and the mitochondrial localization of BCL2L13. Furthermore, we showed that mesenchymal markers [Vimentin and N-cadherin] are upregulated in Bcl2l13$^{-/-}$ cells, indicating acquirement of a mesenchymal phenotype. Our flow cytometry results also showed that apoptosis is significantly reduced in BCL2L13$^{-/-}$ cells compared to wild-type cells indicating anoikis resistance in BCL2L13$^{-/-}$ cells.

Conclusion: Our results highlight a possible role for BCL2L13 in mitophagy and anoikis resistance in NSCLC cells. We will later address how BCL2L13 is involved in regulation of NSCLC phenotype and anoikis resistance and will also address it in NSCLC animal and human model.

Development and Evaluation of a Broad-Spectrum Coronavirus Therapeutic: ACE2-Fc

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Coronavirus outbreaks have been increasing in both frequency and magnitude for the last 20 years. Prior to 2002, there were two identified human coronaviruses. Now in 2022 there are seven, potentially eight. From severe acute respiratory syndrome coronavirus (SARS-CoV) in 2002, to Middle East respiratory syndrome coronavirus (MERS-CoV) in 2012 and SARS-CoV-2 in 2019, coronavirus outbreaks have continued to escalate, causing more infections and more deaths with each successive outbreak. SARS-CoV-2 was declared a pandemic on March 11, 2020 by the World Health Organization. To date, there have been around 460 million cases and over 6 million deaths, worldwide. The need for therapeutic interventions for SARS-CoV-2 is continuing, but even greater is the need for broad spectrum coronavirus therapeutics that may be used for this and other coronavirus outbreaks. Due to the critical role of ACE2 in the replication of SARS-CoV, SARS-CoV-2 and human coronavirus (HCoV)-NL63, ACE2 has become a prime target for therapeutic intervention. Groups have tested chimerics of ACE2 fused to an Fc protein: ACE2-Fc as a decoy molecule that would bind the receptor binding domain (RBD) of SARS-CoV-2 with the same or greater affinity than ACE2 on the surface of cells. Here is described the production and evaluation of seven ACE2-Fc chimerics, and the down-selection to one lead candidate as a potent inhibitor of SARS-CoV-2 cell entry. All ACE2-Fc chimerics were sequence verified, their expression was confirmed in HEK293Ts using western blots and their relative neutralization capacity was tested in *in vitro* neutralization assays. Evaluation of these constructs led to the identification of a lead construct, which was a potent inhibitor of a SARS-CoV-2 D614G pseudotype luciferase reporter virus. Additional testing of the lead ACE2-Fc construct in wild-type SARS-CoV-2 also demonstrated potent neutralization. Further evaluation of this protein *in vivo* will allow for determination of dose range, and how well this protein is tolerated in a live host. ACE2-Fc would fill a potential gap in early treatment and would have the unique benefit of being a broad-spectrum therapeutic able to target infection with multiple different coronaviruses.

Pregnant and Early Parenting Indigenous Women Who Use Substances in Canada: A scoping review of health and social issues, supports, and strategies

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Introduction: This study reviews and synthesizes the literature on Indigenous women who are pregnant/early parenting and using substances in Canada to understand the scope and state of knowledge to inform research with the Aboriginal Health and Wellness Centre of Winnipeg in Manitoba.
Methods: A scoping review was performed searching ten relevant databases, including one for grey literature. We analyzed 54 articles/documents.
Results: Themes include: 1) cyclical repercussions of state removal of Indigenous children from their families; 2) compounding barriers and inequities; 3) prevalence and different types of substance use; and 4) intervention strategies.
Conclusions: Recommendations for future research are identified and discussed.

Investigating the Relationship Between Infant Feeding Practices and Basal Immune Biomarkers of One-Year-Old Infants in the CHILD Cohort Study

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Introduction: Human milk consumption has been associated with infant immune system development; however, the mechanisms governing this relationship remain unclear. Nuances in infant feeding practices - such as human milk feeding duration, exclusivity, and method (e.g. directly from the breast, or pumped and bottled) - have been associated with gut microbiota composition and infant health outcomes. This study aims to understand the relationship between infant feeding practices and immune system development in the first year of life

Methods: We will use data from a subset of 670 infants from the CHILD Cohort Study. Hospital records and questionnaires completed by parents at 3, 6, and 12 months were used to obtain measures of infant human milk feeding duration, exclusive human milk feeding duration, and method of human milk feeding. The Olink Target 96 Inflammation assay was used to measure the normalized expression of 92 inflammation-associated biomarkers in serum collected at one year of age. The collective expression of these biomarkers indicates immune system activity and reflects immune system development. Associations will be investigated using descriptive statistics, multivariate regression modeling, Spearman rank correlation, and principal component analysis. Infant (e.g. sex, birth weight), maternal (e.g. age, BMI), and early life (e.g. birth mode, siblings) factors will be considered as confounders, effect modifiers, or explanatory variables.

Results: Biomarker data was intensity normalized and batch corrected to produce a working dataset on a log2 scale. Of the 92 biomarkers, 76 were detectable in >50% of infant samples and included in analysis. In preliminary analyses, 6 biomarkers show potential associations with human milk feeding duration (FGF-21, EN-RAGE, CD6, CD244, IL-10RB, CCL20).

Significance: Findings from this project will advance knowledge about how different infant feeding practices are related to immune system development. Ultimately, this research will help us understand how human milk shapes the infant immune system and how to provide the best start to life for infants.

Multifaceted Regulation of Viral Infection by mRNA Translational Control

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Viral pathogens pose ongoing threats to public health and our cells utilize sophisticated anti-viral programs to defend against these pathogens. The transcriptional arm of this defense system has been extensively studied. However, although mRNA translation plays key roles in different cellular processes, its role in this anti-viral defense system is poorly characterized. Here, we provide a time-resolved survey of translating ribosomes during infection of three clinically relevant enteroviruses: Poliovirus, a singlestranded RNA (ssRNA) virus and the causative agent of poliomyelitis with an effective vaccine and two non-polio enteroviruses, Enterovirus D68 (EV-D68) and Coxsackievirus B3 (CVB3), with links to acute flaccid myelitis and myocarditis conditions respectively and a worldwide upswing in the 21st century. We profiled both transcriptome and translatome of these three viruses during one cycle of viral replication, from early infection of the host cells to the appearance of cytopathic effects as a sign of cell demise. During early infection, we identified a significant number of genes with differential translation efficiency. Among these genes were several known mediators of anti-viral immunity such as NCOA7. To understand the functional role of these translationally regulated genes in host infection, we intersected the virus translatome with previously published genome-wide CRISPR perturbation data and identified over 30 genes common in both datasets. Targeting these genes could provide a new approach for antiviral interventions. For example, we show that the genetic inhibition of a chromatin remodeling factor inhibits replication of a diverse group of viruses. In summary, our data reveals post-transcriptional mechanisms for the tight regulation of pro and anti-viral genes and uncovers novel regulatory nodes for the translational arm of the innate immunity.

Selective or Specific M1 Muscarinic Receptor Antagonists Exhibit Biased Agonism at the M1 Receptor via β-Arrestin Signaling

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Introduction: Selective (pirenzepine; PZ) and specific (muscarinic toxin 7; MT7) antagonists of the M1 muscarinic receptor (M1R) reverse nerve degeneration in rodent models of peripheral neuropathy. These drugs drive axonal outgrowth of adult sensory neurons and signaling is mediated via extracellular-regulated protein kinase (ERK) and β-arrestin. In order to understand the pharmacological mechanism of action of PZ and MT7, we investigated whether these drugs possess biased agonism at the M1R mediated via Beta-arrestin signaling.

Methods: Nano bioluminescence resonance energy transfer (NanoBRET) assay was employed to measure ligand-induced β-arrestin 2 recruitment to the hM1R in HEK293 cells co-expressing hM1R-Nluc and Halo-tagged β-arrestin 2. Intracellular IP1 levels were measured using IP-One assay (Cisbio Bioassays, USA) in both HEK293 and DRG sensory neurons. ERK phosphorylation was measured by immunoblotting. To determine phosphorylation sites at hM1R, HEK293 cells were transfected with hM1R-Halo, treated with PZ (1μM, 30min), and hM1R were pulled down, purified, and enriched on SDS-PAGE and sent for proteomic analysis. M1R internalization assay was performed using Nano-Glo® HiBiT Extracellular Detection System (Promega).

Results: Both carbachol and muscarine induced Halo-tagged β-arrestin 2 recruitment to hM1R-Nluc in a dose-dependent manner at 5 min of treatment. Pirenzepine and MT7 induced Halo-tagged β-arrestin 2 recruitment to hM1R-Nluc in a dose-dependent manner at longer time points (e.g. after 30 min). Pirenzepine and MT7 treatments did not generate IP1 and significantly inhibited muscarine-induced IP1 generation in HEK293 cells and DRG neurons. Both drugs increased ERK phosphorylation in a time-dependent manner. Pirenzepine treatment was associated with high levels of phosphorylation at residues S251 and T254 within the ICL3 of hM1R. Unlike carbachol, PZ and MT7 increased surface expression of hM1R.

Conclusion: This study for the first time provides molecular evidence to support a role for selective/specific muscarinic receptor antagonists acting as biased agonists at the M1R. This novel signaling pathway may contribute to driving axonal regeneration in adult sensory neurons and could be mobilized for nerve repair in neuropathic disease.

Validation of Algorithms to Identify Human Immunodeficiency Virus (HIV) Cases Using Administrative Data in Manitoba

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Introduction: HIV is a sexually transmitted and blood-borne infection which, if left untreated, will progress to AIDS within 10 years and death within 15 years. The occurrence of HIV infections and AIDS-related deaths are influenced by inequities in health care, human rights, education, and wealth. Evidence-based action against inequities is required to end the AIDS pandemic. To date, most HIV/AIDS administrative data algorithms were validated using US Medicare/Medicaid data. Two Canadian studies (Ontario and BC) validated HIV administrative data algorithms, but the prescription and laboratory data used varied from those available in Manitoba and the reference standard used in Ontario was not population-based.

Methods: The objective of this study was to validate algorithms to identify Manitobans living with HIV using population-based physician visit, hospitalization, and antiretroviral prescription data. Fifteen algorithms requiring two or three years of data were evaluated. ICD-9-CM codes 042-044, V08, 795.71, and 079.53 and ICD-10-CA codes B20-B24, R75, Z21, F02.4 and O98.7 were used to identify HIV-related hospitalizations. ICD-9-CM codes 042-044 and V08 were used to identify HIV-related physician visits. Confirmatory HIV tests from Cadham Provincial Laboratory, available between 2007 and 2018, were the reference standard.

Results: The validation cohort included 1,454,010 Manitobans with at least three years of continuous health insurance coverage between 2007 and 2018. Of these individuals, 1,589 were HIV cases and 1,452,421 were HIV non-cases. Algorithm sensitivity ranged from 81.1% to 96.5%. PPV ranged from 44.1% to 96.0%. Specificity and NPV were very high for all algorithms. Youden's J Statistic ranged from 0.81 to 0.96. Kappa ranged from 0.61 to 0.91. AUC ranged from 0.91 to 0.98.

Conclusion: One or more physician visits for HIV, one or more hospitalizations for HIV, or two or more antiretroviral prescriptions in two years is best to identify all possible HIV cases, without concern for the number of false positives. Six or more physician visits in two years is best to identify as many true positive HIV cases as possible with minimal false positives. Three or more physician visits in two years will most accurately distinguish between HIV cases and HIV non-cases.

Genetic-Wide Association Study of Pediatric Obsessive-Compulsive Behaviors, the Correlation with Imaging Endophenotypes

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Introduction: Obsessive-compulsive disorder (OCD) is a common mental health disorder that involves persistent intrusive thoughts and/or repetitive behaviors. Obsessive-compulsive behaviors (OCB) are the core features of OCD. Here, we focus on OCB measured via the Child-Behavior Checklist OC-Subscale (CBCL-OCS) which has been shown to be highly heritable in pediatric twin studies with high sensitivity and moderate specificity. Moreover, a large body of literature supports the involvement of the cortico-striato-thalamo-cortical model in the pathophysiology of OCD based on magnetic resonance imaging. The overall goal is to identify genetic variants associated with OCB and imaging endophenotypes and determine the relationship between brain activity and childhood OCB.

Methods: Genotyping analysis was performed on non-related pediatric subjects (age 8-25), including cases with OCB and healthy controls. Four different genome-wide arrays were used. Quality control (QC) and association analysis were carried out using PLINK software. Genome-wide association study (GWAS) was performed using linear regression. CBCL-OCS total score was considered as a quantitative trait in the association analysis. Later the imputation analysis was performed using Minimac4. Consequently, other post-GWAS analyses in process. Further, we conducted imaging analysis to identify putative neuroimaging endophenotypes, including cortical thickness and myelin water fraction images.

Results: After QC, 627 samples with corresponding CBCL-OCS scores, and 1.8 million markers (single nucleotide variants (SNV)) passed the QC filters. Although none of the SNVs passed the p-value threshold for genome-wide significance, a number of markers were observed to be close to significance. The top variants were identified for further quantitative trait loci analysis. Initial SNV-based and gene-based analyses showed that observed two genes from 4p13 and 12p11 loci have differential gene expression in brain tissue. Analyses of imaging endophenotypes are in progress.

Conclusion: We have deep phenotype-genotype data for unrelated pediatric subjects. Quantitative GWAS was carried out. The sample size, low for GWAS, is likely the main reason that none of the markers passed stringent p-value significance threshold, and we are addressing this by genotyping additional participants. This is a unique study that will be the largest to date to report genetic markers, and imaging endophenotypes of susceptibility to OCB in a pediatric-clinic-based population.

The Spinal Targets of Subpopulations of Serotonergic Neurons

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Introduction: The descending serotonin (5-Hydroxytryptamine, 5-HT) system is known to have multiple roles in modulating sensory mechanisms, motor control, and autonomic functions. The 5-HT system is thought to inhibit sensory systems while facilitating motor control, but evidence has shown that the actions of 5-HT depend on the originating nuclei and receptor subtype at the spinal target. The innervation of the spinal cord by 5-HT fibers and the location of 5-HT receptors on interneurons has been well studied, with recent evidence of 5-HT boutons on motoneurons and interneurons, both excitatory and inhibitory. However, it is still unknown whether the same descending 5-HT neurons that terminate on motoneurons collateralize and also terminate in the dorsal horn; nor is there a clear consensus on the preferential distribution of 5-HT fibers targeting inhibitory versus excitatory interneurons. We characterized the cervical, thoracic and lumbar terminations of 5-HT fibers from discrete brainstem regions and terminations near inhibitory and excitatory synapses.

Methods: An adeno-associated viral vector (rAAV) tagged with a fluorescent reporter was injected into subpopulations of 5-HT neurons in genetically modified rats (Tph2-iCre). In this rat model, Cre recombinase is restricted to 5-HT neurons and thus the fluorescent reporter is only expressed in the presence of Cre. After transcardial perfusion following rAAV-construct injections, thin spinal cord sections (16-50 µm) were used with antibody against the fluorescent reporter for 5-HT. This was combined with antibodies targeting excitatory and inhibitory terminals. Quantification of the co-localized 5-HT terminals and excitatory and/or inhibitory synapses was analyzed using IMARIS software.

Results: Comparison of cervical, thoracic and lumbar tissue from the same animals showed extensive collateralization in all segments examined. Terminals were found in both dorsal and ventral locations within the spinal gray matter. Preliminary analysis of colocalization is underway.

Conclusion: Small subpopulations of 5-HT influence neural activity at various levels of the spinal cord based on the collateralizations observed here. Ventromedial 5-HT neurons can link activity of multiple segments throughout cervical and lumbar regions. Future research will determine whether these collateral fibers have a functional role in synchronizing activity.

CTRP8 is a Novel Interaction Partner of Delta-like Protein Homolog 1 (DLK1)

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Background: The adiponectin/C1Q-tumor necrosis factor related protein (CTRP) family has functions in metabolism, immunity, and cancer. Among the CTRP family, C1Q-tumor necrosis factor-related peptide 8 (CTRP8) remains the least well characterized in terms of function, receptors and other binding partners. We previously described a novel CTRP8 and relaxin receptor RXFP1 ligand-receptor signaling system that impacts actin cytoskeletal dynamics, proliferation and DNA repair in the fatal brain tumor, glioblastoma (GBM). Here we investigate a new interaction between CTRP8 and the transmembrane EGF-like protein Delta-like homolog 1 (DLK1). DLK1 is a non-canonical NOTCH1 inhibitor that impacts proliferation, migration, differentiation and is considered a marker of stemness in several cancers. In GBM, elevated DLK1 expression increases tumor aggressiveness and is correlated with poor prognosis. DLK1 signaling is complex and versatile, thus there is growing interest in further understanding its regulatory mechanisms in a cancer context.
Hypothesis: CTRP8 may interfere with the ability of DLK1 to bind to and inhibit NOTCH1. CTRP8-DLK1 may indirectly augment NOTCH1 signaling to regulate GBM stemness.
Methods: A yeast-two hybrid screen (Y2H) using human placental cDNA library was used to screen for potential interaction partners of CTRP8. Co-immunoprecipitation with HEK293 cells expressing full-size and truncated DLK1 was used to confirm protein-protein interaction with CTRP8. We treated DLK1 expressing cells with recombinant human CTRP8 to observe the effect of CTRP8 on DLK1 protein processing and downstream signaling.
Results: The Y2H identified DLK1 as a novel CTRP8 interaction partner. Using co-immunoprecipitation we identified that full-size DLK1 and a truncated form of DLK1 interact with CTRP8. In DLK1-expressing cells, NOTCH1 activation was suppressed as indicated by reduced cleavage of NOTCH1 receptor. Treatment with CTRP8 increased cleavage of NOTCH1 receptor, suggesting that CTRP8-DLK1 interaction prevents the inhibitory effect of DLK1 on NOTCH1 activation
Significance: This is the first study investigating CTRP8 as a potential novel regulator of NOTCH1 signaling through DLK1. Our studies may reveal a novel regulatory system in GBM involving CTRP8 and DLK1 with the potential for the identification of novel therapeutic targets.

Vitamin D Signaling Regulates the Expression and Function of Aire in Thymic Epithelial Cells

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Vitamin D plays important roles in the immune system. It stimulates cytokine and antimicrobial peptide production by innate immune cells, and suppresses of pro-inflammatory T-cell responses. Hormonal vitamin D (1,25-dihydroxyvitamin D; 1,25D) is generated from circulating 25-hydroxyvitamin D by the enzyme Cyp27b1. Binding of 1,25D to the vitamin D receptor (Vdr) induces its nuclear translocation and binding to vitamin D response elements (VDREs) to initiate target gene transcription. Vitamin D deficiency is associated with increased risk of infection as well as autoimmune diseases such as type 1 diabetes. Autoimmunity arises from a failure to establish T-cell tolerance to self antigens, which originates during T-cell development in the thymus through interactions with medullary thymic epithelial cells (mTECs). mTECs present tissue restricted antigens (TRAs) and induce apoptosis in overly self-reactive cells. Transcription of most TRA genes is dependent on the transcription factor, Aire (autoimmune regulator). Although vitamin D deficiency has been linked to an increased risk of autoimmunity, very little is known about the potential molecular basis of vitamin D action. We hypothesize that vitamin D signaling in the thymus regulates transcriptional events critical for self-tolerance. Vdr and Cyp27b1 mRNA and protein expression in Aire+ TECs and other thymic populations was confirmed by RT/PCR, microscopy and flow cytometry, and robust vitamin D signaling was observed in organotypic thymic slices and isolated TECs. Moreover, the number of Aire+ TECs was increased 2-fold in 1,25D treated thymic slices, and Aire mRNA expression was upregulated. Conversely, in Cyp27b1-/- mice, there was a reduction of Aire+ cells, Aire mRNA, and TRA mRNA expression. Thymi from Cyp27b1-/- mice presented defects in antigen presentation, thymic architecture, and mTEC differentiation, consistent with impaired non-canonical NF-κB signaling, which is critical for mTEC differentiation and Aire expression. Importantly, we also found that the Vdr recruited Aire to target genes, where it acted as a Vdr coactivator. Thus, vitamin D signaling is robust in TECs, controls TEC differentiation and induces Aire expression. We propose that vitamin D signaling may function to limit autoimmunity by enhancing TRA presentation to developing T-cells, thus promoting more efficient deletion of overly self-reactive cells.

Contribution of IRE1 Signaling to Cytokine/Chemokine Production in Paclitaxel Treated Triple-Negative Breast Cancer Cells

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Introduction: Every year over 26,000 Canadians are diagnosed with Triple-Negative Breast Cancer (TNBC). Currently, the main treatment for TNBC is neoadjuvant chemotherapy followed by tumour resection. While successful in the short term, a significant number of individuals experience tumour relapse within 3-5 years. Relapsed tumours tend to be highly metastatic and unresponsive to further treatment. To improve outcomes for individuals suffering from TNBC we need to understand why relapse occurs and use this knowledge to design new therapeutic approaches. Recent studies have shown that the activity of IRE1, an endoplasmic anchored receptor, is upregulated in TNBC cells upon treatment with chemotherapeutics including paclitaxel. Elevated IRE1 signaling has been shown to promote the production of cytokines/chemokines supportive of tumour relapse. A small-scale screen conducted in our lab identified a range of cytokines/chemokines (1) increased by paclitaxel and (2) in a manner dependent upon IRE1. To build upon this work I will confirm candidate targets (CXCL1, CXCL11, Resistin, CCL19) are increased by paclitaxel treatment and in an manner dependent upon IRE1 activity.

Methods: The TNBC cell line MDAMB231 will be treated with low dose paclitaxel (10nM) +/- IRE1 RNase inhibitor (MKC8866, 20uM) for 72 hours after which conditioned medium and cells will be collected. Immunoblotting will be used to confirm paclitaxel mediated activation of IRE1 dependent signaling and functionality of the IRE1 RNase inhibitor MKC8866 by assessing splicing of XBP1 (IRE1 target). Conditioned medium will be quantified for expression of target cytokines using R&D ELISA duosets selective for CXCL11, CXCL1, CCL19 and Resistin.

Results: Preliminary data I have generated confirms paclitaxel increases IRE1 RNase activity in MDAMB231 cells, as shown by increased XBP1 splicing which is prevented by addition of IRE1 inhibitor MKC8866. Analysis of candidate cytokines/chemokines in conditioned medium from paclitaxel +/- MKC8866 treated MDAMB231 cells is ongoing. To date, I have confirmed that CXCL1 is upregulated in response to paclitaxel treatment and in a manner partially dependent upon IRE1 RNase signaling.

Conclusion: The current findings indicate a functional relationship between chemotherapy-induced cytokine/chemokine upregulation and IRE1 expression.

CANFLAX: Can flaxseed “Milk” Prevent Broken Hearts in Women With Breast Cancer?

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Introduction: Cardiovascular disease and cancer are major public health concerns worldwide. These two diseases are intricately linked, as the treatment of cancer may cause detrimental effects to the heart. Despite the beneficial effects of the anti-cancer agents Doxorubicin and Trastuzumab (DOX+TRX) for improving overall survival in women with breast cancer, cardiotoxicity remains a serious challenge for these drugs. Although recent basic sciences studies have demonstrated the cardioprotective role of flaxseed (FLX) in the prevention of DOX+TRZ mediated cardiotoxicity, little is known on the effects of this nutraceutical in the clinical setting.

Objective: The aim of the CANFLAX study is to investigate whether consumption of FLX “milk” can prevent heart failure in women with breast cancer treated with DOX+TRZ.

Methods: In this prospective randomized clinical study, prior to initiation of DOX-based chemotherapy, women with breast cancer will be randomized to either placebo oat fiber “milk” or FLX “milk” for a total of 4 months. Serial demographic data, echocardiography, and blood work will be measured at baseline, 4-months, 6-months and 12-months post intervention.

Results: Of a total 10 women (mean age 41±2 years with an average body mass index of 25±1 kg/m2) enrolled between June 2021 and Feb 2022, a total of 5 were randomized to oat fibre “milk” and 5 were randomized to FLX “milk”. The prevalence of underlying cardiovascular risk factors was low in both groups. In total, 1 (9%) participant had hypertension and 2 (18%) participants had a family history of premature coronary artery disease (CAD). None of the participants has diabetes mellitus, hyperlipidemia nor smoking history. There was no difference in the location and size of breast cancer, axillary lymph node involvement, or radiation use between the two groups. The majority of the patients 10 (91%) received 4 cycles of adriamycin and cyclophosphamide (AC), whereas 1 (9%) received 6 cycles of 5-fluorouracil, epirubicin, and cyclophosphamide (FEC). The baseline left ventricular ejection fraction values were 62±5% and 60±6%, for the oat fibre “milk” and FLX “milk” groups, respectively.

Conclusion: This ongoing clinical study will promote new standards of care that may include supplementation of FLX as a cardioprotective agent in all women receiving chemotherapy in the breast cancer setting.

Examining Risk Factors Accelerating Time-to-Chronic Obstructive Pulmonary Disease (COPD) Diagnosis among Asthma Patients

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Introduction: Asthma patients may have an increased risk for diagnosis of COPD. However, risk factors accelerating time-to-COPD diagnosis are unclear. This study aims to estimate risk factors associated with the incidence of COPD diagnosis in asthma patients.

Methods: Canada's Population Data BC was used to identify asthma patients without prior COPD diagnosis between January 1, 1998, to December 31, 1999. Patients were assessed for time-to-incidence of COPD diagnosis from January 1, 2000, to December 31, 2018. The study estimated the effects of several risk factors in predicting the incidence of COPD in asthma patients during the 18-year follow-up period. The log-logistic mixed-effects accelerated failure time model was used to estimate the adjusted failure time ratios (aFTR) and 95% Confidence Interval (95% CI) for factors predicting time-to-COPD diagnosis among asthma patients.

Results: We identified 68,211 asthma patients with a mean age of 48.2 years included in the analysis. Risk factors accelerating time-to-COPD diagnosis included: male sex (aFTR: 0.62, 95% CI:0.56–0.68), older adults (age > 40 years) [aFTR: 0.03, 95% CI: 0.02–0.04], history of tobacco smoking (aFTR: 0.29, 95% CI: 0.13–0.68), asthma exacerbations (aFTR: 0.81, 95%CI: 0.70, 0.94), frequent emergency admissions (aFTR:0.21, 95% CI: 0.17–0.25), longer hospital stay (aFTR:0.07, 95% CI: 0.06–0.09), patients with increased burden of comorbidities (aFTR:0.28, 95% CI: 0.22–0.34), obese male sex (aFTR:0.38, 95% CI: 0.15–0.99), SABA overuse (aFTR: 0.61, 95% CI: 0.44–0.84), moderate (aFTR:0.23, 95% CI: 0.21–0.26), and severe asthma (aFTR:0.10, 95% CI: 0.08–0.12). After adjustment, MA ≥0.80 was significantly associated with 83% delayed time-to-COPD diagnosis [i.e. aFTR =1.83, 95%CI: 1.54–2.17 for PDC]. However, asthma severity significantly modifies the effect of MA independent of tobacco smoking history.

Conclusion: The targeted intervention aimed to mitigate diagnosis of COPD in asthma patients should prioritize enhancing medication adherence, highlight smoking cessation programs, and address weight management in male sex.

Measuring the Adherence to Best-Practice Guidelines by Interprofessional Teams in Cardiac Surgery: Development, validation, and pilot test of an extraction tool

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Background: To ensure the delivery of high-quality patient care, a high level of adherence to best-practice guidelines by interprofessional teams practicing in postoperative cardiac surgery is needed. However, until today, no valid instrument has been developed to measure and quantify the level of adherence to best-practice guidelines by interprofessional teams.

Purpose: This study aims to develop an extraction tool to measure and quantify the level of adherence to best-practice guidelines by interprofessional teams in postoperative cardiac surgery.

Method: Development: A systematic review of randomized controlled trials and an extensive search in the literature have been conducted to retrieve the best-practice guidelines in the care given to patients and families by interprofessional teams after a cardiac surgery.

Validation: Content validation with an expert committee of ten experts (clinicians, researchers, managers) is underway. Criterion validation based on the performance measures of the Society of Thoracic Surgeons is planned.

Pilot test: The instrument will be pilot-tested from 30 patient health records who underwent a cardiac surgery in a tertiary health care center in Québec, Canada.

Results: A total of 12 best-practice guidelines were retrieved. Best-practice guidelines related to the pharmacotherapy (e.g., prescription of beta blockers), laboratory tests (e.g., monitoring of potassium), and postoperative assessments (e.g., pain management) performed by interprofessional teams with nurse practitioners have been included in the extraction tool. The literature search identified several confounding variables influencing the level of adherence to best-practice guidelines by interprofessional teams. Confounding variables related to patient (e.g., severity of illness) and interprofessional team characteristics (e.g., consultations in nutrition) have been included in the extraction tool. The content and criterion validation of the tool and the pilot test will be completed during the Winter 2022.

Conclusion: The extraction tool will support subsequent quantitative studies to measure the level of adherence to best practice guidelines by interprofessional teams and identify the effect of the adherence to these guidelines on patient, interprofessional, and organizational outcomes.

Building Capacity in Occupational Therapy Delivery: Supporting Intraprofessional Collaboration Competence in Canada

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Introduction: Occupational therapy (OT) provides unique opportunities to economically improve patient and system outcomes while decreasing health costs. An OT shortage and COVID-related pressures have exacerbated worker and resource scarcities intensifying the prevalence of unregulated health professionals, such as OT-assistants (OTAs) working in Canada. Critical to the safety, effectiveness, and efficiency of OT services is the need for enhanced intraprofessional collaboration (IntraPC) between OTs and OTAs in Canada. Enhanced professional collaboration strategically and economically improves healthcare access, quality, and outcomes. In response, educators in OT and OTA programs are tasked with addressing IntraPC in their curricula to support effective OT-OTA collaboration and supervision practices. OTs and OTAs are educated separately and interprofessional education does not prepare these disciplines for the supervisory nature of their relationship. OT-OTA IntraPC interventions in the United States have enhanced actual and perceived collaboration and are a priority for capacity-building of OT services in Canada. It is not yet known what OT-OTA IntraPC preparation is taking place in Canadian post-secondary education, and, how to best prepare OTs and OTAs to collaborate optimally. Addressing OT-OTA IntraPC is critical given the projected shortage of OTs, increased prevalence of OTAs in healthcare, known OT-OTA collaboration issues impacting quality and cost of care, and recognized benefits of IntraPC education practices.

Methods: This sequential, three-phased mixed-methods applied-research project involves an environmental scan, and the development and subsequent evaluation of a resource to support OT-OTA IntraPC preparation in entry-to-practice education in Ontario. The environmental scan involves an online survey, followed by focus groups to explore the survey results.

Results: Frequency distributions, summary, and inferential statistics will be analyzed to report on the current landscape of OT and OTA IntraPC preparation. Survey results will inform the focus group questions, which will delve deeper into the current landscape to identify faculty and graduate recommendations for educational interventions.

Conclusion: This is the first project to investigate OT-OTA IntraPC education practices in Canada, with the goal to create, evaluate and disseminate innovative resources that will support OT-OTA IntraPC preparation in OT and OTA entry-to-practice education.

Transition to Independent Practice in Pathology: A Competence by Design Approach

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Introduction: The Royal College of Physicians and Surgeons of Canada has implemented Competence by Design Program for the training of medical residents in Canada. This represents a significant shift from knowledge-based education to competency assessment and from time-based residence to competency attainment-based programs.

Methods: This presentation will show preliminary results of an ongoing mixed-methods phenomenological study which seeks to understand how the so called Big Five personality traits (conscientiousness, extraversion, agreeableness, openness, and neuroticism) may impact transition from residency programs to independent medical practice in pathology. In addition, it seeks to understand how feedback moderates residents performance based on the personality traits. The research questions for this study are: 1. What is the incidence of difficulty transitioning from residency to independent practice amongst pathologists? 2. What is the psychological traits profile for pathologists in general, and is there a difference between pathologists with difficulty and pathologists who do not experience difficulty transitioning? 3. How do pathologists perceive the effect of performance-based feedback in their learning? Data is being collected through a quantitative psychometric tool and phenomenological interviews. A t-test will be used to compare the mean scores of personality traits for groups of pathologist that experience difficulty transitioning to practice and those who do not report difficulty. Also, a logistic binary regression will be completed to identify if any personality trait may impact difficulty to transition. Phenomenological interviews will be conducted to understand the phenomenon of transitioning to independent practice and perception of feedback impact.

Results: Preliminary results seem to indicate that agreeableness is the personality trait that scored the highest amongst pathologist followed by conscientiousness, openness extraversion, and neuroticism. Data is currently being collected.

Conclusion: This study aspires to contribute to continuous improvement of the Competence by Design program in three main ways. Provide a better understanding difficulty in transitioning for residents; recognizing how feedback impacts trainees based on salient personality traits; and enhancing the preparation of residency supervisors and faculty in pathology on how to provide effective feedback.

The Use of *in vitro* Antibody Blockades Targeting Immune Checkpoint Inhibitors LAG-3 and PD-1 on iNKT Cells: Potential Implications for HIV Therapeutics

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Background: Invariant Natural Killer T (iNKT) cells are innate lymphocytes critical in combatting viral infection by bridging the innate and adaptive immune systems. Our lab showed that in HIV infection, expression of lymphocyte activation gene 3 (LAG-3), an inhibitory immune checkpoint marker, is elevated on iNKT cells and is correlated with decreased cellular functionality. Another checkpoint molecule, program cell-death-1 (PD-1), is also shown to be increased on iNKT cells in chronic HIV infection, correlating to decreased function. LAG-3 and PD-1 expression kinetics and relationship to iNKT cellular function is not well characterized, and we hypothesize that blocking LAG-3 alone, or in conjunction with PD-1 via immunotherapeutic blockades, will restore iNKT function and immune effectiveness.

Methods: Utilizing peripheral blood mononuclear cells (PBMCs) from HIV-uninfected donors (n=4), surface expression of LAG-3 and PD-1 on iNKT cells was assessed via a multi-day *in vitro* stimulation (24hr, 48hr, 4, 7, 10 day). Further, the efficacy of anti-LAG-3 and/or anti-PD-1 immune therapeutics were assessed in PBMCs via a 10-day assay (n=9), with enhanced proliferation as the main outcome monitored.

Results: Percent and median fluorescence intensity (MFI) of both LAG-3 and PD-1 peaked at Day 7 (LAG-3: 88.5%, 6163.8 MFI; PD-1: 80.5%, 7731.8 MFI), with a steep decrease by Day 10, when iNKT proliferation was at its peak. When iNKT cells were stimulated in the presence of the anti-LAG-3 or anti-PD-1 antibody blockades, there was an average 13.3-fold increase and 59.2-fold increase of the iNKT population, respectively, compared to the no blockade control. Combining anti-LAG-3 and anti-PD-1 blockade systems resulted in an average 82-fold increase in proliferation.

Conclusions: This study is the first to report the kinetics of LAG-3 and PD-1 expression on iNKT cells, and it also provides proof-of-concept for LAG-3 and PD-1 as immunotherapeutic targets, by restoring iNKT cellular proliferative ability. This blockade system will be applied *in vitro* to HIV-positive samples to assess if HIV-mediated dysregulation of iNKT function can be reversed. Future goals of these therapeutics are to ameliorate immune responses to various opportunistic infections, as well as boost viral control in a functional HIV cure approach.

Spinal Cord Injury in Mice Impacts Central and Peripheral Pathology in a Severity-Dependent Manner

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Introduction: Chronic pain is a common medical complication experienced by those living with spinal cord injury (SCI) and leads to worsened quality of life. The pathophysiology of SCI pain is poorly understood, hampering the development of safe and efficacious therapeutics. We therefore sought to develop a clinically relevant model of SCI with a strong pain phenotype and characterize the central and peripheral pathology after injury.

Methods: Mice were anesthetized using a ketamine:xylazine:acepromazine cocktail (50:5:1 mg/kg), and a partial laminectomy was performed at the vertebral levels T10-11. Moderate contusion (50 kdyn) injury with or without sustained compression (60 seconds) of the spinal cord was carried out on female C57BL/6J mice; sham-injured mice only received a laminectomy. Luxol Fast blue was used to assess demyelination in the spinal cord. Changes in CD11b and GFAP immunostaining were also quantified. Flow cytometry was used to assess myeloid and lymphoid immune cell infiltration into the spinal cord and L4-6 dorsal root ganglia. 16S sequencing was used to quantify changes in the microbiome after injury for the three groups.

Results: Mice with compression of the spinal cord exhibited significantly greater heat and mechanical hypersensitivity starting at 7 days post-injury, concomitant with reduced locomotor function, compared to those without compression. Immunohistochemical analysis of spinal cord tissue revealed significantly less myelin sparing and increased macrophage activation in mice with compression compared to those without. As measured by flow cytometry, immune cell infiltration and activation were significantly greater in the spinal cord (phagocytic myeloid cells and microglia) and dorsal root ganglia (Ly6C+ monocytes) following compression injury. We also decided to investigate the gastrointestinal microbiome, as it has been shown to be altered in SCI patients and has recently been shown to play a role in immune system maturation and pain. We found increased dysbiosis of the gastrointestinal microbiome in an injury severity-dependent manner.

Discussion: The use of this contusion-compression model of SCI may help advance the preclinical assessment of acute and chronic SCI pain and lead to a better understanding of mechanisms contributing to this pain.

Uncovering the Role of a Novel E3 Ubiquitin Ligase in B cell Antibody Diversification

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Introduction: Specialized and diverse repertoires of antibodies are critical for effective adaptive immune responses. For this to be possible, B cell lymphocytes undergo antibody diversification events where DNA mutations are intentionally accumulated to generate variation in the antibody repertoire. However, how mutations are permitted to accumulate has remained a long-standing unanswered paradox as the maintenance of genomic integrity is a fundamental rule in all organisms to prevent cellular dysregulation and tumorigenesis.

Methods: Through a CRISPR-Cas9 loss-of-function screen performed in our lab, we have identified a novel factor, FAM72A, which plays a critical role in the balance between mutagenic and faithful DNA repair.

Results: Our lab has published work showing that FAM72A plays a role in the degradation of uracil DNA glycosylase 2 (UNG2), a key enzyme in base-excision repair, thereby compromising an important DNA repair pathway that impacts the efficiency of antibody diversification. However, we have shown that FAM72A cannot be directly responsible for UNG2 degradation as it lacks the ability to ubiquitinate or degrade substrate directly. This leads me to my project where I have discovered a novel factor through our CRISPR-Cas9 screen potentially functioning in the same pathway as FAM72A to achieve UNG2 degradation. My current work provides evidence to show that this novel factor phenocopies FAM72A in antibody diversification and DNA repair.

Conclusion: This project offers insight into an important, novel pathway governing the choice between mutagenic and faithful DNA repair that directly impacts B cell antibody maturation and humoral immunity.

In the D2.mdx mouse model of Duchenne muscular dystrophy, restoring mitochondrial creatine metabolism is associated with partial improvements in muscle quality

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Introduction: Duchenne muscular dystrophy (DMD) is a fatal muscle wasting disease with limited treatments. We recently reported that skeletal muscle mitochondrial creatine metabolism is impaired in a mouse model of DMD (D2.*mdx*). Rescuing this pathway might improve muscle health given creatine can amplify ADP's ability to stimulate mitochondrial ATP production while attenuating mitochondrial reactive oxygen species production. The objective of this study was to identify whether the mitochondrial-targeted drug Olesoxime (TRO19622) would improve mitochondrial creatine-dependent bioenergetics and muscle health in D2.*mdx* mice.

Methods: Male D2.*mdx* mice received a daily oral gavage of Olesoxime (DRUG) (30mg/kg b.w.) or corn oil (VEH, vehicle) from days 10-28 of age. Age-matched wildtype (WT) animals served as a healthy control. Quadriceps and diaphragm muscle were collected, and mitochondrial bioenergetics were assessed using high resolution respirometry and high resolution spectrofluorometry in permeabilized fibre bundles. Creatine sensitivity, an index of mitochondrial creatine kinase (mtCK) activity, was assessed by comparing conditions saturating mtCK (20mM creatine) to conditions lacking creatine.

Results: The ability of creatine to stimulate respiration in WT quadriceps (+37-62% across 100- 500μM ADP) and diaphragm (+39-55%) was lost in VEH only for quadriceps but rescued by DRUG (+18-28%). Likewise, the ability of creatine to attenuate mitochondrial H_2O_2 emission in WT quadriceps (-30 to -38%) and diaphragm (-33 to -34%) was lost in VEH but was rescued by DRUG in quadriceps (-42 to -43%) and diaphragm (-23 to -26%). These improvements in creatine metabolism were related to lower serum creatine kinase (-54%, DRUG vs VEH; muscle damage marker), recovery from fatigue in quadriceps (+25-38% vs VEH), longer cage hang time (+53%), and a small increase in whole body lean volume (+3.0%) and hindlimb muscle volume (+6.5%) as assessed by microCT. Decreases in grip strength, voluntary wheel running distance, maximal stimulated force in quadriceps (*in situ*) and diaphragm (*in vitro*) observed in VEH were not improved by DRUG.

Conclusion: In summary, short-term treatment with Olesoxime rescued mitochondrial creatine metabolism which occurred in parallel to partial improvements in some indices of muscle health in D2.*mdx* mice.

The Usefulness of Machine Learning and Explainable Artificial Intelligence for Clustering of Primary Mitral Regurgitation Severity Phenotypes and Risk Stratification

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Introduction: Primary mitral valve regurgitation (MR) is a highly frequent and heterogeneous cardiovascular disease. Echocardiographic parameters are integrated using guideline-driven recommendations to identify disease severity and guide clinical management of these patients (either close clinical follow-up or surgical referral). Our objectives were to explore novel datadriven approaches using machine learning (ML) and explainable artificial intelligence (AI) to delineate phenotypes of primary MR and evaluate its prognostic value for risk stratification.

Methods: We explored 400 patients from two prospective cohorts with primary MR followed up in France (n=243; development cohort) or Canada (n=157; validation cohort). Unsupervised and supervised ML and explainable AI were used to integrate 24 standard and commonly used echocardiographic parameters to identify clusters of MR severity (i.e. phenogroups). We evaluated and compared the phenogroups' incremental prognostic value over conventional MR classification. This study endpoint was the composite of all-cause mortality and heart failure hospitalization.

Results: In the France Cohort, we identified a High Severity (HS) phenogroup (n=118) who had improved event-free survival following surgery (p<0.001) versus those without surgery, and a Low Severity (LS) phenogroup (n=125) without significant event-free survival improvement irrespectively of surgery occurrence (p=0.89). The validation Canadian cohort confirmed these observations for both HS (p=0.003, n=90) and LS (p=0.06, n=67) phenogroups. The echo-derived phenogrouping provided high predictive performance (78% of sensitivity and 89% of specificity, p<0.001). For patients conventionally classified to have moderate-to-severe or severe primary MR, the phenogrouping approach presented incremental long-term prognostic value with Harrell's Cstatistic, net reclassification improvement and integrated discrimination improvement analyses. The use of explainable AI specified how and the degree to which each echocardiographic parameter contributed to phenogroup distribution.

Conclusions: Our study reveals that novel data-driven phenogrouping approaches using explainable AI aids the integration of echocardiographic data in identifying clusters of patients with primary MR who benefit from surgical intervention. The implantation of ML-AI approaches in clinical practice could enhance risk stratification and therapeutic management of patients with primary MR.

QTc Interval During Acute Exercise in Childhood Acute Lymphoblastic Leukemia Survivor

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Introduction: Anthracycline-related cardiotoxicity is a major cause of mortality and morbidity in childhood acute lymphoblastic leukemia (ALL) survivors. Heart rhythm complications and cardiac autonomic dysfunction are both known to be developed. Current methods for detection of cardiotoxicity have limitations, particularly due to their lack of sensitivity for early detection of subclinical cardiac dysfunction. Detection of subclinical cardiac dysfunction remains a cardiologist's challenge and is essential to allow optimal therapeutic intervention. This study aimed to observe ventricular repolarization during a maximal cardiopulmonary exercise test (CPET) in childhood ALL survivors. We hypothesized that cancer treatments lead to changes in ventricular repolarization that persist over time, and that the use of CPET allows the unmasking of electrophysiological abnormalities.

Methods: A total of 250 childhood ALL survivors underwent a maximal CPET on an ergocycle, and their direct oxygen uptake was measured. All survivors were monitored continuously during the test using a 12-lead electrocardiogram. Measurements of the QT interval were completed at rest, at the end of each stage of the CPET, and during recovery. The QT interval was defined as the period from the onset of the Q-wave to the end of the T-wave, measured linearly. Values during exercise and recovery were corrected (QTc) using a specific group equation.

Results: All survivors (median age: 21 years, 51.5% male) included in the final analysis (n=200) performed a validated maximal CPET. At rest, the QTc interval was 395.8±29.6ms. The QTc interval at peak exercise was 369.7±18.6ms. The mean QTc interval at peak exercise was not different between two groups according to the median of survivors' cardiorespiratory fitness (32.0 $mL.kg^{-1}.min^{-1}$) (369.8±17.6ms and 369.6±19.7ms, p=0.930). Group A (≥32.0 $mL.kg^{-1}.min^{-1}$) had a longer QT interval at low to moderate exercise intensities.

Conclusion: Cancer and anthracycline treatments have an impact on the cardiorespiratory system. Low cardiorespiratory fitness in childhood ALL survivors is associated with longer ventricular repolarization during exercise. These differences may be an indicator of altered cardiac function. This shows the importance of studying the response to exercise to improve early cardiac dysfunction detection.

ARK5 Regulates Subcellular Localization of hnRNP A1 During Hypertonic Stress

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The hnRNP A1 is a nucleocytoplasmic shuttling RNA-binding protein that plays an important role in nucleic acid metabolism and gene expression regulation. The function of hnRNP A1 is, in part, determined by its specific location within the cell. Although some work has been done to elucidate the signalling pathways that regulate the cellular localization of hnRNP A1, the precise mechanism(s), including physiological and pathophysiological conditions that alter hnRNP A1 localization, are not known. We have previously conducted an unbiased RNAi-based kinome-wide screen to identify kinases that regulate hnRNP A1 localization during hypertonic stress. One of the hits from this screen is an AMPK-related protein kinase 5 (ARK5). Here we validate ARK5 as the kinase responsible for hnRNP A1 subcellular localization in response to hypertonic stress. We find that ARK5 directly interacts with and phosphorylates hnRNP A1 on the serines within the F-peptide region. We further show that the M9 motif of hnRNP A1 is essential for the ARK5-hnRNP A1 interaction and phosphorylation. Finally, the silencing of ARK5 increases the expression of an anti-apoptotic protein Bcl-xL and consequently delays caspase activation during hypertonic stress.

Identifying Novel Pathophysiologically-Relevant Plasma Biomarkers in Relapsing-Remitting Multiple Sclerosis

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Introduction: Relapsing-remitting multiple sclerosis (RRMS) is a chronic immune-mediated inflammatory disease characterized by central nervous system (CNS) demyelination and axonal damage. Under current guidelines, a MS diagnosis most often occurs over the course of months and requires clinical assessment, MRI, and a lumbar puncture. As a result, this process leaves patients waiting for critical healthcare services. The objective of our research is to identify novel candidate biomarkers in blood plasma of MS patients and elucidating pathophysiological disease mechanisms in RRMS.
Methods: Blood was collected from healthy control individuals and consenting participants. Patient demographic and clinical variables, including past relapse activity, were recorded upon enrollment. Using an ELISA, circulating levels of IL-1RA, IL-18, IL-1β, and CXCL10 were measured in plasma. IL-1RA expression was also investigated *in vitro* and in situ using primary human macrophages and microglia and post-mortem MS tissue, respectively. Peripheral blood mononuclear cells (PBMCs) and extracellular vesicles (EVs) were stained and quantified by flow cytometry using antibodies against common EV markers and immune cell subsets.
Results: Following multiple regression analysis, plasma IL-1RA, an endogenous antagonist of IL-1β, correlated with disability scores in RRMS independent of all other clinical variables. Plasma IL-18, IL-1β and CXCL10 were not associated with most clinical or demographic variables – plasma CXCL10 was significantly correlated with disease duration. *In vitro*, induction of the NLRP3 inflammasome, a pathological hallmark within MS lesions, led to increased release of IL-1RA from primary human microglia and macrophages. In the brain, IL-1RA+ macrophages/microglia were present at the rim of mixed active/inactive MS lesions. Flow cytometry analysis of EVs in plasma revealed a significant increase in several EV populations, including T cell, B cell and monocyte derived EVs in RRMS compared to controls.
Conclusions: Immune-relevant factors can be measured in blood plasma of RRMS patients and represent a rich source of biomarkers for investigation. Here, we have identified two putative inflammation-related biomarkers relevant in RRMS that are associated with disability and/or disease activity. Longitudinal studies are ongoing to assess the clinical utility of these biomarkers in terms of monitoring DMT efficacy, disease progression/activity, and how their levels compare with more widely used biomarkers that are currently under investigation.

Machine-Learning Methods to Investigate Heterogeneity in Longitudinal Patient-Reported Outcome Measures

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Introduction: Patient-reported outcome measures (PROMs) are multi-item self-report scales used by patients to describe their health and quality of life. A challenge of using PROMs to measure health status change over time is that patients may change their PROM interpretations, an effect known as response shift (RS). Ignoring RS could lead to incorrect conclusions about variations in health status over time. Item Response Theory (IRT) models have been used to identify individual PROM scale items that contribute to RS. Unsupervised machine-learning methods can aid in identifying clusters of individuals with similar RS patterns. Our research aims to develop a new longitudinal model that combines IRT with unsupervised machine-learning techniques and compare this newly-developed model with conventional IRT models that use covariates (e.g., age) to detect heterogeneity in RS.

Methods: The conventional IRT model implementation will follow these steps: establish the measurement model for the latent (unobserved) construct (mental and physical health); test for an overall RS effect; test for RS on each PROM item; estimate the final RS model and the true change in the latent construct scores. Conventional IRT models that assume homogeneity in parameter estimates over time may result in imprecise RS if heterogeneity exists. The unsupervised IRT model will be implemented by applying recursive partitioning to the conventional IRT model residuals. Models will be compared using computer simulations by manipulating sample size, number of item response categories, RS effect size, and correlation magnitude over time. We will apply IRT models to real-world data (from the Winnipeg Regional Health Authority Joint Replacement Registry) about PROMs from patients with hip and knee replacement surgery; RS will be tested for one month before and year post-surgery.

Results: Model performance for RS detection will be presented with measures of bias and error. We hypothesize that unsupervised IRT models will be more sensitive than conventional IRT models to detect heterogeneity in RS.

Conclusion: Our research will lead to valid and sensitive PROMs and advance the use of unsupervised machine-learning methods to detect RS in PROMs. Valid analytic techniques will contribute to a better interpretation of the patient's perspective on their own health.

Exploring New Targeted Therapies for Sonic Hedgehog (SHH) Medulloblastoma

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Introduction: Medulloblastoma is the most common malignant primary pediatric brain tumor and is currently divided into 4 molecular subgroups that exhibit different genomic alterations, gene expression profiles and response to treatment. The 4 subgroups are WNT, Sonic Hedgehog (SHH), Group 3 and Group 4. SHH medulloblastoma is characterized by activation of the SHH pathway and is comprised of very high-risk groups of both children and infants. Personalized therapies for SHH medulloblastoma are lacking and targeted therapies that have the potential to reduce toxicity and improve survival are urgently needed. Our lab has recently identified novel roles for the MAPK signalling pathway in contributing to SHH medulloblastoma growth and tumor progression. The MEK inhibitor selumetinib crosses the blood brain barrier and is currently in clinical trials for the treatment of other brain cancers. Our lab found that treatment with selumetinib reduced tumorsphere size, stem cell proliferation, migration and survival *in vitro* while also increasing survival *in vivo* providing the first evidence that the MEK/ERK pathway is a therapeutic target in human SHH medulloblastoma.

Aim: The "best in class" MEK1/2 inhibitor trametinib is blood brain barrier penetrant and is currently undergoing clinical testing in other pediatric brain tumours. However, trametinib has not been assessed in SHH medulloblastoma. A major objective of my MSc project is to evaluate the effect of trametinib on SHH medulloblastoma cell properties both *in vitro* and *in vivo*.

Results: My preliminary data demonstrate that trametinib treatment reduces tumorsphere size, stem/progenitor cell proliferation and survival *in vitro* while also increasing survival *in vivo*. These effects are observed in the nanomolar range relative to the micromolar range for selumetinib.

Conclusion: These new findings reveal novel roles for MEK inhibitors in reducing SHH medulloblastoma tumor properties *in vitro* and *in vivo*. The most promising lead will ultimately be translated into early clinical development to generate a new and innovative targeted therapy for future SHH medulloblastoma patients.

Obesity Research Inspired by the Ophthalmic Drug Bimatoprost's Side Effects: From Deepened Eyelid to Less Lipid to Opening the Lid on the Obesity Mystery

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Background: The anti-ocular hypertension medicine Bimatoprost (Bim.) presents the side effect of upper eyelid deepening, by shrinking the eye socket fat cushion. This has triggered research into its biological analog prostaglandin compound prostamide F2alpha (PMF2α). Both molecules inhibit adipogenesis; the process of preadipocytes differentiation into fat-storing adipocytes; the major constituents of white adipose tissue (WAT). Adipocyte size increase (hypertrophy) is a main characteristic of abdominal obesity and its associated chronic inflammation and the metabolic syndrome.

Research question: How does Bim./PMF2α curb adipogenesis?

Hypothesis: Since peroxisome proliferator-activated receptor gamma (PPAR-γ) is the key adipogenic transcription factor, our compounds might block its activity.

Methods: We utilized 3T3-L1 mouse preadipocytes highly reliable model of adipogenesis, and we extracted proteins for western blotting (WB) analysis.

Serendipitous empirical observation: While I was optimizing WB experiments, I noticed an unexpected and unprecedented biological phenomenon! Bim./PMF2α-treated samples had much bigger cell pellets than vehicle controls.

Additional research hypothesis/question: Do these compounds induce preadipocyte proliferation?

Chasing the potential new discovery: We measured proliferation by MTT and crystal violet assays, and cell counting. We investigated the underlying mechanisms, via the pharmacological inhibition of the PMF2α receptor and the main cellular proliferative pathway, mitogen-activated protein kinase (MAPK). Moreover, through qPCR, we monitored gene expression levels of several cell cycle promoting and inhibiting proteins, namely, cyclin-dependent kinases (CDKs), and their inhibitors (CDKIs), respectively. Additionally, Bim./PMF2α effect on PPAR-γ activity was determined by transcription reporter assays.

Results: Bim./PMF2α curbed PPAR-γ activity, and increased preadipocytes proliferation (hyperplasia). Furthermore, they reduced the expression of the CDKIs p21 and p27. The key mechanisms might involve MAPK signaling stimulation, which might also be implicated in an inhibitory phosphorylation of PPAR-γ, that we are currently investigating.

Conclusions: Besides inhibiting adipogenesis, Bim. and PMF2α induce preadipocyte multiplication, which might supply WAT with a ready-to-differentiate cellular reserve for hyperplasia. This could explain why eyelid deepening is quickly reversed upon Bim. cessation. We further, provide novel insights into PMF2α's endogenous activity, as a regulator of WAT growth mechanisms, potentially promoting hyperplasia over hypertrophy, to maintain WAT storage capacity and preventing ectopic fat deposition on vital organs, such as the heart.

Body Compression Reduces Upright Blood Pressure Oscillations in Patients with Postural Orthostatic Tachycardia Syndrome

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Introduction: Blood pressure oscillations increase with upright posture in patients with Postural Orthostatic Tachycardia Syndrome (POTS). Compression garments reduce orthostatic heart rate and symptoms in patients with POTS, but the effects of compression garments on blood pressure oscillations are unknown. We sought to determine the effects of body compression on blood pressure oscillations in patients with POTS.
Methods: POTS patients (n=29) completed 2x10min head up tilt tests (HUT) with full lower body compression (FULL) or no compression (NONE), in a random order. Continuous ECG and blood pressure were recorded, with advanced hemodynamics from Modelflow. Oscillation magnitude was defined by Systolic Blood Pressure [SBP] Range (SPR; Max–Min SBP) within 5 minute windows. Low frequency mean arterial pressure (LF-MAP) power was calculated using spectral analysis (for baseline and last 5 min HUT). Data are presented as mean±SEM.
Results: Baseline SBP was higher in FULL than NONE (121±3 mmHg vs. 116±2 mmHg; p=0.01), as was HUT SBP (115±3 mmHg vs. 106±3 mmHg; p<0.001). Baseline SPR was not different between FULL and NONE (40±4 mmHg vs. 33±2 mmHg; p=0.1), but HUT SPR was lower with FULL than NONE (36±2mmHg vs. 45±3 mmHg; p=0.008). Baseline LF-MAP was not different between FULL and NONE (p=0.7). HUT LF-MAP was lower in FULL than NONE (4.8±0.5 $mmHg^2$ vs. 7.8±0.9 $mmHg^2$; p<0.001). There was a positive correlation between SPR and LF-MAP during baseline (r=0.63; p<0.001) and HUT (r=0.60; p<0.001). Baseline stroke volume (SV) was not different between FULL and NONE (98±3 mL vs. 96±3 mL; p=0.2). HUT SV fell less, and was higher, in FULL than NONE (78±3 mL vs. 65±3 mL; p<0.001). There was a negative correlation between LF-MAP and SV during HUT (r=-0.27; p=0.04), but not during baseline (r=0.18; p=0.16).
Conclusions: Full lower body compression in POTS patients reduced SBP oscillations when upright. FULL compression reduced LF-MAP. There was strong correlation between LF-MAP and SBP oscillation magnitude, and a weak correlation between LF-MAP and stroke volume. These findings suggest that increased SBP oscillations may be driven by lower stroke volume, leading to increased sympathetic vascular tone.

Involvement of mTOR and the Unfolded Protein Response in Oligodendrogenesis and Remyelination in Multiple Sclerosis

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Multiple sclerosis (MS) is an autoimmune disease of the central nervous system (CNS), characterized by demyelination that leads to loss of oligodendrocytes and axons resulting in progressive neurodegeneration. Replacing myelinating oligodendrocytes and remyelination are essential for repairing the CNS in MS. While remyelination occurs efficiently in the acute phases of MS, as the disease progresses, this process becomes a challenge due to the impaired differentiation and maturation of oligodendrocyte precursor cells (OPCs) into myelinating oligodendrocytes. Elucidating the mechanisms underlying impaired OPC maturation in progressive MS can aid in identifying novel treatments for MS. Our findings from two mouse models of MS, the experimental autoimmune encephalomyelitis (EAE) and the cuprizone-induced demyelination, show that mTOR activity is increased transiently during acute demyelinating and remyelinating stages through the complex mTORC1 and its downstream effector pS6K. This suggests a role for mTOR pathway in acute stage of demyelination and remyelination. Additionally, we found that unfolded protein response (UPR) is upregulated in EAE and cuprizone mice, and its activity persists in the chronic stages. Taken together, our *in vivo* data suggests a simultaneous activation of mTOR and UPR suggesting a role for both pathways in demyelination and repair of myelin. However, the role of these pathways and their cross talk are not fully understood. Our initial direct *in vitro* data shows that mTOR activity is required for the proliferation and maturation of OPCs and thus dysregulation of the mTOR pathway may be an underlying cause for remyelination failure in MS. Our findings are an interesting starting point for elucidating the role of these pathways in regulating oligodendrocytes in chronic, progressive MS.

Children with Medical Complexity in the Canadian Maritimes: A Mixed Methods Study Protocol

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Introduction: Ongoing developments in the medical field have improved survival rates and long-term management of children with complex chronic health conditions. While the number of children with medical complexity is small, they use a significant amount of health resources across various health settings and sectors. Research to date exploring this pediatric population has relied primarily on quantitative or qualitative data alone, leaving significant gaps in our understanding of this population. The objective of this research is to use health administrative and family reported data to gain an in-depth understanding into patterns of health resource use and health care needs of children with medical complexity and their families in the Canadian Maritimes.

Methods: An explanatory sequential mixed methods design will be employed. Phase One of this research will leverage the use of health administrative data to examine the prevalence and health service use of children with medical complexity. Phase Two will employ case study methods to collect multiple sources of family-reported data to generate a greater understanding of their experiences, health resource use, and health care needs. Two cases will be developed in each of the three provinces. Cases will be developed through semi-structured interviews with families and their health care providers and health resource journaling. Findings will be triangulated from Phase One and Two using a joint display table to visually depict the convergence and divergence between the quantitative and qualitative findings.

Results: This study will be completed summer of 2022.

Conclusions: There is a current disconnect between the Canadian health care system and the needs of children with medical complexity and their families. By combining both health administrative and family reported data, this study will unveil critical information about children with medical complexity and their families to more efficiently and effectively meet their health care needs. Results from this research will be the first step in designing patient-oriented health policies and programs to improve the health care experiences, health system utilization, and health outcomes of children with medical complexity and their families.

Assessing the Impact Reduced EMI1 Expression has on Chromosome Instability in Colorectal Cancer

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Introduction: Colorectal cancer (CRC) is the third most diagnosed and second most lethal cancer in Canada. Chromosome instability (CIN), or ongoing changes in chromosomal complements, is a driver of genetic and cell-to-cell heterogeneity. CIN is associated with ~85% of CRCs and is a suspected driver of CRC pathogenesis; however, the molecular determinants giving rise to CIN (i.e., aberrant genes, proteins and pathways) remain poorly understood. Our preliminary data suggest that aberrant expression of a ubiquitylation gene, EMI1, induces CIN but its relevance in CRC remains unclear. Accordingly, we seek to gain novel insight into the impact reduced EMI1 expression has on CIN in a CRC context.

Methods: To determine the impact reduced EMI1 expression has on CIN, siRNA-based silencing was employed in karyotypically stable, malignant (HCT116; SW48), and non-malignant (1CT; A1309) colonic epithelial cell lines. Single cell quantitative imaging microscopy approaches were employed to evaluate the effects reduced EMI1 expression has on CIN phenotypes, including changes in nuclear areas (i.e., large-scale DNA changes) and increases in micronucleus formation (i.e., extranuclear DNA-containing bodies). Semi-quantitative western blots were employed to assess endogenous protein levels of established EMI1 targets (e.g., RAD51) following silencing.

Results: Initial results reveal statistically significant increases in nuclear area distributions and micronucleus formation following EMI1 silencing in all four cell lines. More specifically, HCT116 exhibited a ~4-fold increase in nuclear areas and ~7-fold increase in micronucleus formation relative to the non-targeting control (siControl). In 1CT and A1309, there is a >1.5-fold increase in nuclear areas and >3-fold increase in micronucleus formation, while SW48 exhibit a 1.2-fold increase in nuclear areas and >2-fold increase in micronucleus formation relative to siControl. Finally, initial semi-quantitative western blots revealed reduced EMI1 expression corresponds with a ~3-fold increase in RAD51 abundance in HCT116, confirming an on-target and functional impact following EMI1 silencing.

Conclusion: Collectively, these findings show that reduced EMI1 expression induces CIN phenotypes in four colonic cellular contexts, which suggests EMI1 is a novel CIN gene with potential pathogenic implications in CRC.

Eosinophils Decrease Pulmonary Metastatic Mammary Tumor Growth

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Introduction: Metastatic breast cancer remains incredibly challenging to treat, highlighting the need for an improved understanding of host factors that prevent metastasis. The lungs, which are a common site of breast cancer metastasis, are host to a variety of immune cell subsets, including eosinophils, which are innate immune cells that target pathogens via degranulation. Though the presence of eosinophils within solid tumors and metastases has been cited for decades, their role in breast cancer metastasis is poorly understood.

Methods: To study the role of eosinophils in pulmonary breast cancer metastasis, we utilized transgenic mice that over-express the cytokine IL-5, which results in systemic eosinophil expansion (IL5Tg mice), as well as eosinophil-deficient mice (dblGATA mice). EO771 mammary carcinoma cells were injected intravenously (i.v.) to seed the lungs. Flow cytometry and immunohistochemistry were used to quantify lung immune cell infiltrate and tumor burden.

Results: We found that IL5Tg mice had significantly lower EO771 pulmonary metastasis compared to WT mice. Additionally, both eosinophil-deficient dblGATA mice and WT mice treated with anti-Siglec-F to deplete eosinophils exhibited accelerated metastatic progression compared to both WT and isotypetreated mice injected i.v. with EO771 cells. We also found that WT and dblGATA mice had an increased number of EO771 tumor cells compared to IL5Tg mice merely 5 days post-i.v. injection, and that eosinophils degranulated next to EO771 cells in the lungs of IL5Tg mice, indicating that eosinophils may play a role in both initial tumor cell seeding and subsequent metastatic nodule progression. Importantly, we found that lung eosinophils killed tumor cells via degranulation ex vivo, and that eosinophils expressed markers of degranulation (CD63) and activation (CD69, CD35) following pulmonary colonization of EO771 cells.

Conclusion: These results highlight a role for eosinophils in preventing tumor cell colonization to metastatic sites and suggest that developing drugs to trigger eosinophil degranulation may serve as a viable therapeutic option to treat metastatic disease.

Sympathetic Nervous System and Integration from V3 Neurons in Spinal Cord Injury

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Introduction: Approximately 80,000 individuals live with spinal cord injury (SCI) in Canada. Dysregulation of sympathetic function is common after SCI because connections between autonomic centres in the brainstem are severed from spinal sympathetic preganglionic neurons (SPNs) that regulate sympathetic outflow to target organs. SPNs located in intermediate lamina (IML) of T1-L2 spinal cord remain active after injury, and lumbar electrical stimulation increases thoracic sympathetic output and improves cardiovascular function after cervical SCI. The source of this excitatory neuronal input to SPNs is unknown. We hypothesize that ascending propriospinal interneurons (INs) located in lumbar spinal cord provide excitatory inputs on thoracic SPNs.

Methods: We examined the extent of SPN innervation from a class of propriospinal neurons – V3 INs, which have a known role in locomotion in adult male and female Sim1CreTdTomato mice. This mouse line allows for visualization of V3 INs under fluorescent microscopy. To determine if lumbar V3 INs show distinct distribution patterns, BDA was injected into L1-L5 lumbar segments. To investigate which V3 IN populations were responsible for input to the T8 spinal segment, CTB tracer targeting the IML was injected. One week post-surgery, mice were euthanized and spinal cords were harvested and analyzed through immunohistochemical staining. IMARIS Bitplane software was used to create 3-dimensional reconstructions of SPNs and their contacts to quantify our results.

Results: Of all excitatory input apposed to thoracic SPNs, ~20% arose from V3 IN projections (TdTom+/VGlut2+, n=3). L2 injections resulted in 2x more V3 contacts in rostral (T1-6) versus caudal (T6-T12) segments. Interestingly, L4/5 BDA injections resulted in 2.6x more contacts in caudal segments (BDA+/TdTom+) of the spinal cord. Injections of CTB targeting T8 IML revealed that over half of V3 INs in L1-6 provided input to this region, with a slightly higher contribution from contralateral V3 INs (65% of V3D; 55% V3V ipsi- versus ~70% contralateral, n=2).

Conclusion: We continue to investigate SPN innervation by genetically defined spinal neurons, but this is the first demonstration that lumbar V3 INs provide direct excitatory synaptic input onto thoracic SPNs (~20%). This suggests that locomotor-related lumbar neurons may activate sympathetic output during movement.

Developing an Aptamer-Base Tool Targeting SARS-CoV-2 S Protein for COVID-19 Diagnostic and Therapeutic Use

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Introduction: The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) epidemic has highlighted the need for developing both deployable diagnostic and therapeutic tools for emerging diseases. Development of aptamers, nucleic acid based biomolecules that specifically bind to viral protein targets, could be an approach that is as reliable and rapid as other options like antibody-based lateral flow assays and RT-qPCR, increasing the flexibility to respond to the current and future pandemics.

Methods: The SARS-CoV-2 viral surface receptor S protein is a prime candidate target for both diagnostic and therapeutic purposes. Commercially available recombinant spike receptor binding site (S-RBD) protein was used to screen and select suitable candidates from the random single stranded DNA (ssDNA) 40mer Bank40 aptamer library (EURx, Poland). S-RBD specific aptamers is being selected by successive cycles of systematic evolution of ligands by exponential enrichment (SELEX), resulting in aptamer candidates with high affinity to the target. Good binders will be further screened by electrophoretic mobility shift assay (EMSA), and the binding affinity, dynamics, and mechanism of selected candidates will be analyzed using in silico simulations, supported with experimental protein interaction analysis. To explore the therapeutic potential of the aptamer candidates, selected candidates will also be tested *in vitro* against a recombinant vesicular stomatitis (VSV) pseudovirus expressing SARS-CoV-2 S protein to examine their ability to neutralize viral infectivity. Since the initiation of our study, variants of concern and viral mutations have become another important area to consider. We plan to evaluate the binding efficiency of aptamer candidates against S-RBD of variants of concern, further investigating the aptamer interaction with viral protein with critical amino acid mutations.

Conclusion: We propose that the aptamer candidates could fill both diagnostic and therapeutic gaps. The aim of the study is to ultimately develop aptamer-based tools that have high antiviral potential and high sensitivity and specificity to detect SARS-CoV-2 in *in vitro* and clinical samples.

Scleraxis is Required for Induction of GLS1 Expression in Cardiac Myofibroblasts

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Cardiac fibrosis is an aberrant wound healing process involving activation and conversion of fibroblasts to myofibroblasts and excessive deposition of extracellular matrix proteins which interfere with the systolic and diastolic function of the heart, leading to heart failure and death. Fibrosis is a very energy intensive process, and in liver and lung fibrosis, glutaminolysis has been reported to supply these increased energy demands. In contrast, little is known about how the metabolic requirements are met in cardiac fibrosis. The rate-limiting enzyme of the glutaminolysis pathway is glutaminase (GLS1), which catalyzes the conversion of glutamine to glutamate which then is converted to α-ketoglutarate to enter the TCA cycle to generate cellular energy. Here we examined if GLS1 plays a role in the activation of fibroblasts to myofibroblasts, and how its expression is regulated. Fibroblasts are activated by pro-fibrotic growth factors such as TGFβ or through passaging, and under both circumstances GLS1 expression was highly induced in myofibroblasts compared to fibroblasts. Scleraxis expression was also induced by about 9 fold in cardiac myofibroblasts compared to fibroblasts. Our lab has previously shown that scleraxis is required for the transition of fibroblasts to myofibroblasts though transactivation of various profibrotic genes, thus we explored if scleraxis regulates GLS1 expression as well. While over-expression of scleraxis induced GLS1 expression by about 20 fold, downregulation or knockout of scleraxis attenuated GLS1 expression by 76% and 70% respectively, and TGFβ could not induce GLS1 expression in the absence of scleraxis. Further, both glutamine and glutamate cellular levels from wild type and scleraxis knockout fibroblasts treated with or without TGFβ also showed results that are consistent with elevated glutaminolysis after TGFβ treatment, and attenuation by scleraxis loss. Using luciferase assay, scleraxis was found to transactivate the human GLS1 promoter primarily by binding to an E-box (scleraxis putative binding site) and this was further validated by chromatin immunoprecipitation assay. These results suggest that scleraxis plays a major role in regulating GLS1 expression to in turn regulate energy production required for the conversion of fibroblasts to myofibroblasts, potentially contributing to cardiac fibrosis.

TRPM3 Agonists Activate AMPK and Mitochondrial Function in Adult Sensory Neurons Increasing Neurite Outgrowth

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Peripheral neuropathy affects approximately 50% of the population with diabetes mellitus depending on age and disease severity. It is associated with substantial morbidity and is characterized by induction of pain and loss of sensory function beginning distally in the lower extremities. Recent studies suggest that molecular cascades maintaining mitochondrial function and calcium homeostasis are effective therapeutic targets for diabetic peripheral neuropathy. Interestingly, our lab has recently reported that muscarinic acetylcholine type 1 receptor (M1R) antagonists stimulated neurite outgrowth, in part, by activating Ca2+/calmodulin-dependent protein kinase kinase II (CaMKKII) and mobilization of AMP-activated protein kinase (AMPK). This augmented mitochondrial function in sensory neurons imparts protection against small and large fiber neuropathy in various rodent models of peripheral neuropathy (including diabetes). Transient receptor potential melastatin receptor 3 (TRPM3) is a TRP type cation channel that triggers Ca2+ influx. We hypothesized that opening of TRPM3 could activate CaMKKII and induce neurite outgrowth and may mimic antimuscarinic drug effects. Dorsal root ganglion (DRG) neurons were isolated from adult control and streptozotocin-induced type 1 diabetic male Sprague–Dawley rats. A significant dose-dependent elevation of neurite outgrowth was observed in response to pregnenolone sulphate (PS) or CIM0216 (selective and specific TRPM3 agonists, respectively) These TRPM3 agonists also increased AMPK activation and triggered an increase in the calcium influx which was detected with Flou-4AM using confocal imaging. Mitochondrial membrane potential (ΔΨm) (using JC-1 dye) was significantly increased in response to pretreatment of PS and CIM. Our investigations of TRPM3 activation and its downstream molecular cascade will hopefully lead to potential therapeutic targets for impeding detrimental effects of peripheral neuropathy.

Overlapping HIV-1 Transmission Networks Among Men Who Have Sex with Men and Female Sex Workers Accessing the Sex Worker Outreach Program (SWOP) in Nairobi, Kenya

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Background: HIV prevention is increasingly targeting key populations at risk of HIV even within generalized epidemic settings. Both paid and unpaid sex with women is commonly reported among African MSM and anecdotal reports suggest that MSM sex workers and female sex workers (FSW) share clients at common hotspots. Therefore, overlapping sexual networks could play an important role in shaping the wider HIV epidemic, but transmission dynamics remain poorly understood. Here we utilize phylogenetic analysis to better understand HIV transmission among MSM and FSW accessing the Sex Worker Outreach Program in the central business district of Nairobi, Kenya.
Methods: Blood was collected in consecutively sampled MSM (n= 165) and FSW (n= 746) living with HIV as part of routine treatment provided by SWOP between 2017-2019. HIV pol gene was sequenced using an in-house HIV drug resistance mutation genotyping assay. Phylogenetic clusters were inferred using patristic distance between sequences measured on phylogenetic trees. Effective population size estimates were inferred using an MCMC analysis as implemented in BEAST v1.10.4.
Results: We amplified 511 HIV pol sequences of the 911 (56.1%) available specimens. A majority of MSM sequences (65.2%) were part of a cluster while only 22.0% of FSW sequences clustered. A total of 58 clusters were inferred ranging from 2-9 individuals in size. Most clusters were exclusively FSW (n=29) or a mixture of FSW and MSM (n=25). Only four clusters were exclusively MSM. Effective population size estimates suggest that HIV among MSM grew exponentially around 2006, peaked in 2008, and remained stable until 2019. A similar trend was observed among FSW except the initial phase of growth occurred around 2009.
Conclusion: Clustering between MSM and FSW sequences suggests a significant overlap between key population transmission networks. HIV transmission within MSW and FSW follow similar trajectories suggesting transmission occurred mainly before SWOP's existence.

Brain Blood Flow and Infarct Volume Measurement from Routine Acute Stroke CT Imaging

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Introduction: Rapid reperfusion treatment of stroke can substantially reduce disability from this lethal cerebrovascular disease. Treatment candidates are those with a small to moderate volume of irreversibly damaged brain (infarction) detected at admission neuroimaging. Infarct volume can be measured by automated analysis of CT perfusion (CTP) imaging, which detects reduced brain blood flow by monitoring the passage of x-ray dye from 25 time-resolved contrast-enhanced brain CT scans. This advanced diagnostic scan requires a trained radiographer, who may not be emergently available at rural hospitals that benefit most from automated diagnosis. In this study, we investigate the feasibility of using ubiquitous routine CT scans in place of CTP to calculate brain blood flow and measure infarct volumes.

Methods: Patients who presented to our emergency department with a suspected stroke and received routine CT and CTP were included in this retrospective study. Presence of stroke was determined from clinical notes. Routine CT comprising a non-contrast CT and three time-resolved contrast-enhanced CT images were combined to form a CTP-like time series. Quantitative maps of brain blood flow were calculated from both CTP and routine CT using a prototype commercial CTP software. Infarct was considered brain with blood flow less than 10% of the normal brain hemisphere. Routine CT classification accuracy of small (<10 ml), moderate (10-50 ml), and large (>50 ml) infarct volumes was determined using CTP volumes as reference. Pearson correlation coefficient and mean ± standard deviation of differences between routine CT and CTP-derived infarct volumes were calculated.

Results: Of twenty included patients, 14 (70%) had a stroke. Both the routine CT and CTP-derived blood flow maps correctly identified a negligible lesion (<5 ml) in the 6 non-stroke patients. With respect to CTP infarct volume in stroke patients, the routine CT approach correctly identified 9/10 small, 2/3 moderate, and 1/1 large infarcts. Pearson coefficient was 0.88 ($p<0.001$) and the mean infarct volume difference (CTP minus routine CT) was 0.7±8.7 ml across all included patients.

Conclusion: Routine CT-derived brain blood flow maps may be sufficient for automated infarct volume measurement at rural hospitals. Validation on a large patient cohort is warranted.

Definition of DEPTOR Role in Development and Function of Brown Adipose Tissue

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Introduction: Brown adipose tissue (BAT) is a key organ to maintain body temperature. In response to cold, it increases its volume and energy expenditure to produce heat. This increase of energy expenditure following activation is of great interest since it could be a vector of improvment of metabolic health in obesity. Mechanistic Target Of Rapamycin (mTOR) is part of mTOR complexes 1 & 2, which control several biological processes promoting anabolism, cell growth and proliferation. Both of these complexes play important roles in BAT development and function. DEP domain containing mTOR-interacting protein (DEPTOR) is associated with mTOR and modulates its activity. Preliminary works have demonstrated that DEPTOR expression is increased in brown preadipocytes differentiating into adipocytes and within the BAT of cold-exposed mice. Knowing its influence on mTOR activity, DEPTOR could be involved in the development and function of the BAT.

Methods:: Myf5-Cre mice have been crossed with Deptor-LoxP mice to suppress DEPTOR expression within BAT progenitors. These mice were then exposed to 10°C or 30°C for two weeks to study the effects of DEPTOR loss in activated and unactivated BAT, respectively. Rectal temperatures of the mice were measured during the first hours of exposition to cold and thermoneutrality. The expression of genes involved in lipogenesis, thermogenesis and BAT metabolism was studied as well as the expression and phosphorylation of proteins involved in lipogenesis and BAT metabolism.

Results: DEPTOR knock-out (KO) in BAT induces a more important decrease of body temperature following cold exposure. At thermoneutrality, BAT DEPTOR KO increases the expression of genes and proteins involved in lipogenesis associated with an increase of Akt phosphorylation. This could be due to an overactivation of mTORC2 pathway, inducing BAT whitening.

Conclusion:: DEPTOR loss within the BAT leads to an increase of its pro-lipogenic capacities to the detriment of its thermogenic capacities.

Mice Possess a More Limited Natural Anti-Factor VIII Antibody Repertoire than Humans that is Generated Primarily by Marginal Zone B cells

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Introduction: 30% of severe hemophilia A (HA) patients mount an immune response to therapeutic Factor VIII (FVIII), resulting in neutralizing anti-FVIII antibodies. Studies have reported the presence of naturally-occurring non-neutralizing antibodies (NNAs) specific to FVIII in some patients and healthy donors. This study aims to characterize and determine the significance of this natural anti-FVIII antibody repertoire in mice and humans.

Methods: Natural anti-FVIII NNAs were quantified in naïve wild-type and HA C57Bl6 mice (FVIII- /-) as well as healthy human donors. To measure antibody production, murine B cells were harvested from the spleen and cultured ex vivo in lipopolysaccharide. Marginal zone B cells (MZBs) and follicular B cells (FOBs) were sorted based on CD21 and CD23 expression. MZBs were depleted via intraperitoneal injections of anti-CD49 and anti-CD11a antibodies. Anti-FVIII antibodies were detected by ELISA and quantified against purified immunoglobulin standards. Inhibitory activity of the anti-FVIII antibodies was measured via Bethesda assay.

Results: Non-neutralizing anti-FVIII IgM, but not IgG nor IgA, was detectable in naïve wild-type and FVIII-/- mice. These antibodies were significantly higher in FVIII-/- compared to wild-type mice ($p<0.001$) and increased with age ($p<0.001$). Splenic B cells isolated from naïve FVIII-/- mice produced anti-FVIII IgM ex vivo when stimulated with lipopolysaccharide and increased with age ($p<0.05$). Total ex vivo IgM production remained consistent across ages. Anti-FVIII IgM was shown to bind FVIII *in vivo*, as intravenous administration of FVIII reduced plasma levels by 44% after 24 hours ($p<0.01$). Following depletion of MZBs in naïve FVIII-/- mice, anti-FVIII IgM was significantly reduced compared to mice receiving isotype control antibodies ($p<0.001$). When sorted and cultured with lipopolysaccharide, MZBs produced almost all anti-FVIII IgM compared to FOBs ($p<0.01$). In six human donors, FVIII-specific IgM was detected at the highest concentration, followed by IgG then IgA. No inhibitory antibodies were detected.

Conclusion: FVIII-specific NNAs are present in both mice and humans. MZBs are the predominant source of natural anti-FVIII IgM in mice. Anti-FVIII IgM levels are higher in HA mice, rise with age and fall following exogenous FVIII administration, suggesting MZB-derived IgM may be involved in the immune surveillance of FVIII.

Non-Optimal Bacteria Species Induce Neutrophil-Driven Inflammation and Epithelial Barrier Disruption in the Female Genital Tract

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Introduction: The mucosal surface of a healthy female genital tract (FGT) is comprised of physicochemical, immunological and microbial components that serve as a rapid, first line of defense against infections. Alterations in any of these components have been associated with higher HIV acquisition risk. Analysis from >700 women from the CAPRISA004 cohort show that women with a non-Lactobacillus dominant vaginal microbiome were at significantly higher risk of sexual HIV acquisition, and that this strongly correlated with vaginal epithelial barrier disruption, inflammation and neutrophil accumulation. However, how FGT barrier function is impacted by changes in the vaginal microbiota, and a mechanistic understanding of mucosal neutrophils in this process, remains unclear. Here, we utilized microscopy and proteomic approaches to better define the interplay between vaginal microbial species, epithelial barrier function and neutrophil activation *in vivo*.

Methods: Balb/c mice were intravaginally inoculated with either PBS, *L. crispatus* (CT-1), *Mobiluncus mulieris* (CT-4) or *Gardnerella vaginalis* (CT-3/4). Cervicovaginal lavage (CVL) was collected on day 0, 2, 4, and 7 to confirm bacteria colonization through 16S rRNA sequencing and assess protein expression profile through mass spectrometry. Vaginal tissues were collected for immunohistochemical analysis. To determine neutrophils contribution to barrier disruption, barrier integrity was assessed in the presence or absence of neutrophils by intravaginally inoculating mice with lucifer yellow (0.45Da) and harvesting tissues to measure dye penetration into the epithelium by immunohistochemistry.

Results: Immunohistochemistry analysis demonstrate a larger neutrophil influx within the vaginal epithelium and the submucosa of mice inoculated with *M. mulieris* and *G. vaginalis* but not *L. crispatus*. High levels of inflammatory cytokines and neutrophil-related factors were also identified by proteomic analysis of collected CVL samples. Additionally, permeability assay revealed a significant loss of barrier integrity in mice inoculated with *M. mulieris* and *G. vaginalis*. However, upon depletion of neutrophils *in vivo*, vaginal barrier integrity was restored even in the presence of these non-optimal bacteria.

Conclusion: Our study demonstrates that the presence of non-optimal bacteria species in the FGT results in genital inflammation, high neutrophil activation and increases barrier breakdown. Excitingly, we show that neutrophils response to these non-optimal bacteria species directly impacts FGT barrier function *in vivo* and ongoing work will determine whether these changes in barrier function directly can enhance HIV acquision.

Rare Congenital Disorders: An Urgent Need of Therapy

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Rare congenital disorders such as SRD5A3 congenital disorder of glycosylation or Cori disease are two examples of rare congenital diseases for which no treatment is available. As a result, there is need to develop new relevant models of the disease in order to better understand the pathological molecular substratum. Simple *in vivo* models such as nematodes (*C. elegans*) have been proven convenient for unveiling insights into the pathogenicity of human genetic disorders. With a fully sequenced genome and a high degree of genetic conservation with mammals including humans, they are an attractive model to study complex human disorders. In this project, we took advantage of *C. elegans* combined with modern genetic editing techniques such as CRISPR/Cas 9 to develop patient-specific genetic avatars. On one hand, Cori disease is caused by a mutation in AGL gene coding for glycogen debranching enzyme which is involved in the glycogenolysis pathway. This dysfunction leads to significant glycogen buildup which appears with liver hyperplasia, hypoglycemia, mental retardation, and myopathy. The *C. elegans* orthologue of the Human AGL gene is agl-1. Our work with whole gene deletion (agl-1 (deletion)) and point mutation strains (agl-1 (W1044X), agl-1(S1444R)) showed significant movement impairments, and interestingly, a glycogen accumulation phenotype which make them suitable for drug screening experiments. On the other hand, SRD5A3-CDG is caused by a mutation in steroid 5 alpha-reductase 3 gene (SRD5A3). Clinically, SRD5A3-CDG patients have significant psychomotor, cognitive, and visual impairments. The *C. elegans* ortholog of SRD5A3 is called B0024.13. We characterized two mutant strains: B0024.13(deletion) and B0024.13(W6X) point mutation. B0024.13 *C. elegans* strains show significant motor impairments, as well as neurodevelopmental delay.

Psychiatric and Cognitive Features of Binge Eating in Early Versus Late Adulthood

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Introduction: Binge eating disorder (BED) is the most common eating disorder. Evidence has demonstrated that BED is not solely a concern for young adults; rather, the prevalence is comparable in young and old. Cultivating a population-specific understanding of the presentation is important for effective treatment and management, yet there is a dearth of research on BED in older adults. We investigated the relationship between binge eating and commonly associated psychiatric (depressive symptomatology) and cognitive (reward sensitivity, executive control) features in young and old. We predicted that depression would demonstrate a stronger contribution to binge eating severity in younger than older adulthood based on the higher rates of depression in young. In contrast, cognition would be a more robust predictor in older adults given the normal age-related cognitive changes that are characteristic of older adulthood.

Methods: A community sample of 128 younger (18-34 years) and 177 older (58-92 years) adults were included (57% female). Behavioural assessments of binge eating (Binge Eating Scale (BES)), depression, and cognition were drawn from a collaborative data collection initiative at York and Cornell University. Bivariate correlations and linear modelling (simple and multilevel) were applied to the data to test the relationship of BES with depression and executive control in young and old.

Results: 'Binge eaters' comprised 3.61% of the sample. Female sex was a significant predictor of BES in young ($R2 = 8.2\%$), but not old. Consistent with predictions, depression was a significant predictor of BES in adults ($r = .33$); however, the relationship was more robust in young ($R2 = 18.3\%$) than old ($R2 = 5.8\%$). Notable trends were observed in support of a positive relationship between reward sensitivity and BES; however, this effect was only significant in older adults ($b = -.08$). Effects of executive control were null.

Conclusion: This is the first study to explore age contrasts in BES and comorbidities at the poles of the adult age spectrum. Important discrepancies were observed highlighting the potential nuances in BED presentation in younger versus older adulthood; thus, emphasizing the need for health practitioners to be attuned to unique patient context to engage in effective care.

Whole Genome Sequencing Reveals Insights into the Challenges of Identifying *Vibrio cholerae* Amongst Environmental *Vibrio* Species in Shellfish

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Introduction: The genus *Vibrio* represents a group of bacteria that are ubiquitous in marine environments worldwide. Some *Vibrio* species are pathogenic to humans and are of the utmost concern including *V. cholerae*, *V. parahaemolyticus* and *V. vulnificus*. The main sources of *Vibrio* infection in Canada are shellfish. Human disease surveillance and food and environmental monitoring for *Vibrio* species and resulting illnesses they cause are essential for assessing the risks related to public health. However, these activities are limited in Canada. Whole genome sequencing (WGS) combined with powerful bioinformatics tools provides the greatest taxonomic resolution for differentiating among *Vibrio* species and has the potential to replace phenotypic methods, and can build or enhance existing surveillance systems.

Purpose: This research aims to utilize a full suite of proteomic, genomic and bioinformatics tools to uncover the true identity of isolates that were previously characterized as *V. cholerae*. The hypothesis is that the previous gold standard phenotypic methods are insufficient for distinguishing *V. cholerae* from other *Vibrio* species in shellfish.

Methods: In this study, a population of *Vibrio* species that were previously isolated from Canadian retail and harvested shellfish and characterized as *V. cholerae* were utilized. Isolates were analyzed in depth using matrix assisted laser desorption/ionization-time of flight (MALDI-TOF) mass spectrometry (MS), WGS and bioinformatics tools to compare methods and determine an accurate species-level classification. A maximum likelihood tree was built using a reference free approach in IQ-tree. Results were analyzed and visualized in RStudio using the ggtree package.

Results: Out of 55 isolates, only 20% were identified as *V. cholerae* by proteomic, genomic and bioinformatics tools. WGS and bioinformatics analysis illustrated a comprehensive view of *Vibrio* species diversity in shellfish samples. Taxonomic classification revealed the presence of known pathogenic species to humans (*V. alginolyticus*, *V. cholerae*, *V. parahaemolyticus*, *V. vulnificus*), coral (*V. mediterranei*), shellfish (*V. aestuarianus*), and a potentially novel *Vibrio* species.

Conclusions: WGS provided accurate species-level identification of *Vibrio* bacteria that were previously masked by other taxa. Without accurate identification tools we do not have a complete picture of the risks posed by this organism and its potential threat to Canadians in the future.

The Impact of Waiting for Surgery on Patient Outcomes in Hip and Knee Replacement: A Scoping Review

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Background: Total hip and total knee replacement are some of the most performed elective surgeries in Canada. As such, patients can face significant wait times for these procedures, a situation worsened by surgery delays due to the COVID-19 pandemic. Most patients undergo joint replacement to treat severe osteoarthritis which causes pain, stiffness, and loss of function. Patients who are waiting for surgery over prolonged periods may experience deterioration in their health and quality of life while on the wait list. The purpose of this review is to describe the changes in health that patients experience while waiting for hip and knee replacement and the impact of wait time on outcomes after surgery.

Methods: This review was conducted following the Preferred Reporting Items for Systematic reviews and Meta-Analyses extension for scoping reviews with additional guidance from the JBI Manual for Evidence Synthesis. EMBASE, Medline, PubMed, CINAHL, Scopus, and Cochrane electronic databases were searched for primary research articles published in 2000 or later. Studies were eligible for inclusion if they included adult patients, diagnosed with osteoarthritis, undergoing primary hip or knee arthroplasty and they assessed the change in patient health outcomes while waiting for surgery. Two reviewers independently screened titles and abstracts of articles identified in the search and reviewed the full text of relevant studies for inclusion in the review. We extracted data from relevant articles and described the study characteristics, conceptualization of wait time, outcomes assessed before and after surgery, and factors associated with change in health during the wait period.

Results: In 7 extracted studies, 3 reported only on the wait period before surgery, and 4 also reported on outcomes after surgery. The most commonly used outcome measure was the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC), n=4. Four studies found that longer wait times were associated with worsening outcomes during the wait period and 3 studies reported that wait time was not associated with worse outcomes while waiting and after surgery.

Conclusion: Better understanding of how patient health may deteriorate while waiting for surgery can inform decision making for prioritizing patients for joint replacement.

Whole Genome Sequencing Reveals High Level of Genetic Diversity in Antifungal Resistant *Candida Glabrata* Isolates in Canada

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Introduction: *Candida glabrata* is a major human fungal pathogen that can exhibit reduced susceptibility towards antifungal drugs. Here, whole genome sequencing (WGS) was utilized to elucidate the genomic epidemiology of antifungal resistant *C. glabrata* isolates.

Methods: The ten provincial public health laboratories in Canada provided 142 *C. glabrata* isolates. Resistant isolates from invasive infections were prioritized. Of 142 isolates, 80 (56%) were resistant to at least one antifungal drug (fluconazole, micafungin, amphotericin B) and 110 (77%) were collected from sterile sites. Sequencing libraries were prepared using the Nextera XT DNA Library Preparation Kit and sequenced on the NextSeq 550 platform. The Single Nucleotide Variant Phylogenomics pipeline version 1.1 was used to generate a maximum likelihood phylogenomic tree based on single nucleotide variants in the core genome. CBS138 was used as the reference strain for this phylogeny.

Results: The phylogenomic tree represented 68% of the *C. glabrata* core genome. Of 142 isolates, 127 (89%) grouped into one of 16 genetically related clusters. The five largest clusters accounted for 61% of the isolates (cluster 3, 15.5%; cluster 7, 10.6%; cluster 11, 10.6%; cluster 13, 8.5%; cluster 16, 15.5%). Few clusters grouped based on province of isolation, with the exception of cluster 9, which consisted of isolates exclusively from the same province in Central Canada. Some clusters grouped according to site of isolation, for example clusters 2, 5, 8, 9, and 10 were isolated from sterile body sites with the majority from bloodstream infections. Resistant isolates were present throughout the tree and all clusters contained at least one resistant isolate. Cluster 14 (n = 6) consisted entirely of isolates that were resistant to fluconazole. Cluster 16 (n = 22) had the largest number of resistant isolates with 15 resistant to fluconazole, three resistant to micafungin and one resistant to amphotericin B.

Conclusion: WGS revealed that there was a high level of genetic diversity in antifungal resistant *C. glabrata* isolates circulating in Canada. Only two branches were enriched for antifungal resistant isolates, suggesting that, in most cases, antifungal resistance may arise spontaneously due to selective pressure during treatment rather than dissemination of resistant clones.

Muscle Weakness Precedes Atrophy during Cancer Cachexia and is linked to Muscle-Specific Mitochondrial Stress

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Background: Muscle weakness and wasting are defining features of cancer-induced cachexia. Mitochondrial stress occurs before muscle atrophy in certain muscles, but the heterogeneity between muscles and across time remains unclear. This investigation compared the effects of cancer on quadriceps and diaphragm muscle force, atrophy, and mitochondrial bioenergetics at 2 and 4 weeks of tumour growth in a mouse model of cancer cachexia.

Methods: Colon-26 (C26) carcinoma cells or phosphate-buffered saline (PBS) were injected in the hind flank of 8-week-old male CD2F1 mice. Tumours developed for 2 or 4 weeks in separate groups. Muscle force, fibre cross-sectional area and the mitochondrial electron transport chain (ETC) protein contents were assessed in quadriceps and diaphragm. Mitochondrial bioenergetics were measured by high-resolution respirometry and spectrofluorometry (H_2O_2 emission) in permeabilized muscle fibres.

Results: At 2 weeks, the presence of small tumours had no effect on body or muscle mass, while force production was lower in both quadriceps (-52.7%) and diaphragm (-13.4%) vs control. Pyruvate-supported ADP-stimulated mitochondrial respiration was lower in quadriceps at 2 weeks (-27.9%) while mitochondrial H_2O_2 emission was elevated in diaphragm (28.1%) without changes in ETC protein contents in both muscles. At 4 weeks, tumour size was larger compared to 2 weeks and corresponded to lower tumour-free body mass, muscle mass, and cross-sectional area of quadriceps and diaphragm fibres. Force production in quadriceps was similar to control but remained lower in diaphragm (-13.4%). Mitochondrial respiration was increased in both muscles vs control across a range of [ADP] (34.3% quadriceps, 32.9% diaphragm) despite lower ETC protein content in quadriceps (-31.8%) and unchanged contents in diaphragm.

Conclusion: These findings indicate muscle weakness precedes atrophy in quadriceps and diaphragm in the C26 model of cancer cachexia. This early weakness is associated with heterogeneous mitochondrial responses whereby pyruvate oxidation is reduced in quadriceps and H_2O_2 emission is elevated in diaphragm. Muscle-specific compensations in force production and mitochondria occur thereafter which demonstrates how the effects of cancer on one muscle do not necessarily predict the response in another muscle. Exploring the heterogeneity of cachexia across time and muscle type may reveal distinct mechanisms that confer unique sensitivities, or resistance, to cancer.

The Phosphorylation of AMPKβ1 is Critical for Increasing Autophagy and Maintaining Mitochondrial Homeostasis in Response to Fatty Acids

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Introduction: Fatty acids are vital for eukaryotic cell survival, however, when present in surplus chronically, can lead to metabolic disturbances. The AMP-activated protein kinase (AMPK) is an important regulator of cellular metabolism – governing energy homeostasis through the phosphorylation of enzymes involved in glucose uptake, carbohydrate metabolism, fatty acid and cholesterol synthesis, lipid oxidation, and mitochondrial network dynamics. AMPK is a required heterotrimeric complex protein consisting of an activating alpha, and regulatory beta and gamma subunits; with multiple isoforms for each. Recently, our lab has discovered a new fatty acid sensing axis involving long chain acyl-CoA esters and the β1 subunit isoform of AMPK. Although it is well documented that both synthetic and natural molecules can activate AMPK through a mechanism involving the Ser108 residue within the β1 subunit, its physiological significance *in vivo* has not been assessed.

Methods: In our study, we developed a mouse model with a single amino acid residue knock-in mutation of Serine-108 to Alanine-108 (S108A-KI), making the site unable to be activated.

Results: We found that S108A-KI mice had impairments in fat oxidation in response to exogenous lipids on a regular diet. S108A-KI mice also had greater glucose intolerance and liver steatosis on a high-fat diet, indicating the importance of this site for metabolic health in response to elevated lipids *in vivo*. Subsequent studies from extracted tissues and cultured hepatocytes from the livers of mice indicated a defect in the ability of S108A-KI mice to recycle (through the process of autophagy) and replenish (through the process of biogenesis) mitochondria in response to the presence of excess fatty acids.

Conclusion: These data demonstrate an important physiological role for AMPK β1 Ser108 in promoting mitochondrial biogenesis and autophagy to maintain cellular energy homeostasis and support the concept that pharmacological targeting of AMPK through this binding site may exert favorable metabolic effects.

A Nonrandomized Trial of a Five-Session Brief Mindfulness-Based Intervention for Emerging Adults Experiencing Anxious and Depressive Symptoms

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Introduction: Emerging adults (EAs) experience elevated distress and mental illness, particularly depressive and anxious symptoms (Ferro et al., 2015). Despite increased mental health needs, EA symptoms often go untreated on university campuses (McGorry et al., 2011). Therefore, additional preventative, evidence-based treatment is needed to enhance coping among EAs (Hetrick et al., 2008). Recent studies have illustrated the effectiveness of brief mindfulness-based interventions (bMBI) in reducing depression, anxiety, stress, and improving well-being for clinical and non-clinical EA student populations (Burgess et al., 2021; Nyklíček et al., 2014; Song & Lindquist, 2015). We tested the effectiveness of a bMBI for EAs experiencing anxious and depressive symptoms. Specifically, we examined: 1) whether there are improvements in mental health outcomes among participants at post-intervention and in a one-month follow-up, and 2) whether initial health variables predict overall mental health improvement over the intervention.

Methods: Forty-five undergraduate students (18-29 years old) who were experiencing mood and/or anxiety symptoms participated in a five-session, in-person mindfulness group. Data on anxious symptoms (Generalized Anxiety Disorder-7), depressive symptoms (Patient Health Questionnaire–9), mental well-being (Warwick-Edinburgh Mental Well-Being Scale), perceived stress (10-item Perceived Stress Scale), and self-compassion (12-item Self-Compassion Scale – Short Form) were collected at baseline, immediately prior to the beginning of the intervention, at the beginning of session three, immediately following the intervention and at one-month follow-up. To examine the predictions relating to outcome change, and to gain a better understanding of within-individual differences in the development of mental health outcomes over time, a series of linear mixed effects models were conducted (Curran et al., 2010).

Results: The preliminary analysis indicated that this bMBI for EAs was effective in decreasing psychological distress and increasing well-being, with improvements continuing at the follow-up. Additionally, the moderation findings illustrated that pre-bMBI self-compassion scores–but not pre-bMBI perceived stress–moderated anxious, depressive and well-being scores at mid-bMBI, post-bMBI, and one-month follow-up.

Conclusions: The current findings lend support for an effective intervention to figure in an EA university-wide mental health prevention strategy. The findings provide direction for increased services and preventative strategies in post-secondary education and future research investigating bMBIs in EA populations.

Modelling of Electrical Fingerprints Reveals Transcriptomic Profiles Linked to α-Cell Dysfunction in Type 2 Diabetes

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Introduction: Diabetes affects close to half a billion people worldwide. Every 24 hours, nearly 500 Canadians are diagnosed with a form of diabetes. Commonly, diabetes is diagnosed either as Type 1 (T1D), an auto-immune disorder that destroys insulin-producing cells, or Type 2 (T2D), a condition of combined impairments in the production and action of insulin. In the healthy pancreas, β-cells secrete insulin to lower elevated blood glucose levels (preventing hyperglycemia) while pancreatic α-cells secrete glucagon, insulin's counterbalancing hormone, to raise diminished blood glucose levels (preventing hypoglycemia). The heterogeneity of β-cells and their dysfunction in diabetes receives more attention than pancreatic α-cells, even though the malfunction of α-cells is well documented in multiple forms of diabetes. Therefore, we developed approaches to study the heterogeneity of human islet cell electrophysiologic behaviour and applied these to α-cell phenotypes in T2D.

Methods: Machine learning methods were applied to patch-clamp electrophysiology to generate classifier models that empirically predict and score canonical α- and β-cell phenotypes in humans. We then linked the model scores to single-cell RNA sequencing data (patch-seq). Next, we applied the model to score α- and β-cells from T2D donors and investigated T2D α-cell dysfunction by correlating their scores with their respective single-cell transcriptomes.

Results: The model's scoring, without *a priori* knowledge of cell type, intuitively correlates with the expression of relevant lineage and identity markers in non-diabetic (ND) donors, thus validating our model. In T2D, we find impaired α- and β-scores compared to ND, indicative of a loss of electrophysiological identity. Our correlation analysis identified that pathways involved with the mitochondrial respiratory chain complex assembly are enriched in T2D α-cells with the most dramatically impaired scores. Further, we discover that T2D α-cell exocytosis is selectively impaired in α-cells enriched in lineage and maturity factors such as ISL1, NEUROD1, NKX2-2, and ARX.

Conclusion: We generated modelling approaches and combined them with electrophysiological patching and single-cell sequencing techniques to improve the understanding of human islet cell phenotypes. We advocate important links between α-cell mitochondrial function, lineage, maturation state, and susceptibility to dysfunction in human T2D.

Examining Copper Susceptibility Testing Methods to Screen Hospital Sink Drain *Pseudomonas Aeruginosa* Isolates Carrying GI-7, a Copper Tolerant Genomic Island

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Introduction: Copper is an important antimicrobial metal that inhibits the growth of both Gram-negative and Gram-positive bacterial species. However, its efficacy is threatened by emerging copper tolerance mechanisms in opportunistic pathogens like Pseudomonas aeruginosa. The genomic island GI-7 is of interest as it contains several known copper tolerance genes and has been identified in P. aeruginosa sink drain isolates across the world. However, our ability to test copper tolerance in isolates that contain GI-7 is limited due to a lack of experimental consistency in measuring copper tolerance. Standard broth microdilution assays of copper salts result in media acidification that may not support bacterial growth or survival. Here, we will examine the presence of GI-7 in ICU sink drain isolates, and use susceptibility testing and transcriptomics to examine the effects of media acidification and copper exposure on P. aeruginosa.

Methods: KAT sect was used to identify the presence of GI-7 in ICU sink drain isolate genomes. To determine the effect of acidification on copper susceptibility testing, we compared *P. aeruginosa* strains growth in MHB containing CuSO4 and in MHB adjusted to its acidic pH values at the same copper concentration. It was determined that copper and pH have an additive effect on growth inhibition, as P. aeruginosa strains survived in media adjusted to pH 5.5, but not in copper-supplemented media at the same pH. Finally, we exposed *P. aeruginosa* strains to pH 5.5 or 2 mg/mL CuSO4 and performed transcriptomics to compare the transcriptional response to these types of stress.

Conclusions: GI-7 is an important marker of copper tolerance, and surveillance of this genomic island may be important in mitigation of sink drain related outbreaks of *P. aeruginosa*. Additionally, current copper susceptibility testing must be reviewed due to the effects of pH on perceived copper susceptibility and transcriptional responses.

Exploiting the SCF Complex to Identify Novel Therapeutic Targets in High-Grade Serous Ovarian Cancer

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Introduction: High-grade serous ovarian cancer (HGSOC) is the most commonly diagnosed histotype of epithelial ovarian cancer and remains the most lethal gynecological malignancy. Unfortunately, >80% patients are diagnosed at stages III or IV, when chemotherapy may be the only therapeutic option. As ~70% of patients eventually succumb with drug resistant disease, novel drug targets are urgently needed to improve HGSOC patient outcomes. Recently, we determined that reduced expression of each core member (SKP1, CUL1, RBX1) of the SCF complex, an E3 ubiquitin ligase, induces chromosome instability (CIN), or ongoing changes in chromosome complements. As ~88% of HGSOCs harbour alterations within SKP1, CUL1 or RBX1, we now seek to therapeutically target these defects using a synthetic lethal (SL) paradigm.

Methods: To identify putative SL interactors (i.e., drug targets) of SCF complex member genes, we performed an siRNA-based screen of the DNA damage response library (239 genes) within heterozygous SKP1, CUL1 and RBX1 CRISPR/Cas9 knockout clones. Briefly, quantitative imaging microscopy was employed to assess the number of cells (nuclei) relative to a negative control (siControl). Genes were prioritized based on the strength of the SL interaction, reproducibility between the knockout clones and the availability of small molecule inhibitors.

Results: Overall, the screen identified 180 putative SL interactors, 84 of which were shared between the various knockout clones. Of these, CHEK2 and PARP1 were selected for further direct testing. First, semi-quantitative western blot analyses were performed to assess the silencing efficiencies of 4 individual siRNA duplexes or a pool (comprised of all 4 individual duplexes), which reduced endogenous CHEK2 and PARP1 levels to ~5% and ~1%, respectively. Next, to assess the reproducibility of SL phenotypes, the two most efficient individual siRNA duplexes and the pool were utilized within the RBX1+/- clones. Initial results show decreases in the number of nuclei within the RBX1+/- clones relative to controls and suggest that CHEK2 and PARP1 are novel SL interactors of RBX1.

Conclusions: Collectively, these data suggest that CHEK2 and PARP1 are novel SL interactors of RBX1 that may warrant further pre-clinical study to ultimately improve HGSOC patient outcomes.

The Impact of the Built Environment on Adherence to Cancer Prevention Guidelines and Cancer Risk, Cancer Incidence and Cancer Mortality in a Cohort of British Columbians

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Background: Several health agencies have recommended the adoption of lifestyle modifications that adhere to specific cancer prevention guidelines including maintaining a good body weight, being physically active, eating a healthy diet, limiting alcohol consumption and cessation of smoking. However, findings in Canada show that only a few people adhere to these guidelines, possibly due to lack of awareness, low communication, built neighbourhood characteristics and other factors that influence lifestyle behaviour. This underscores the importance of research into factors that promote adherence to the cancer prevention guidelines. It is not clear what role the built environment could play in promoting compliance with such guidelines. This study will evaluate the association between objectively derived neighbourhood built environment characteristics and adherence to the cancer prevention guidelines as well as the impact of the adherence on cancer risk, cancer incidence and cancer mortality.

Methods: The study included 29,179 participants from the British Columbia Generations Project (BCGP), a prospective cohort study of adults aged 35 to 69 years recruited between 2009 and 2013 and followed for a median of 9 years for cancer risk or incidence. The BCGP has been linked to the Canadian Urban Environmental Health Research Consortium (CANUE) and the British Columbia cancer registry (BCCR), to provide geospatial environmental data on different indices of the built environment as well as confirm all cancer diagnoses. Covariate-adjusted generalized linear regression models were used to estimate the associations between neighbourhood characteristics (walkability, green space, community deprivation) and adherence to the cancer prevention guidelines. Multi-level Cox proportional hazards regression was used to assess the impact of adherence to cancer prevention guidelines on the risk of cancer (overall and by cancer type). Propensity score matching was done to help establish the robustness of the main finding.

Results & Conclusion: In both men and women, adherence to the Candian Cancer Society cancer prevention guidelines was associated with reductions in all cancer incidence, specific site cancer incidence, cancer mortality and total mortality. These findings suggest that, after accounting for cigarette smoking, adherence to a set of healthy behaviours may have considerable health benefits.

Tuning Muscle Energy Homeostasis: The Role of Nix in Muscle Metabolism

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Introduction: Early in diabetes, skeletal muscle ceases to respond to insulin signalling and as the disease progresses, the positive effects from muscle on whole body metabolism during exercise are diminished. The protein Nix is a central signalling protein in muscle metabolism that can regulate insulin signaling, mitochondrial turnover, and muscle growth and development. The objective of this research is to answer the questions: What aspects of muscle metabolism is Nix critical for, and what are the effects on muscle and whole-body metabolism in the absence of Nix?

Methods: To determine the effect of Nix, muscle-specific deletion of Nix in mice was achieved using Cre-lox recombination and used in a series of histological, biochemical, and physiological tests. To assess cellular mechanisms, a cell culture model of C2C12 myotubes was coupled with fluorescent microscopy.

Results: Deletion of Nix in muscle caused the appearance of ragged red fibers (N=3, $p<0.001$), a diagnostic marker of accumulated dysfunctional mitochondria, which was confirmed by electron microscopy. In the cellular model, we observed that depleting Nix levels resulted in impaired mitochondrial clearance which supports the phenotypes observed *in vivo*. Array-based analysis of kinase activity (kinomics) indicated that Nix-knockout mice could have deficiencies in activity of enzymes in aerobic respiration. Lastly, physiological tests showed that Nix-knockout mice had reduced exercise endurance, increased CO2 output during treadmill running suggesting greater reliance on anaerobic respiration, and impaired glucose tolerance.

Main Findings: Together these data provide evidence that deletion of Nix results in impaired clearance of dysfunctional mitochondrial, decreased exercise tolerance, and diminished ability to regulate blood glucose levels. In conclusion, Nix plays no small role in muscle metabolism through the mitochondria, and the absence of Nix results in multi-faceted disruption muscle metabolism including systems important to the development (ie. insulin resistance) and management (ie. exercise) of type 2 diabetes.

A HSP40 Co-Chaperone Modulates the Function of a Specific miRNA Silencing Complex in *C. Elegans*

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microRNAs (miRNAs) are small non-coding RNAs of about 21 nucleotides long that regulate gene expression post-transcriptionally. To be active, microRNAs need to associate with an Argonaute (AGO) protein to form the microRNA-Induced-Silencing-Complex (miRISC). The miRISC can target mRNAs sharing partial sequence complementarity and silence them through translational inhibition and/or targeted degradation. microRNAs have been shown to be key regulators of various cellular processes, which explain why they are frequently misregulated in numerous diseases. Although the interaction between various miRISC and their mRNA targets are similar, the binding outcome of the miRISC is mediated by multiple factors. Notably, AGO protein function can be regulated through post-transcriptional modifications and interaction with specific partners. In *C. elegans*, more than 20 AGO proteins can interact with multiple types of small RNAs. To answer what confers the specific loading of miRNA onto specific AGO and how AGOs are differentially modulated, we performed a yeast two-hybrid screen to identify specific binding partners of ALG-1, one of the two AGOs responsible for miRNA-dependent silencing. Among the new interactors identified, we focus our work on a DnaJ co-chaperone protein called DNJ-12. Immunoprecipitation experiments confirm that DNJ-12 interacts with ALG-1 but not with the other AGOs. Various genetic assays indicate that the DnaJ chaperone is important for the miRNA pathway in animals. Notably, altering DNJ-12 expression in animals leads to improper alae formation and aberrant seam cells number, two developmental processes mainly controlled by miRNAs. Molecularly, we observe that modulating DNJ-12 level affects AGO ALG-1 stability and causes a decrease of several miRNAs, which both can explain the presence of miRNA-related phenotypes in animals. Furthermore, our data suggest that the presence of DNJ-12 is essential for the interaction between ALG-1 and HSP70, an heatshock protein previously shown to be required for proper miRNA loading *in vitro*. Experiments are ongoing to determine how the alteration of DNJ-12 causes the degradation of ALG-1 in animals. At term, this study will provide important insights on how small non-coding RNAs such as miRNAs are loaded onto specific AGO proteins.

Let's (Not) Talk About Sex: A Multimethod Examination of Inflammatory Bowel Disease Patient Experiences Obtaining Healthcare for their Sexual and Intimate Lives

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Introduction: Inflammatory Bowel Disease (IBD) is a chronic gastrointestinal condition with varied biopsychosocial sequelae. One area of well-being in IBD that lacks high-quality research or clinical attention is sexuality. Despite sexual difficulties being elevated among, and of major concern to, IBD patients, they are seldom addressed in healthcare. We sought to fill these gaps in the literature using a multimethod approach. Objectives 1 and 2 aimed to examine the proportions of participants who wanted and ultimately received information, respectively, on sexual functioning from a healthcare provider (HCP). Objective 3 aimed to elucidate individuals' lived experiences accessing information and help related to their sexual and intimate lives in the healthcare system.

Methods: An international sample of n = 480 adults with IBD completed an online survey. Data for Objectives 1 and 2 were gathered using Yes/No questions and were analyzed using frequency analyses. Written responses were obtained from participants for Objective 3 and data were analyzed using reflexive thematic analysis.

Results: 39.2% of participants reported wanting information on sexuality from an HCP, whereas 60.8% did not. Only 10.8% of participants received information on this topic, with 89.2% never having received any. Five major themes emerged in participants' written responses, including: 1) lack of supportive healthcare experiences (i.e., a dearth of care or understanding felt from HCPs and the healthcare system regarding sexuality); 2) lack of valuable information (i.e., a shortage of available, relevant, or holistic information on sexuality); 3) patient factors affecting support (i.e., not wanting information from HCPs due to a lack of need or barriers such as embarrassment); 4) I have to be my own support (i.e., feeling the need to find one's own information and help for sexuality outside of the healthcare system); 5) support is out there (i.e., positive experiences accessing information and help in the healthcare system).

Conclusion: IBD patients who want information on sexuality from an HCP rarely receive it. Potential reasons for not wanting this information from an HCP include embarrassment and past negative experiences. Improving communication between patients and HCPs surrounding sexuality is essential to improve the holistic care of IBD patients.

Mumps: A Truly Endemic Pathogen

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Despite the fact that the Mumps vaccine has been in use in Canada for over three decades, the mumps virus (MuV) has not been eliminated. Recent years have seen a re-emergence of mumps outbreaks in vaccinated populations, particularly in young adults. These outbreaks have been almost exclusively comprised of genotype G infections. It is unclear what is driving these outbreaks, but some possible explanations are it could be due to a vaccine escape strain, waning immunity, or both. Through the use of whole genome sequencing of Canadian outbreaks, combined with epidemiological data such as age, sex, vaccination status and geographical location we aim to identify if there is an endemic MuV strain circulating in Canada. This will also allow us to identify if there are certain groups who may be more at risk for future outbreaks. Investigating the Manitoba 2016-2018 outbreak (3000 cases) and the Newfoundland/Nova Scotia 2018 outbreak (150 cases) with the use of a novel whole genome sequencing method, we have sequenced 294 full genomes directly from clinical samples. Based on the phylogenetic and Bayesian analysis of these genomes, we see that the provincial outbreaks diverged separately from one another. When analyzed with archival Canadian sequences, it is clear that mumps is endemic in Canada, and these outbreaks are not due to new importations. From this data we have identified a number of significant mutations, which will be tested for association with epidemiological factors. Overall this project will generate data that will be used to inform future vaccination and outbreak policy surrounding mumps. It will also identify characteristics of the mumps outbreaks that will allow us to combat future outbreaks more efficiently.

Examining How Mitochondrial Dysfunction May Contribute to Right Ventricular Fibrotic Damage Seen in Early-Onset Duchenne Muscular Dystrophy

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Introduction: Duchenne muscular dystrophy (DMD) is a X-linked recessive neuromuscular disorder that is perpetuated by mutations to the DMD gene, which is responsible for encoding the dystrophin protein. It is a muscle-wasting disease whereby the absence of functional dystrophin protein can hinder the extracellular matrix from connecting to the actin cytoskeleton. This can lead to sarcolemmal instability and reductions to force transduction by making muscle fibres more susceptible to contraction-induced membrane damage. DMD affects males beginning in childhood and can culminate at fatal outcomes. Although DMD is associated with loss of ambulation and respiratory failure, dystrophin deficiency also renders the heart more susceptible to left ventricular (LV) fibrotic injury. Several studies have confirmed the presence of LV damage, including interstitial fibrosis and inflammation at a later age in the course of the pathology (>7 weeks). The degree to which fibrosis occurs in the remaining cardiac chambers remains unknown, particularly at early stages of the disease. Furthermore, mitochondrial dysfunctions are emerging as a potential contributor to cell death in cardiac fibres. We hypothesize that fibrotic damage to the remaining chambers of the heart early in the disease's progression may precede LV dysfunction and could even contribute to the dilated cardiomyopathy commonly seen in DMD patients later in life.
Methods: Our study design compared D2.mdx mice to wildtype (WT) mice at 28 days of age. Cardiac fibrosis was characterized by four-chamber histopathology (picrosirius red staining), Western blotting, and serum-based ELISA kits for circulating cytokine markers.
Results: Preliminary data from our study suggests significant elevations to left atrial and right ventricular fibrosis (n=7-10) and trending elevations to right atrial and LV fibrosis (n=7-10). Our findings will be corroborated by a two-pronged mitochondrial bioenergetics approach, in which permeabilized chamber-specific fibre bundle respiration and reactive oxygen species emission rates will be conducted and compared to mitochondrial-linked caspase 9/3 apoptotic signaling.

Conclusion: This project aims to investigate how chamber-specific distribution of collagen deposition in a young D2.mdx age group may link mitochondrial dysfunction to cardiomyocyte death. The work will provide foundational knowledge that may inform future development of mitochondrial therapeutics to treat cardiomyopathy in early-onset DMD.

Evaluation of Environmental and Aerosol Exposure Risk of SARS-CoV-2 during Intensive Care Interventions Using an African Green Monkey Model

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SARS-CoV-2, the etiological agent of COVID-19, continues to cause an unprecedented global pandemic associated with over 450 million cases and 6 million deaths as of March 15, 2022. As the pandemic has progressed, there have been shifts in infection prevention and control guidelines as new data on infection, isolation and transmission becomes available. Current belief has exposure occurring in any of three principal ways; 1) Through deposition of larger respiratory droplets on exposed mucous membranes; 2) Through inhalation of very fine aerosol particles; 3) Through the touching mucous membranes with hands either directly soiled with respiratory fluids, or indirectly by touching surfaces or objects (fomites) with virus on them. While few argue with the role of large particle droplets, the roles of smaller particles and surface fomites is less clear. The World Health Organization (WHO) advises aerosol generation mainly occurs during aerosol generating procedures (AGPs) including positive pressure ventilation, tracheal intubation, airway suctioning, nebuliser treatments, high-flow oxygen delivery and bronchoscopy procedures. Given the large potential risks associated with aerosol and droplet transmission, especially during high-risk intensive care interventions, it is important to assess the degree AGPs produce aerosolized and droplet particles and the degree to which these may transmit. Therefore, we aimed to quantify the spread of droplet and potential aerosol generation of SARS-CoV-2 during intubation and bronchoscopy procedures using an African green monkey (AGM) model. We found that droplet contamination occurred during AGPs in three of nine AGMs finding SARS-CoV-2 viral RNA on the veterinary technician's gloves and face shield. To evaluate aerosol contamination, both CSU low volume air samplers as well as a 6-stage Andersen bioaerosol cascade impactor were used. However, throughout all of the procedures on the nine AGMs no air contamination was detected. With these findings, we agree with other literature that larger droplet contamination is the primary mode of transmission for SARS-CoV-2.

Protein Translation is Compromised in the Bowen-Conradi Ribosomopathy

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Bowen-Conradi Syndrome (BCS) is an inherited ribosome assembly disorder, or ribosomopathy, present in the Hutterite population at a rate of 1/355 births. BCS presents with severe developmental delay, a failure to thrive, and death in infancy. BCS is due to a D86G variant in the pseudouridine methyltransferase and SSU processome protein Emg1, required for both ribosome assembly and methylation of a pseudouridine in the decoding P site of the small subunit of the ribosome. This 18S rRNA pseudouridine is post-transcriptionally hypermodified and critical to the translational fidelity of the ribosome in mRNA decoding. As with other ribosomopathies (such as Diamond-Blackfan anemia and Treacher-Collins syndrome), the consequences of ribosome mis-assembly on translational fidelity and alterations to the proteome have been under-investigated. Here, we investigate suspected translation defects in BCS. Using a yeast model system of the disorder, the translation terminating antibiotic puromycin was first used to probe the molecular effects of BCS on translation. The puromycylation assay revealed a decrease in overall translational capacity, along with a likely change in translational preferences. Our results reveal, for the first time, that BCS cells are translationally compromised. This is consistent with recent results suggesting that the rRNA target site of Emg1 is hypomodified in all cancers. Ongoing experimentation using dual luciferase translation reporters will further characterize the nature of the translational defect – which we expect includes a decrease in translational fidelity due to the location of Emg1's target residue in the decoding P site – along with changes to the proteome.

Detection of Respiratory Syncytial Virus (RSV) in Wastewater Using RT-qPCR and the Cepheid GeneXpert System

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Introduction: Respiratory Syncytial Virus (RSV) is a common respiratory virus that typically affects children under five, the elderly, and immunocompromised individuals. The incidence of RSV was near-zero through 2020 due to prevention strategies meant to slow the spread of SARS-CoV-2, but a resurgence in RSV was observed in late 2021. Wastewater-based epidemiology (WBE) was shown to be an effective tool for the surveillance of outbreaks of SARS-CoV-2. Recent studies have shown that RSV is shed in the feces of young children and can be detected in wastewater, making WBE an option for RSV. WBE is particularly important for remote communities that face significant challenges to accessing clinical testing and experience delays in receiving clinical results. Due to its confirmed accuracy for detecting SARS-CoV-2 in wastewater, a desktop-sized molecular testing device, the Cepheid GeneXpert, was also tested for its ability to detect RSV in wastewater.

Methods: A RT-qPCR assay was developed to detect and quantify RSV genetic material in wastewater. This method was used to assess the stability of RSV in wastewater overtime at two temperatures: 25°C and 4°C. For comparison, we also assessed the stability of other respiratory viruses, including Influenza-A, Influenza-B, and SARS-CoV-2 in wastewater. All samples were also tested on the Cepheid GeneXpert to assess the practicality of using this system for WBE of RSV.

Results: Using RT- qPCR, we found that trace amounts of RSV can be consistently found in wastewater at least 14 days post-inoculation when stored at room temperature and can be found in greater quantities in the solid fraction of wastewater compared to the liquid fraction. The GeneXpert was shown to detect trace amounts of RSV until day 7, after which the signal disappeared.

Conclusion: WBE can be used to accurately identify regions experiencing an increase in RSV cases. Both our newly developed RT-qPCR assay and the GeneXpert can be used to monitor the signal of RSV in wastewater, with RT-qPCR having a lower limit of detection. Using WBE in cities and communities will strengthen the existing public health surveillance systems in Canada to help create a more robust surveillance system for RSV.

Impact of Differential Gene Expression on Early Innate Immune Responses to COVID-19 Vaccination in End Stage Renal Disease Patients

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Introduction: Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has infected more than 315 million people worldwide causing more than 5.5 million deaths since the beginning of the COVID-19 pandemic. End Stage Renal Disease (ESRD) patients are immunocompromised and at an increased risk of infection and severe disease with an adjusted hazard ratio of 2.59 (95%CI, 1.55 – 4.32) for in-hospital mortality from COVID-19. Furthermore, ESRD patients' humoral and B cell memory formation in response to COVID-19 vaccination is significantly reduced. However, early innate immune responses have yet to be explored in this population.

Hypotheses: ESRD patients will have muted expression of early innate immune genes in response to COVID-19 vaccination compared to healthy controls. Innate immune responses will associate with immune parameters (cellular and antibody) at 2 weeks, 6 months, and 1-year post vaccine.

Methods: Blood was collected in PAXgene RNA Blood Tubes before (BD1) and 1-4 days after first dose (PD1) of COVID-19 vaccine (n=17 ESRD; 28 healthy controls) and RNA was isolated for RNA-Sequencing. A detailed questionnaire was collected at enrollment with demographics, medical history, COVID-19 history, and medications, and is being updated at subsequent timepoints. We have approximately 80% power to detect genes differentially expressed 2.5-fold between groups (BD1, PD1, ESRD, healthy control). Significantly differentially expressed genes will be investigate for association with immune parameters (i.e. antibodies, soluble mediators, memory T and B cells, etc.) at 2 weeks, 6 months, and 1-year post vaccine.

Significance: ESRD patients are at an increased risk of SARS-CoV-2 infection and severe COVID- 19 outcomes including hospitalization and death, making them a critical immunocompromised population. However, few studies have investigated the factors driving this phenotype. Furthermore, it is poorly understood what impact this phenotype will have on the longterm success of COVID-19 vaccination in this population. Understanding the differential gene expression in response to COVID-19 vaccination can elucidate which aspects of the immune response are muted and inform policy decisions for vaccination schedule and preventative public health measures.

Characterization of MERS-CoV Monoclonal Antibodies as Antibody-Based Therapeutics

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Introduction: Middle-East Respiratory Syndrome Coronavirus was first identified in Saudi Arabia and has since become endemic to regions of the Middle East. Transmission of MERS-CoV between human hosts is poor, but boasts a high mortality rate (~35%). Research has produced promising therapeutics and vaccine candidates, however, none are currently licensed for commercial use. Antibodies play an important role in our ability to fight off viral infection. Some antibodies prevent entry of viruses into our cells through the process of neutralization and these may be promising therapeutics.

Methods: Conventional hybridoma technology was used to develop murine hybridoma cell lines. Monoclonal antibodies (mAbs) were purified and specificity to the viral spike protein was confirmed using ELISA. Neutralizing activity was confirmed via a microneutralization assay with live MERS-CoV. DNA sequences were determined using Sanger sequencing and compared against an antibody database (IgBlast) to determine germline origin. The protein sequences were obtained by de novo peptide sequencing, in which the proteins are digested and analyzed using mass spectrometry. Antibodies were isotyped using a commercial isotyping kit (IsoStrip™, Roche). Dose-response curves were generated using a plaque-reduction neutralization test (PRNT) with Vesicular Stomatitis Virus-vectored MERS-CoV spike (VSV-MERS-CoV-S). Additionally, the binding affinity was quantified using bio-layer interferometry (BLI) technology. Selection of mAb escape mutants was carried out by passaging VSV-MERS-CoV-S with serially diluted antibody, and mutants sequenced. Lastly, the therapeutic potential will be evaluated, involving the development of a delivery system (e.g., DNA or viral vector) that permits the in situ production of antibody.

Results: 15 antibodies were generated, 6 of which demonstrated neutralizing activity against live MERS-CoV in a microneutralization assay. 5 of 6 antibodies contained very similar amino acid sequences and the neutralizing activity of these antibodies were quite similar. In contrast, one antibody originating from different germline genes showed greater ability to fully neutralize the virus. All antibodies are the IgG 2α isotype with a κ light chain.

Conclusion: The past two years especially have shown us the importance of having effective and readily available therapeutic options for emerging viruses. We hope that our research can contribute to ongoing efforts to develop treatments for MERS-CoV infection.

Increases in Alcohol and Cannabis Use Associated with Deteriorating Mental Health among LGBTQ2+ Adults in the Context of COVID-19: A Repeated Cross-Sectional Study in Canada, 2020–2021

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Background: Lesbian, gay, bisexual, trans, other queer, and Two-Spirit (LGBTQ2+) people are particularly at risk for the psycho-social consequences of the COVID-19 pandemic, though population-tailored research within this context remains limited. This study examines the extent of, and associations between, increased alcohol and cannabis use and deteriorating mental health among LGBTQ2+ adults in Canada during the COVID-19 pandemic.

Methods: Data are drawn from LGBTQ2+ respondents to a repeated, cross-sectional survey administered to adults living in Canada (May 2020–January 2021). Bivariate cross-tabulations and multi-variable logistic regression models were utilized to examine associations between increased alcohol and cannabis use, and self-reported mental health, overall coping, and suicidal thoughts.

Results: Five-hundred and two LGBTQ2+ participants were included in this analysis. Of these, 24.5% reported increased alcohol use and 18.5% reported increased cannabis use due to the pandemic. In the adjusted analyses, increased alcohol use was associated with poor overall coping (OR = 2.28; 95% CI = 1.28–4.07) and worse self-reported mental health (OR = 1.98; 95% CI = 1.21–3.25), whereas increased cannabis use was associated with suicidal thoughts (OR = 2.30; 95% CI = 1.16–4.55).

Conclusion: These findings underscore the need for population-tailored, integrated substance use and mental health supports to address interrelated increases in alcohol/cannabis use and worsening mental health among LGBTQ2+ adults, in the context of the COVID-19 pandemic and beyond.

Postnatal Choline Supplementation Ameliorates Deficits in Hippocampal Synaptic Transmission Following Prenatal Ethanol Exposure

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Introduction: Ethanol is a teratogen that, when present during fetal development, leads to a range of life-long cognitive impairments known as Fetal Alcohol Spectrum Disorders (FASD). FASD affects an estimated 5% of the Canadian population, yet currently there is no effective treatment for FASD. The nutrient choline is one candidate therapy that has shown some promise when used as a supplement in clinical populations, however the mechanism of action remains uncertain. The objective of this study was to determine if postnatal choline supplementation improves learning and memory outcomes following prenatal ethanol exposure (PNEE) utilizing both behavioural and cellular metrics of memory.

Methods: Sprague Dawley rats were administered an ethanol-containing diet from gestational day 1-22, equivalent to moderate ethanol exposure during the first two trimesters of pregnancy in humans. Offspring were injected with choline chloride or saline from postnatal day (PND) 10-30 and then tested from PND 31-36. The radial arm maze was utilized to determine changes in both working- and long-term spatial memory with PNEE and choline treatment. To investigate a role for improved synaptic function we also examined long-term potential, the cellular mechanism of memory, in the hippocampal dentate gyrus of juvenile offspring.

Results: The preliminary data suggests subtle deficits in spatial memory performance using the radial arm maze following PNEE and some select improvements with choline treatment. At a synaptic level, PNEE decreased the overall magnitude of LTP in male offspring. This deficit was rescued with postnatal choline supplementation. In female offspring, while no deficits were seen in magnitudes of LTP following PNEE, there was an aberrant increase in excitability that was ameliorated with choline treatment. These data suggest a potential sex-specific compensatory mechanism for the initial ethanol exposure.

Conclusions: These data uncovered novel sex differences in how the hippocampus is altered following PNEE and aid in understanding that there may be 'neuroprotective' changes that occur in the female brain. These results support the use of choline as a treatment for FASD and encourage future work delving into changes in synaptic plasticity and hippocampal function.

Development of a Transwell Culture for Studying *Legionella Pneumophila* Infections

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Introduction: Legionella has historically been considered a rare etiological agent of pneumonia. Recent studies have shown that the prevalence of Legionella infections has been underestimated due to a lack of awareness, faulty detection methods, and non-specific respiratory clinical manifestation, causing it to be overlooked. According to the CDC, reported cases of Legionella infections in the United States have increased tenfold between 2000 and 2018. It is not known if the increase in cases is due to evolution or more meticulous testing. Nevertheless, this indicates that Legionella is overlooked in the clinic. Legionella is much more common and harder to detect in individuals coinfected with HIV due to nonspecific presentation and other potential causes of pneumonia. In individuals coinfected with Legionella and HIV, mortality rates have been observed to increase to 53% compared to the 20% seen in HIV-infected individuals without Legionella.

Rationale: The goal of this project is to establish transwell cultures as a viable method of studying Legionella infections. Current methods used for studying Legionella fail to recreate the environment of the human lungs during infection, sometimes leading to large discrepancies between what occurs *in vitro* and what occurs *in vivo*. The transwell culture bridges this gap by providing a more biologically relevant environment that allows different cell types to communicate with each other, replicating the immune response.

Methods: To develop the transwell culture for studying *Legionella pneumophila* infections, cell lines will be prepared for mimicking the lung microenvironment: CALU-3 (Lower lung epithelium), A549 (Macrophage), and ATCC (Lung fibroblasts). Firstly, these cells' permissiveness to infection by L. pneumophila Lp02 will be measured using CFU assays to assess bacterial growth and observe the employed immune response using a MILLIPLEX human chemokine panel to detect 12 proinflammatory and anti-inflammatory mediators. These cells will then be reconstituted into a transwell culture, repeating the same assays to verify that the growth kinetics and cytokine response are changed upon using a transwell culture.

Significance: Using the transwell culture will allow interrogation of Legionella-induced inflammation as well as simulation of the cells participating in response to infection and the consequences to the alveolar barrier.

Prevalence and Inequalities of Undiagnosed Hypertension in Bangladesh: Evidence from a Nationwide Cross-sectional Survey

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Background: Although hypertension is a significant risk factor for cardiovascular diseases, 46% of global hypertensive cases are unaware of their elevated blood pressure (BP) level and remain undiagnosed. If undiagnosed hypertension (UNHTN) is not treated promptly, it can lead to complex health conditions. According to the Bangladesh Demographic and Health Survey (BDHS), around 60% of hypertensive cases are undiagnosed in Bangladesh, and socioeconomic inequalities exist in the prevalence of UNHTN.

Objectives: The research objectives are to: 1) estimate the prevalence of UNHTN among Bangladeshi adults (aged ≥18years), 2) identify individual-level risk factors for UNHTN, and 3) test for socioeconomic inequalities in the prevalence of UNHTN.

Methods: This research will use 2017-18 BDHS data, which are representative of the Bangladeshi population. Survey respondents were assessed as UNHTN if they were hypertensive at the time of the survey based on the latest clinical guidelines, were not taking prescribed antihypertensive medication, and had not been diagnosed with hypertension prior to the survey. We will estimate the age- and sex-standardized prevalence of UNHTN using the age-sex structure of the Bangladeshi population. Multivariable logistic regression models will be used to test the association of UNHTN with individual-level risk factors, including sex, age, education, occupation, smoking status, body mass index, and selected comorbid conditions. Adjusted odds ratios and 95% confidence intervals will be reported. We will order respondents by their wealth index and estimate the degree and direction of socioeconomic inequalities in the prevalence of UNHTN using the concentration index.

Significance: Recent clinical guidelines lowered the BP threshold for diagnosing hypertension; this research will provide new information about previously unidentified hypertensive cases. Policymakers and government officials in Bangladesh can use information about UNHTN prevalence, risk factors, and socioeconomic inequalities to design appropriate and effective hypertension screening and early detection programs.

The Corporate Political Activity of the Food Industry and the Nutrition Profession in Quebec: State of Play and Recommendations

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Introduction: While governments have the responsibility to develop public health policies to counter obesity and chronic diseases, registered dietitians (RD)/nutritionists also have a role to play. However, public policy and nutrition practice can be influenced by industry-led strategies known as corporate political activity (CPA). The impact and population health risks associated with the pharmaceutical and tobacco industries' CPA have been well documented in the past. However, from the perspective of nutrition professionals, little data exists regarding the nature of the interactions with industry, the associated risks, and solutions to address this issue. This project aims to 1) explore existing literature on the issue, 2) document existing reflections and actions carried out by the profession in Quebec on this issue, 3) identify the strategies used by the food industry in Quebec to influence RD/nutritionists, and 4) develop a training about the critical analysis of CPA.

Methods: For the literature review, we did a scoping review that covered six databases and followed the steps proposed by Arskey and O'Malley. Secondly, we did an inductive analysis of the interviews made with the RD to classify existing actions and reflections on the issue. Third, a convergent mixed-methods design will combine the analysis of a survey completed by RD, case studies (n=3), and participant observations (n=3) to identify and classify CPA strategies within an existing conceptual framework. Finally, based on these studies and the competence-based approach, a training will be developed and tested with nutrition students.

Results: The scoping review and interviews revealed a variety of industries (e.g., food or pharmaceutical) that may interact with RD through various contact points (e.g., school, hospital, professional body). Acceptability was largely heterogeneous despite the many risks raised. Several solutions have been identified, but we do not have any data on their effectiveness yet.

Conclusion: Research on the influence of industry on nutrition profession has never been conducted in Canada. This project will inform and raise awareness across RD/nutritionists about the issue and prepare them to face CPA during their career. The goal is to enable them to play their role in this public health crisis with independence.

The Validity of Electronic Health Databases for Measuring Smoking Status: A scoping review

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Introduction: Smoking is a risk factor for many chronic diseases. Population-based electronic health databases, such as administrative databases, do not directly capture smoking information. However, many smoking status algorithms based on smoking-related health conditions have been developed for these databases. Our aim was to conduct a scoping review of smoking status algorithms developed from electronic health databases and describe the characteristics and validity of these algorithms.

Methods: The five-step Arksey and O'Malley framework for systematic reviews was adopted. We searched for articles published from 1990 to 2021 in MEDLINE, Scopus, and Web of Science with key terms such as validity, administrative data, electronic medical records (EMRs), smoking, and tobacco use. Abstracts were reviewed by two co-authors for decisions about study inclusion/exclusion. The extracted information included article characteristics (e.g., country of data origin, publication year, years and geographical source of study data), algorithm characteristics (e.g., data structure, data source, implemented techniques), and features of algorithm validation (e.g., type of reference data, accuracy measures). Study data were descriptively analyzed.

Results: The initial search resulted in 513 articles; 27 were selected for full review. Most articles were published in 2016-2021 and used US data from a single state or organization. A total of 65 algorithms were identified; 52 were based on EMR data and 13 were based on administrative data. The smoking status algorithms were primarily constructed using diagnosis codes for smoking-related conditions; prescription drug dispensations and physician service codes were also used. About half of the algorithms were developed using machine-learning models. Sensitivity, specificity, and accuracy of the algorithms were highly variable and ranged from 9%-100%, 58%-100%, and 42%-98%, respectively. Overall, EMR-based algorithms that relied on both structured and unstructured text data had the highest validity; administrative data-based algorithms based solely on diagnosis codes had low validity.

Conclusion: Several algorithms using different data sources and construction techniques have been proposed to ascertain smoking status in electronic health databases. While algorithm validity is influenced by the data source, many algorithms have low sensitivity and accuracy. Future research could develop new algorithms by linking multiple databases that contain smoking-related information.

Changes in Anxiety and Depression from 2-36 Months after Moderate to Severe Traumatic Brain Injury

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Introduction: Moderate-severe traumatic brain injury (TBI) can be considered a chronic health condition that leads to persisting disability and poses enormous challenges in returning to previous roles at work, school, and home. One of the most disabling consequences of TBI is the increased risk of psychiatric disorders. Anxiety and depression can compound the effects of brain injury and have a major impact on functional outcomes and quality of life. Cross-sectional studies have demonstrated elevations of anxiety and depression across stages of recovery, but further research is needed to elucidate change over time. The present study seeks to examine (1) longitudinal trajectories of anxiety and depression following moderate-severe TBI; (2) predictors of these trajectories; and (3) associations with return to productivity.

Methods: This was a secondary analysis of prospective data from the Toronto TBI Recovery Study database (n=148). Adults with moderate-severe TBI completed the Beck Anxiety Inventory (BAI) and Beck Depression Inventory (BDI) self-report questionnaires at 2-, 5-, 12-, and 36-months post-injury. Clinical interviews collected information about demographics, injury characteristics, and return to previous roles (e.g., work, home) by 1-year post-injury. Trajectory analysis was performed using latent class growth mixture modeling of BAI and BDI scores in Mplus. Multinomial regression analyses assessed predictors of class membership. Chi-Square tests examined associations between trajectory sub-groups and 1-year return to previous roles.

Results: Analyses identified four clinically meaningful trajectories of anxiety and depression from 2- to 36-months post-injury. The anxiety model had the following trajectories: 1) stable minimal (67%); 2) stable low (18%); 3) rapidly worsening (7%); and 4) early improving but chronically worsening anxiety (9%). The depression model identified the following sub-groups: 1) stable minimal (70%); 2) stable low (10%); 3) rapidly worsening (8%); and 4) delayed depression (12%). Predictors of worsening anxiety and depression included younger age, being male, and lower education. Return to previous roles by 1-year post-injury was less likely among those with worsening anxiety (5%) or depression (11%) compared to those with stable, minimal-low levels of anxiety (33.6%) or depression (32.4%).

Conclusion: While many individuals have stable, minimal-low levels of anxiety and depression in the sub-acute to chronic stages after moderate-severe TBI, there are some for whom anxiety and depression are worsening. These findings help to develop a better understanding of who is experiencing emotional distress in the chronic stages post-injury, when individuals are less likely to be monitored and are at risk for poorer outcomes. This line of research may improve our ability to predict prognosis and inform the targeting of early prophylactic treatment to certain patient groups.

Phylogenetic Diversity of *Treponema Pallidum* in Manitoba

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Background: Over the last 15 years Manitoba has seen a dramatic rise in the number of syphilis cases, caused by *Treponema pallidum* subsp. Pallidum, along with a demographic shift from MSM to heterosexual groups and women. Another cause for concern is the return of congenital Syphilis, which was unseen in the province for over 30 years but has been increasing since 2015. By the end of 2021 there were over 150 infants that had Syphilis exposure during pregnancy or birth. To our knowledge no whole genome sequencing (WGS) data currently exists for Manitoba. Long read WGS of clinical swabs would aid public health efforts for surveillance and epidemiological investigations. It also provides more accurate analysis of paralogous regions and repetitive sequences.

Methods: A screening PCR will be implemented to identify *T. pallidum* positive samples from mucocutaneous swabs in transport media, previously submitted for HSV testing. The low bacterial load in clinical samples complicates sequencing efforts. To overcome this challenge an extraction to deplete host nucleic acid will be used. Multiple displacement amplification (MDA) will be used to increase the abundance of remaining microbial DNA to the required input level for Oxford Nanopore sequencing. Sample library prep and sequencing will be performed following manufacturers recommendations on the Nanopore Minion. An optimized bioinformatics pipeline will be used to assemble and align the sequencing reads in order to produce whole genomes for the *T. pallidum* bacteria in each sample. This information will enable the creation of a phylogenetic tree specific to Manitoba.

Results: 1053 screened samples 163 were positive for HSV 1 and 98 were confirmed positive for *T. pallidum*. The NEBNext Microbiome DNA Enrichment kit and QIAamp DNA microbiome kit both show the ability to reduce the amount of host DNA but further optimization is required.

Conclusion: The results from this project will enable wider context to the increasing Syphilis case rates in Canada. Specifically, this will provide Manitoba data to be included with the few sequences currently available from the larger Canadian provinces.

Impact of Non-Invasive Transcutaneous Auricular Vagus Nerve Stimulation (NitaVNS) on the Development of Experimental Colitis in Mice

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Introduction: Mucosal changes in inflammatory bowel disease (IBD) are characterized by ulcerative lesions, prominent infiltration of activated immune cells, and alteration in the parasympathetic nervous system, including the vagus nerve. It has been shown that non-invasive transcutaneous auricular vagus nerve stimulation (NitaVNS) has anti-inflammatory effects on the function of intestinal macrophages and the production of inflammatory cytokines. As a proof of concept, I hypothesized that NitaVNS could, in a preclinical setting, decrease the development of acute colitis in mice.

Methods: Acute gut inflammation was induced in C57BL/6 mice (10-12 weeks) by administrating 5% dextran sulfate sodium (DSS) in their drinking water for 5 days. Preventive NitaVNS or sham-paw stimulation (Voltage: 10 V, Pulse Width: 500 µs, and Frequency: 20 Hz) were performed for 10 min on a daily basis starting one day before the induction and continued till sacrifice. Weight loss, stool consistency, blood in the feces, and disease activity index were all evaluated on a daily basis. Three macroscopic parameters, including rectal bleeding, colon bleeding, and fecal consistency in the colon, were determined as the macroscopic score upon sacrifice. Furthermore, pro-inflammatory cytokines such as interleukin (IL)-1β, IL-6, and anti-inflammatory cytokine tumor growth factor (TGF)-β were measured using ELISA in both plasma and colon samples, while C-reactive protein (CRP) was measured only in plasma.

Results: NitaVNS significantly improved gut inflammation in a preventive acute colitic setting. Delayed onset and decreased severity of clinical disease as assessed by stool consistency loss, weight loss, and rectal bleeding were observed in NitaVNS mice as compared to sham mice after the induction of colitis by DSS. The macroscopic score significantly decreased. The levels of IL-6, IL-1β, and TGF-β were not altered in NitaVNS mice colonic samples. In addition, IL-6 and IL-1β were not detected in plasma, but CRP level was significantly lower in NitaVNS mice while TFG-β level was significantly higher.

Conclusion: These findings suggest that NitaVNS possess an anti-inflammatory effect through the upregulation of anti-inflammatory cytokines and down regulation of pro-inflammatory markers.

Effect of Inherited Bleeding Disorder on Postpartum Hemorrhage and Mediation of this Relationship by Anemia

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Objective: Postpartum hemorrhage (PPH) remains the leading cause of maternal morbidity. Inherited bleeding disorders (IBD) contribute to PPH risk; however, studies examining potential pathways linking IBD and PPH are lacking. Therefore, we aimed to assess the effect of IBD on PPH and determine if anemia mediates this relationship.

Methods: We conducted a retrospective, population-based cohort study using the ICES MOMBABY dataset. Deliveries between 2014-2019 were linked with the Discharge Abstract Database (DAD) and Ontario Lab Information System to identify cases of PPH, maternal characteristics and outcomes. PPH was defined as blood loss of ≥500 ml following vaginal delivery or ≥1000 ml following Caesarean section, or as diagnosed. Anemia was defined as hemoglobin <110g/mL, or as diagnosed. Diagnoses and procedures were identified by International Classification of Diseases (ICD)-10 and Canadian Classification of Interventions codes. Validation studies have demonstrated high specificity and sensitivity of the ICD-10 code for PPH in DAD. IBD included von Willebrand disease, hemophilia, and other hereditary coagulation deficiencies. Analyses were restricted to in-hospital, live or stillbirth deliveries. The independent effect of IBD on PPH was determined using multivariate Poisson regression with robust error variance. Meditation analysis was conducted using separate multivariate logistic regression models.

Results: Between 2014-2019, 601,773 women had 779,301 deliveries. The mean age of the cohort was 30.7 years. Overall PPH incidence was 4.9% (29,661/601,773). Among the cohort, 2002 (0.33%) women had an IBD diagnosis. Compared to women without IBD, women with IBD had higher rates of anemia (4.8% vs 1.8%; std difference 0.17), and lower hemoglobin (119.7 g/L vs. 121.9g/L; std difference 0.16), at admission for delivery. There was no difference in rates of placental conditions, parity, or age. Among the cohort, both IBD (aRR = 1.26, 95% CI 1.08,1.46) and anemia at admission (aRR=2.09, 95% CI 2.03, 2.15) were determined to be independent risk factors for PPH. When age and socioeconomic status were controlled for, anemia at admission for delivery partially mediated the relationship between IBD and PPH.

Conclusions: Women with IBD are at increased risk of PPH. Anemia partially mediated this relationship, suggesting that correcting anemia prior to delivery may impact PPH risk.

Characterization of embB406 and Novel Mutations that Confer Variable Ethambutol Resistance in *Mycobacterium Tuberculosis* Infections

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Background: Global antimicrobial resistance is rising in *Mycobacterium tuberculosis* (MTB), the causative agent of tuberculosis. As gold standard phenotypic drug susceptibility testing (DST) strategies are slow and labour-intensive, comprehensive knowledge of molecular predictors of first line anti-tuberculosis drug resistance is essential to inform rapid DST and improve patient treatment outcomes. Ethambutol (EMB) is a bacteriostatic first line drug which targets arabinosyltransferases encoded by the embCAB operon. Mutations in embB, including embB406, convey EMB resistance. However, higher discordance between phenotypic DST and rapid genotypic methods bring into question the clinical significance of the embB406 mutation.

Aim: Decipher implications of embB406 and novel mutations on EMB resistance in MTB.

Methods: MTB isolates screened for the embB406 mutation (n=16) and pan-sensitive control MTB isolates (n=10) were selected from the National Reference Centre for Mycobacteriology culture collection. Confirmation of embB mutations was performed by PCR and Sanger sequencing. Minimum inhibitory concentration DST was performed at a range of EMB concentrations (0, 2, 3, 4, and 5 µg/mL) with reference strain H37Rv as a control. Genomic DNA was extracted and whole genome sequencing achieved on Illumina MiSeq. Phylogenetic analysis was performed using SNVPhyl. Snippy was employed to detect single nucleotide polymorphisms within the embCAB operon and elsewhere in the genome (i.e. efflux pumps). In combination with MyKrobe Predictor, these tools were utilized to generate genotypic resistance profiles to be correlated to phenotypic profiles and determine high vs. low confidence mutations.

Results: Sequencing of the embB region revealed two subgroups of embB406 mutations: gly406asp (n=13) and gly406ala (n=3). Preliminary phenotypic DST data shows variability in minimum inhibitory concentrations among isolates, yet there is no clear relationship between mutation subgroups and resistance levels.

Conclusion: Mutations in embB406 may be low-confidence associated with low-level EMB resistance which are undetectable by the current critical concentration for EMB DST (5 µg/mL). Novel mutations outside embB are predicted to exacerbate variability in EMB susceptibility within strains harbouring identical embB406 mutations.

An Enkephalin-like Peptide with Pain-Relieving and Anti-Depressant Effects

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Objectives: Opioids exert high analgesic efficacy and potency, which makes them essential for pain management. Unfortunately, they are addictive, possess high abuse potential and may cause serious side effects. Opioids account for 69,000 overdose deaths per year globally and were involved in 70% of drug overdoses in the US in 2019. Thus, a safer alternative to opioids for pain treatment is needed.
The endogenous peptide Leucine-Enkephalin (Leu-ENK) is a neuromodulator central for pain reduction, stress response and mood regulation, but its low metabolic stability and membrane permeability makes it inefficient as a drug. Through structural modification, the Leu-ENK-like peptide KK-103 with a greatly improved drug-likeliness was obtained.
Methods: The antinociceptive activity of KK-103 was tested in mice using the hot plate and the formalin test, two well-established models for reflexive and spontaneous pain. Analgesic tolerance, physical dependence, and gastric motility were assessed in mice after multiple doses for 7 days, and acute respiratory depression was determined by video analysis. Antidepressant effects of KK-103 were investigated in the tail suspension and the forced swim test.
Results: KK-103 shows superior plasma stability and antinociception compared to Leu-ENK. On the hot plate, KK-103 improved antinociception 10-fold (142 %MPE·h) compared to Leu-ENK (14 %MPE·h) and reduced the licking and biting time by 50% in the formalin model. The use of selective opioid receptor antagonists showed the activation of the delta opioid receptor by KK-103, and in the tail suspension and the forced swim test the immobility time of mice was significantly reduced by 12% and 21%, respectively. Compared to morphine, no breathing depression, tolerance, or dependence was induced by KK-103 and gastrointestinal transit inhibition was significantly reduced.
Conclusion: KK-103 exhibited significant antinociceptive and mood-improving effects in mice, while common side effects of opioids were greatly decreased. Compared to morphine, significantly less antinociceptive tolerance, physical dependence, respiratory depression, and constipation were induced.

Physical Activity mHealth Tailored to Postpartum Women with Lumbopelvic Pain

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Introduction: Postpartum physical activity (PA) is essential for the health of the mother, their children, and future pregnancies. Unfortunately, postpartum women are at high risk for being physically inactive and face significant barriers to PA participation such as childcare and lack of sleep and time. Up to 67% of postpartum women also suffer from lumbopelvic pain (LPP) which can affect their ability to be active. Mobile health applications (mHealth apps) have great potential for promoting PA amongst postpartum women; however, those currently available lack quality and evidence-based recommendations and have limited effectiveness and engagement. The objective of this research program is to design and develop an evidence-based, effective, feasible, scalable, and low-cost PA promotion mHealth tailored to postpartum women with LPP.
Methods: The mHealth development and design is guided by the IDEAS (Integrate, Design, Assess, and Share) framework and includes four studies. Study 1 is a systematic review of mediators of behaviour change interventions among postpartum women. Study 2 is a cross-sectional survey to determine predictors of PA participation among postpartum women with LPP. Predictors will include reflective, regulatory, and reflexive processes of the multi-process action control framework as well as biopsychosocial factors (e.g., fear of movement, perceived changes in walking/running gait). Study 3 is a focus group study to collect feedback from health practitioners and postpartum women with LPP to inform tailoring of a previously existing PA mHealth to postpartum women with LPP. Study 4 is a feasibility randomized controlled trial to determine the feasibility and acceptability of the tailored PA mHealth among postpartum women with LPP.
Results: Study 1 has been registered with PROSPERO with initial search underway and Study 2 will commence in July, 2022. Study 3 and 4 will follow with an overall projected timeline of three years. **Conclusion:** The findings of this research program will illuminate the unique barriers, needs, and considerations for PA mHealth when intervening with postpartum women with LPP. They will also inform future large scale trials with the ultimate intents of optimizing health and reducing chronic disease for postpartum women themselves, their future pregnancies, and their children.

Role of c-Met in Abnormal Bone Homeostasis Leading to Fracture Non-union

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Introduction: Fracture healing involves a fragile continuum from inflammation to repair and finally ends in bone reestablishment. Fractures that fail to heal progress to a state of nonunion, causing substantial morbidity for the individual. Currently, no effective medical treatments are available to promote fracture repair, and the mainstay of fracture nonunion management is surgery. However, the exact mechanism of fracture nonunion is unknown. In recent studies, it has been shown that HGF/c-Met are closely related to the differentiation of osteoblasts and osteoclasts, which are the prominent cells responsible for callus formation and bone remodelling during fracture repair. We hypothesized that c-Met is the critical factor that regulates osteoblast and osteoclast differentiation and also correlated with the progress of fracture nonunion.

Methods: To validate our hypothesis, human bone samples obtained from participants with fracture union and nonunion were collected to determine the expression of c-MET. To further explore the mechanism of c-Met in the regulation of osteoblasts and osteoclasts, we generated c-Met fl/fl mice possessing loxP sites flanking exon 16 of the c-Met sequence. Removing exon 16 results in the loss of the c-Met catalytic domain and the mice are thus knockout mice. We then crossed the c-Met fl/fl mice with Prx1 cre mice (to target expression in osteoblast progenitors) or Ctsk cre mice (to target expression in osteoclast precursors) and investigated differences in fracture healing following a proximal femur osteotomy.

Results: Our results show that c-Met expression is downregulated in fracture nonunion tissues such as callus and the greater trochanter. In serum and bone samples of patients with fracture nonunion, key osteoblastic and osteoclastic markers were also suppressed. The conditional knockout of c-Met in both cell lines does not cause abnormal bone development. However, we observed impaired osteoblast and osteoclast differentiation after birth, subsequently leading to osteopenia (Prx1 cre c-Met fl/fl) and increased bone formation (Ctsk cre c-Met fl/fl). Knockout of c-Met also delayed the process of fracture healing in the gene-edited mice following fracture surgery.

Conclusion: Collectively, our study shows that deletion of c-Met results in suppression of osteoblastogenesis and osteoclastogenesis, ultimately leading to fracture delayed union.

Targeting CSPG/LAR/PTPσ Axis Enhances Neuronal Replacement and Synaptogenesis by Engrafted Human Neural Precursor Cells and Improves Functional Recovery after Spinal Cord Injury

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Spinal cord injury (SCI) is caused by a traumatic impact on the spinal cord tissue resulting in progressive neurodegeneration of spinal cord neurons and damage to the spinal neural circuitry.

To date, effective replacement of damaged neurons and circuit re-assembly of the injured spinal cord remain challenging. Transplantation of neural precursor cells (NPCs) offers a promising approach for neuronal replacement in SCI due to their potential to produce neurons, in particular spinal neurons, astrocytes and oligodendrocytes. However, our and others studies indicate limited success in neuronal replacement by transplanted NPCs in the injury-induced hostile milieu of SCI. We previously identified that injury-induced upregulation of chondroitin sulfate proteoglycans (CSPGs) hinders long-term survival and integration of transplanted NPCs after SCI. Our *in vitro* investigations revealed CSPGs inhibit NPCs properties mainly through two protein tyrosine phosphatase receptors, LAR and PTPσ. LAR and PTP-σ can be functionally blocked by two intracellular peptides, ILP and ISP, respectively. Here, we have investigated the therapeutic potential of blocking CSPG/LAR/PTPσ, using ILP and ISP peptides, in combination with transplantation of human caudalized directly reprogrammed NPCs (drNPCs) with the capacity to generate spinal specific neuronal subtypes. Our *in vitro* studies demonstrated ILP/ISP co-treatment reverses the inhibitory effects of CSPGs on drNPCs and significantly enhances their neuronal differentiation, neuronal maturation and complexity, synaptogenesis and partially improves spontaneous activity. In rat model of clip compression SCI, systemic delivery of ILP/ISP significantly promoted long-term survival and biodistribution of human drNPCs graft, in particular in the caudal side of the injury. Moreover, ILP/ISP co-administration substantially enhanced neuronal differentiation and complexity of engrafted drNPCs, and promoted their excitatory synapse formation with the host axons that culminated in improved recovery of locomotion after SCI. Mechanistically, our transcriptomic analysis revealed that CSPGs impede neuronal differentiation by

dysregulating Wnt/β-catenin pathway. *In vitro* validation of RNA-seq data confirmed that blocking LAR and PTP-σ improves neuronal differentiation of drNPCs on CSPGs by increasing nuclear translocation of β-catenin complex as a hallmark of Wnt signaling activity. Taken together, we have established a new targeted, translationally feasible strategy with the potential to optimize neuronal replacement, synaptogenesis, and functional recovery after SCI.

Association Between Baseline Bone Mineral Density Testing and the Risk of Fractures in Men Initiating Androgen Deprivation Therapy: Population-Based Study

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Introduction: Androgen deprivation therapy (ADT) is a staple of advanced prostate cancer (PCa) treatment, however several side-effects are associated with its long-term use. Notably, loss of bone mineral density (BMD) is accelerated which increases fracture risk. Although guidelines recommend BMD testing when initiating ADT to properly assess baseline fracture risk, there is scarce data to support this recommendation in the PCa patient population. The objective was to examine the association between baseline BMD testing and the risk of fractures in men initiating ADT for PCa.

Methods: A retrospective observational cohort study using data from Quebec public healthcare insurance administrative databases was conducted. The cohort included PCa patients who initiated ADT from 2004-2015 and who received at least one year of ADT treatment. Baseline BMD testing was defined as a BMD test performed from 12 months prior to ADT initiation and up to 3 months after. The primary study outcomes were incidence of any fracture and incidence of fractures resulting in hospitalization. Inverse probability of treatment weighting was used to adjust for measured baseline characteristics which included patient demographic variables, comorbidities, risk factors for fractures, and use of other medications affecting bone density.

Results: We identified 13,532 patients who initiated ADT, of which 2,070 (15.3%) underwent baseline BMD testing. The unadjusted 5-year incidence of any fracture was 15.1% for patients not receiving baseline BMD and 14.0% for patients receiving baseline BMD testing. In adjusted analyses, baseline BMD testing (hazard ratio [HR] 0.92, 95% confidence interval [CI] 0.76-1.12) was not statistically significantly associated with the risk of fracture. For fractures requiring hospitalization, baseline BMD testing was associated with a lower risk (HR 0.71, 95%CI 0.52-0.98). Furthermore, baseline BMD testing was associated with increased odds of bisphosphonate use within 1 year of ADT initiation among patients who were bisphosphonate-naïve at baseline (odds ratio (OR) 2.03, 95%CI 1.74-2.36).

Conclusion: In our study population, baseline BMD testing was associated with a lower risk of fractures resulting in hospitalization. Given the low uptake of baseline BMD testing, additional efforts emphasizing the importance of BMD testing in guidelines may be needed.

Parenting Pain Away: Development and usability testing of an educational website about infant procedural pain management

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Introduction: All newborns undergo minor painful procedures (e.g., injections). Despite strong evidence supporting parents' efficacy to reduce procedural pain (e.g., breastfeeding), parents remain an underutilized resource. Limited evidence-based resources about infant procedural pain management targeting parents in the perinatal period exist. We co-created Parenting Pain Away, a website to enhance parents' access to information and participation in procedural pain management following birth. This study aimed to conduct iterative usability testing with the perinatal population from PEI, an Atlantic Canadian province, to refine Parenting Pain Away based on target users' identified needs and satisfaction.

Methods: In 2020, parents of healthy newborns or expectant parents from PEI participated in two iterative cycles of usability testing of Parenting Pain Away. Through recorded interviews, participants were directed to use the "Think Aloud" approach (e.g., verbalize what they see, think, feel) as they navigated through the website. Participants completed online questionnaires related to demographics and user satisfaction, measured by the Post Study System Usability Questionnaire (PSSUQ). Descriptive statistics and content analysis were conducted to analyze the data.

Results: In total, there were ten participants with average age of 29.9 years (SD=3.9). Participants identified as mothers (n=7) or fathers (n=3) and were expecting (n=6) or had a newborn (n=4). User satisfaction was near the top of the 7-point scale, with PSSUQ scores of 1.84 (SD=0.55) and 1.34 (SD=0.49) in Cycle 1 and 2, respectively. While no significant difference for PSSUQ scores was found across the cycles (p=.65), the average score in Cycle 2 suggests improved satisfaction. Participants provided positive feedback about the website and suggested major refinements to simplify content and site navigation.

Conclusions: Findings from usability testing cycles refined Parenting Pain Away in response to user satisfaction and feedback. Engaging target users in the development process enhanced this website in preparation for further effectiveness testing.

Panx2 Activation by Stromal-Interacting Molecules

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Pannexins are non-selective ion channels known for their ability to release ATP. Three isoforms have been discovered: Panx1, Panx2 and Panx3. Panx1 is the most studied, known for its involvement in pathophysiology, including stroke, Alzheimer's disease, learning and memory, and development. Interestingly, Panx2, in addition to Panx1, needs to be knocked down to protect mice from stroke induced brain damage. During early development, Panx1 is the major isoform in the brain, while during adulthood Panx2 begins to dominate. Due to these findings, it is surprising that little to nothing regarding the function of Panx2 is known, compared to what is known about Panx1. Previously, our lab has discovered a novel activation mechanism for Panx1. This mechanism involves stromal interaction molecules (STIMs), ER proteins which can move to the membrane, activating Panx1. Sequence conservation between Panx1 and Panx2 and my own preliminary data shows that this same mechanism can activate Panx2 as well. Due to this information, I hypothesize that STIM1/2 activates Panx2 through a physical interaction involving an analogous site preserved within the N-term of Panx2 (Panx223-28). To test this hypothesis, I will perform electrophysiology on human embryonic kidney (HEK) cells expressing both pannexins (1 or 2) and STIMs (1 or 2). I will apply thapsigargin (Tg), an inhibitor of SERCA pumps which maintain ER calcium stores in the cell. Application of Tg will induce STIM-dependant activation of pannexins 1 and 2. I will then aim to identify the minimal region of Panx2 required for STIM-dependant activation by generating deletion mutants of the 53 amino acid n-terminus. This information will be used to identify an antibody specific to this region (commercially available or to be generated by us), producing a STIM-dependant function blocking antibody. Next, using mice hippocampal neurons, I will aim to identify whether ischemic injury (simulated by oxygen-glucose deprivation) influences STIM-dependant activation of Panx2 to link Panx2 to stroke. These studies will characterize a first activation mechanism for Panx2 and provide the basis for developing experimental tools that prevent STIM-dependant function of Panx2, which are essential in order to elucidate the contribution of Panx2 in neurological disorders.

Beyond LGBT - A Mixed Methods Exploration of the Primary Healthcare Experiences of Underrepresented 2SLGBTIQIA+ Young Adults in Winnipeg, Manitoba

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Background: The 2SLGBTQIA+ (Two-Spirit, lesbian, gay, bisexual, trans, queer/questioning, intersex, asexual) population is at increased risk of adverse health outcomes such as problematic drug use, domestic violence, and mental health issues when compared to non-2SLGBTQIA+ individuals. However, the current literature privileges the experiences of gay and bisexual men, binary transgender individuals, and lesbians. This proposed study seeks to close the gap by exploring the primary healthcare experiences of underrepresented 2SLGBTQIA+ young adults in Winnipeg.

Approach: Focus groups, consisting of participants between the ages of 19 and 24, will be facilitated in collaboration with 2SLGBTQIA+ serving community organizations. Participants will be asked about their primary healthcare experiences. The transcripts will be coded using an Interpretive Phenomenological Analysis approach. Information gleaned from the focus groups, along with follow-up feedback from participants, will be used to develop a survey appropriate for 2SLGBTQIA+ young adults. The survey will ask about positive and negative primary healthcare experiences, current and past health statuses, and frequency of accessing care and will be distributed to 2SLGBTQIA+ young adults in partnership with community organizations.

Anticipated Results: Data will be collected from both LGBT (lesbian, gay, bisexual men, transgender) participants and underrepresented 2SLGBTQIA+ participants. It is expected that underrepresented 2SLGBTQIA+ participants will report more instances of negative primary healthcare experiences, more negative health statuses, and less frequency of accessing care than LGBT participants. Further, it is anticipated that the types of negative primary healthcare experiences will vary between the two groups. Data collection from the 2SLGBTQIA+ community has historically not been inclusive of all gender, sexual, and romantic identities. This process of developing a culturally relevant survey through community consultation serves as a template for future research with this community.

Conclusion: This first of its kind study in Manitoba is a step forward in collecting data that is inclusive and representative of the 2SLGBTQIA+ community. Results will inform the development of clinical practice guidelines to create a primary healthcare experience that is more equitable, comfortable, and accessible for 2SLGBTQIA+ patients.

Pro-Survival Role of NRG1 in HER2+ Breast Cancer Brain Metastasis

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Introduction: HER2+ (ErbB2+) breast cancer (BC) patients have a high incidence of 30-50% to develop brain metastases (BM). Despite the success of ErbB2 targeting therapies in the management of primary BC, BM remain a fatal complication. The median survival after diagnosis of BM is only 2-24 months. Limited drug permeability across the blood-brain barrier and factors derived from the brain tumor microenvironment determine the low efficacy of ErbB2 targeting therapies in the brain. Neuregulin-1 (NRG1) is a member of the epidermal growth factor family that can bind to ErbB3 (HER3) and ErbB4 (HER4) receptors, which can both dimerize with ErbB2.

Methods: We established a novel ErbB2+ overexpressing and ErbB1+/ErbB3+/ErbB4+ breast cancer brain metastasis model (BCBM-94) by intracardial xenografting of patient BM cells in mice. BCBM-94 cells were treated in culture with the reversible dual EGFR/ HER2 receptor tyrosine kinase inhibitor Lapatinib (100nM or 250nM) for up to 72h. We assessed ErbB receptor activity (Western blot), cell viability (WST assay), and apoptosis (WB, caspase-3/7 activity) over time. We used recombinant human Neuregulin-1 (rhNRG1) at 5ng/mL to determine the responsiveness of BCBM-94 to this ErbB3/ErbB4 ligand known to be secreted by neurons, microglia, and activated astrocytes in the brain. siRNA was used to silence ErbB3 and ErbB4 in BCBM-94 cells.

Results: Treatment of BCBM-94 cells with Lapatinib (250nM) resulted in a 40% decrease in viability. rhNRG1 attenuated the Lapatinib effects on viability. rhNRG1 was able to retain strong phosphorylation of kinase-deficient ErbB3 under Lapatinib, which suggests the involvement of ErbB3 in promoting viability in BCBM-94 under Lapatinib. This coincided with the rhNRG1-mediated rescue of Akt phosphorylation under Lapatinib. rhNRG1 blocked Lapatinib-driven upregulation of cleaved PARP and activation of pro-apoptotic caspases 3/7. The role of ErbB3 in the anti-apoptotic effects of rhNRG1 was confirmed by siRNA-mediated knockdown of the receptor which abrogated rhNRG1-mediated rescue of BCBM-94 cells from Lapatinib-induced apoptosis. rhNRG1 rescued surviving protein expression and retained Bad phosphorylation suggesting possible mechanisms to counteract apoptosis in BCBM-94 cells.

Conclusion: We developed a novel patient-derived HER2+ BCBM cell model. Our data indicate an important role of NRG1 in promoting resistance to ErbB2 targeting therapies.

The Role of Gut Microbiota in Type 1 Diabetes

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Introduction: Type 1 diabetes mellitus (T1D) is a devastating disease in which the β-cells in the pancreas produce little to no insulin due to their autoimmune destruction. The gut microbiome has emerged as a potential driver of autoimmunity against the beta-cell in T1D. A recent clinical study showed that in new-onset T1D individuals, autologous fecal microbiota transplantation (FMT), preserved β-cell function for over 12 months (de Groot et al, 2020). To gain insight into the mechanism underlying this protective effect, we will assess the impact of human FMT in the non-obese diabetic (NOD) mouse model. We hypothesize that FMT from human will lead to durably improved beta-cell function and less insulitis in NOD mice.

Methods: In order to determine whether diabetes incidence in NOD mice is affected by FMT from individuals with T1D, feces from human T1D recipients of FMT, including both responders (those whose diabetes was delayed) and non-responders, will be transplanted into 6-week old NOD mice pre-treated with antibiotics. Blood glucose will be monitored and following development of diabetes or at 21 weeks of age the microbiota and pancreas of these mice will be assessed by metagenomic analysis and histology, respectively. Beta-cell function will be followed by glucose tolerance test and proinsulin: C-peptide ratio. Quantification of beta cell mass and other inflammatory cell infiltrations within the pancreatic islets will be done by immunostaining.

Results: Prior to commencing NOD mice experiments, we measured serum C-peptide and proinsulin levels from T1D individuals using enzyme immunoassays at different timepoints before, during and after FMT. The proinsulin to C-peptide ratio (PI:CP), as a measure of β-cell dysfunction, revealed that even before FMT, T1D individuals who responded well to FMT treatment had significantly lower PI:CP ratios ($p<0.05$). These data suggest that PI:CP ratios could be a marker of how well persons with T1D will respond to FMT.

Conclusion: As we continue this study, we aim to further understand the effects of FMT on pancreatic tissues, revealing at the molecular level how FMTs lead to improved β-cell function. A better understanding of the role of the microbiome in T1D pathogenesis could lead to the development of microbiome-based therapies for T1D.

Single-Implant Overdentures Retained by a Novel Attachment: A mixed-methods crossover RCT

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Introduction: Single-implant mandibular overdentures (SIMO) are one of the least invasive implant treatments for edentulism. The new Novaloc® attachment system can improve the clinical performance of implant-retained overdentures, but has not been previously tested for SIMOs.

Objectives: To compare Novaloc® to a "gold standard' system (Locator®) for SIMOs in an edentate elderly population in terms of patient-reported outcomes, and device and treatment-related complications.

Methods: In this single-center cross-over RCT, 10 edentulous participants received an implant in the lower midline and had their lower complete dentures converted to SIMOs. The participants received each attachment system for 3 months in a randomized order, followed up by the patient satisfaction and oral health-related quality of life (OHRQoL) measurement using the MDSQ and the OHIP-EDENT questionnaires, respectively. Complications were registered throughout the RCT. Patients were interviewed for their experiences with SIMOs and preference for one of the attachment systems. Quantitative analysis employed mixed linear models and chi-squared tests ($\alpha=0.05$), whereas interview data underwent thematic analysis and, in turn, integration into the quantitative data (mixed-methods explanatory design).

Results: All ten randomized participants completed the trial. Mean general satisfaction (±SD) was 92 (±8) % with Novaloc® vs 85 (±13) % with Locator® (mean difference: 9%; 95%CI: 1 to 17%). For the specific MDSQ items, only denture stability was significantly increased for Novaloc®. Seven participants preferred Novaloc® over Locator® at the end of the RCT (chi-squared, $p=0.045$). No difference was found between the attachments in terms of OHRQoL using the OHIP-EDENT and complications. Thematic analysis revealed high patient satisfaction with SIMOs, with denture stability as the main criterion for their satisfaction and attachment preference.

Conclusion: Among elder edentulous patients wearing SIMOs, Novaloc® led to increased patient satisfaction and preference. Better patient-perceived denture stability may explain this result. Both tested attachment systems exhibited similar short-term maintenance needs. The results of this mixed-method study can be used by clinicians when choosing which attachment system to use for SIMOs. Results suggest that edentulous patients prefer attachments with a better-defined seating position than that found with a nylon matrix on a metallic abutment, as observed with the Novaloc® system.

Screening Virus-Host Dependency Factors via Functional Genomics *in silico* and *in vitro* for Drug Targeting

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Introduction: Viruses are obligate intracellular pathogens that require many host cell components to establish and maintain infection. Multiple genome-wide knockout/knockdown studies have identified host proteins that are essential for viral replication. These host dependency factors (HDF), are good candidates to develop novel antivirals, but defining which may make good targets and which may lead to drug toxicity is challenging. One opportunity to identify good target genes is by accessing large human genome sequencing resources and finding genes that harbour homozygous loss of function polymorphisms. Intersecting genes found to be essential for viral replication but non-essential for host function may open new avenues for development of therapies.

Methods: We performed a literature review to identify genome-wide studies of viral HDFs for multiple viruses. We identified 27 studies covering HIV, Hepatitis C, Hepatitis D, SARS-CoV-2, SARS-CoV, Ebola, Influenza A, Zika, Dengue and West Nile viral models. These HDFs were then intersected with the genome aggregation database (gnomAD) using an in-house bioinformatics pipeline, the genome non-essentiality and loss-of-function identifier (gNALI). The gnomAD resource contains >125,000 human exome and >15,000 whole-genome sequences from healthy individuals with specific information on genes harbouring loss-of-function polymorphisms.

Results: A total of 3248 HDFs were identified across all viruses, with 320 genes implicated in more than 1 virus and 9 HDFs implicated in 4 viruses. We did not observe any genes shred by more than 4 viruses. Using gnomAD data, we found that when an HDF is implicated in more than one virus, it tends to be highly intolerant to a loss-of-function mutation suggesting they are highly conserved within the host. Pathway analysis of the combined gene list showed that the majority of the HDFs are involved in processes such as phagosome acidification and RNA processing/transport. Select candidate genes will be disrupted in the future, via CRISPR gene editing, for further analysis on the rate of infection.

Conclusion: Better understanding of the interaction of viruses with non-essential host genes can aid in the development of new drug targets potentially leading the way for development of novel broad acting antivirals.

Rare Variant Analysis Approach to Finding Inborn Errors of Immunity in Blastomycosis

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Introduction: Blastomycosis is a severe pulmonary disease caused by Blastomyces dermatitidis, a dimorphic fungus endemic to northwestern Ontario and Manitoba. Infection occurs through the inhalation of aerosolized spores with nearly half of all cases progressing to severe disease. This variability in disease severity is only partially explained by risk factors such as age, sex, and immunodeficiency. Previous host genetic studies of blastomycosis suggest that polymorphisms in genes of the immune system or inborn errors of immunity (IEI) can influence severity of disease particularly in the genes: GATA2, IL6, and VDBP. Our study attempts to find known and novel IEI involved in blastomycosis through exome sequencing.

Methods: We conducted a literature review to identify IEI known to alter susceptibility and severity of dimorphic fungal, fungal, and mycobacterial infections. Exomes from 18 individuals previously diagnosed with blastomycosis and 9 household controls with similar risk factors were sequenced on the Illumina MiSeq. Sequencing reads were aligned to the reference human genome (build 38) and genetic variants were identified and filtered according to the GATK best practices workflow. Variants were annotated to determine their corresponding gene and predicted functional impact using ANNOVAR. Individual variant enrichment in cases and controls was assessed using logistic regression. The rare variant analysis method, binary optimized SKAT, was used to test gene level variant enrichment in IEI identified in the literature review.

Results: We identified 60 genes previously implicated in relevant infection models through review of the literature. A total of 105,746 high quality exonic variants were identified from 18 cases and 9 controls. Individual variant analysis did not identify any significantly associated variants exceeding the threshold for study-wide significance ($P<5x10-7$). Preliminary rare variant analysis of high-priority gene candidates identified variants in GATA2 and TLR1 to be significantly enriched in controls with p-values of 0.0034 and 0.049, respectively.

Conclusion: Our preliminary analysis suggests a novel association with TLR1 and an association with GATA2 in the opposite direction than expected. These may represent important genes involved in the immunological response against *B. dermatitidis.* Our results can help to inform therapeutics directed at improving clinical outcomes of blastomycosis.

Visuospatial and Executive Dysfunction in Patients with Acute Kidney Injury, Chronic Kidney Disease, and Kidney Failure: A multilevel modeling analysis

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Introduction: Neurocognitive impairment is a common finding across the spectrum of kidney disease, and carries important consequences for quality of life. We previously demonstrated that robotic technology identifies neurocognitive impairments not readily detectable by traditional testing in patients with acute kidney injury (AKI) and chronic kidney disease (CKD). However, to date, no studies have examined the differences in neurocognitive declines between patients with varying kidney disease severities. Therefore, the present study aimed to assess whether quantifiable deficits in neurocognition differ based on a diagnosis of AKI, CKD, or kidney failure.

Methods: This was a cross-sectional analysis of participants enrolled in an observational study. Adults ≥17 years of age with AKI, CKD, or kidney failure were enrolled at a tertiary academic hospital in Kingston, Canada. Each participant underwent robotic neurocognitive assessment using the Kinarm: an interactive robotic device that uses a series of behavioral tasks involving movement of the upper limbs to precisely quantify neurocognitive impairment across a variety of domains. Multilevel modelling was used to determine the effect of Kinarm task type, kidney diagnostic group (AKI vs. CKD vs. kidney failure), and the interaction between the two, on neurocognitive performance.

Results: 104 participants with AKI, CKD, or kidney failure were enrolled. Averaging across all kidney diagnostic groups, participants performed worst on the Kinarm tasks measuring visuomotor, executive function, and attention: Visually Guided Reaching (VGR; b = .28 [95% confidence interval .07, .49]), Reverse VGR (RVGR; b = .64 [.42, .85]), and Trail Making (b = .50 [.28, .72]). Averaging across all tasks, patients with AKI, CKD, or kidney failure did not perform significantly differently from one another. However, diagnostic group and task type interacted to determine performance, with patients with AKI performing worse than those with CKD or kidney failure on the RVGR task.

Conclusion: Although patients with AKI, CKD, and kidney failure exhibit similar degrees of global neurocognitive impairment, patients with a recent history of an AKI event demonstrate significantly worse executive functioning and visuomotor control than patients with CKD or kidney failure. This may have implications for activities of daily living, driving safety, medication adherence, and medical decision-making.

Insulin-Induced Cardiomyocytes Hypertrophy That Is Prevented by Taurine via β-alanine-Sensitive Na+-Taurine Symporter

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Although insulin-induced cardiac hypertrophy is reported, very little information is available on the hypertrophic effect of insulin on ventricular cardiomyocytes and the regulation of sodium and calcium homeostasis. Taurine is a non-essential amino acid synthesized by cardiomyocytes and the brain and is present in low quantities in many foods, particularly seafood. The purpose of this study was to investigate whether chronic exposure to insulin induces hypertrophy of ventricular cardiomyocytes that are associated with changes in Na+ and Ca2+ homeostasis and whether taurine pre-treatment prevents these effects. Our results showed that chronic treatment with insulin leads to cardiomyocyte hypertrophy that is associated with an increase in basal intracellular Na+ and Ca2+ levels. Furthermore, long-term taurine treatment prevents morphological and ionic remodeling induced by insulin. In addition, blocking the Na+-taurine co-transporter prevented the taurine antihypertrophic effect. Finally, the insulin-induced remodeling of cardiomyocytes was associated with a decrease in the ratio of phospho-CREB (pCREB) to total cAMP response element binding protein (CREB); taurine prevented this effect. In conclusion, our results show that insulin induces ventricular cardiomyocyte hypertrophy via downregulation of the pCREB/tCREB level and that chronic taurine treatment prevents this effect.

Anxiety Disorder as Self-Disorder: The Role of Disrupted Self-Specifying Processes

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Even before the pandemic's arrival in March of 2020, anxiety disorders were the most common psychiatric disorders and are associated with a high burden of illness. And as the reports of anxiety symptoms continue to rise given the state of the world, anxiety disorders are due additional analysis that might aid our descriptions and explanations. I propose that a phenomenological approach to anxiety disorders can do just that. Specifically, we ought to examine the ways in which the self plays a role in anxiety disorders. Borrowing the notion of self-disorder from phenomenological pathology, I argue that anxiety disorders similarly exhibit an alteration to our most fundamental experience of a self-immersed-in-the-world via disordered/disrupted organization of self-specifying processes. To substantiate my claims, I refer to empirical work on anxiety from clinical psychology and cognitive science regarding disruptions in experience of selfhood, on the one hand, and corresponding alterations of worldly experience, on the other. Next I propose that interoception, which plays a fundamental role in forming our basic sense of self, is a good place to start when looking for the disruption of self-specifying processes in anxiety disorder. There are reasons to believe that anxiety patients might have poor interoception. For example, although they report being attentive to their interoceptive signals, this increased sensitivity does not amount to increased interoceptive accuracy. Moreover, poor interoception has been shown to lead to dissociation and derealization, which are common symptoms of another self-disorder within phenomenological pathology, that is, depersonalization/derealization disorder. Finally, I motion to possible causal explanations for this disruption in interoception by drawing from emotion theory and recent work in neuroscience.

Barriers and Facilitators for use of New National Recommendations for Preoperative Endoscopic Localization of Colorectal Neoplasms According to Gastroenterologists and Surgeons in Winnipeg, Manitoba

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Background: Many patients undergo repeat endoscopy before surgery for colorectal tumours in Winnipeg. Non-standard documentation and variable tumour marking are the primary causes. Repeat endoscopy delays surgery and puts patients at risk of colonoscopy-related complications. Recommendations for localizing and documenting colorectal lesions were recently developed. This study aimed to identify barriers and facilitators to following these new recommendations in Winnipeg.

Methods: Gastroenterologists and surgeons were purposely sampled from various practices in Winnipeg. Participants were introduced to the recommendations and asked for their perspectives in semi-structured interviews. The Consolidated Framework for Implementation Research (CFIR) guided data collection. Deductive content analysis was used to map participant perspectives to CFIR constructs. Perceived barriers were linked to possible solutions according to the Expert Recommendations for Implementing Change (ERIC) framework.

Results: Ten surgeons and eleven gastroenterologists participated. There were many facilitators. The recommendations were viewed as highly adaptable, and compatible with local skills. Implementation was perceived as important, and preferable to alternate solutions. The simplicity of the recommendations and the manner of presentation were strengths. The central organizational structure for endoscopy, strong interdisciplinary communication networks, and the learning climate were all facilitators. Some barriers were identified. Familiarity with the evidence basis for certain recommendations was lacking. No relevant formal feedback processes exist. External reimbursement structures incentivize guideline non-compliance, and there were no internal organizational incentives. Key resources required to maximize guideline integration were unavailable in some settings. Participants requested educational materials and tools to facilitate use which do not currently exit. Finally, some surgeons reported they were likely to repeat endoscopy in select circumstances regardless of guideline uptake. According to the ERIC framework, the most compatible recommendations identified for addressing barriers identified were: 1. 'Conduct educational meetings', 2.

'Alter incentive/allowance structures', 3. 'Access new funding', 4. 'Develop educational materials', 5. Audit and provide feedback', and 6. 'Distribute educational materials'.
Conclusions: We identified barriers and facilitators to implementing new recommendations for documenting and marking colorectal neoplasms at endoscopy. Barriers were mapped to expert recommended practices to enhance guideline use. Future research is needed to test proposed implementation strategies' effect on guideline uptake and repeat endoscopy rates.

Stress Sensitivity and Trajectories of Psychopathology in Adolescence: An Investigation Across the COVID-19 Pandemic

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Introduction: Psychiatric illnesses in adolescence are associated with long term impairments, impacting both mental and physical health across the lifespan. Therefore, understanding predictors of youths' trajectories of psychiatric distress during the COVID-19 pandemic, which has threatened youths' wellbeing, is critical for the development and dissemination of effective interventions. There is reason to believe that individual differences in stress sensitivity, defined as the tendency of an individual to respond more or less strongly to stress, could be associated with longitudinal trajectories of internalizing symptoms (e.g., anxiety, depression; the most prevalent classes of mental illness in adolescence). Historically, however, researchers have operationalized stress sensitivity by assessing ether objective or subjective responses to stress. We posit that the relative discordance between subjective (e.g., affective) and objective (e.g., biological) responses to stress is a critical metric of stress sensitivity.
Methods: Thus, we examined whether discordance-based indices of stress sensitivity were related to one another and to internalizing psychopathology across the pandemic using a latent growth curve modelling approach. In other words, we examined whether discordance indices of stress sensitivity placed Canadian youth on a pernicious trajectory during the pandemic.
Results: Findings indicated that acute social-evaluative stress sensitivity was associated with internalizing symptoms at baseline ($\beta = 0.47$, $p = .011$) and over time (linear slope, $\beta = -0.59$, $p = .022$; quadratic slope, $\beta = 0.56$, $p = .038$). Greater discordance between subjective (i.e., affective) and objective (i.e., biological) responses to an acute interpersonal stressor was associated with higher internalizing symptoms at baseline and an initial downward trend in symptoms, followed by a greater quadratic growth in symptoms across the pandemic (i.e., an accelerated increase in internalizing symptom growth trajectory).
Conclusion: Taking a within-persons lens, these findings identify the relative discordance between objective and subjective experiences of stress as a predictor of a pernicious growth trajectory of internalizing symptoms across a ubiquitous and naturalistic stressor (i.e., the COVID-19 pandemic) among Canadian youth. This work not only advances current methodologies and contributes to theoretical understanding, but also has implications for policy and practice by identifying a key vulnerability factor placing youth on pernicious trajectory during the current pandemic.

Defining CD8+ T Cell Exhaustion Development Using scRNA-seq Analysis

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Introduction: CD8+ T cells are key in mediating immune responses to viral infections. Viral infections can be acute, in which the infection is resolved, or chronic, in which antigen persists in the body. Several studies have illustrated that CD8+ T cells adopt distinct functional states in each infection type. In acute infections, naïve CD8+ T cells become activated, undergo vigorous clonal expansion and differentiate into cytotoxic CD8+ T cells (CTLs). 90% of CTLs induce apoptosis of virus-infected cells by releasing cytolytic molecules such as granzymes, defensins and perforins. 5-10% of CTLs are maintained as memory CD8+ T cells following clearance of infection. In chronic infections where the antigen persists, activated CD8+ T cells adopt an altered state known as functional exhaustion. Exhausted CD8+ T cells (Tex) lose effector functions and show high expression of inhibitory receptors such as PD-1.

Gap in Knowledge: While several studies characterize exhaustion after this state has developed, it remains undefined how exhaustion is specified.

Methods: We use the Lymphocytic Choriomeningitis (LCMV) mouse model of acute and chronic infection to comparatively analyze antigen-specific CD8+ T cell differentiation during acute vs. chronic infection at multiple time-points post infection. We apply single-cell RNA sequencing analyses to identify transcriptional differences influencing CD8+ T cell differentiation into protective vs. exhausted T cells at early and late times after infection.

Results: Preliminary data shows that there are distinct differences in CD8+ transcriptional profiles between acute vs. chronic infection, observed as early as Day 4 post infection.

Conclusion: A comprehensive understanding of how CD8+ T cell exhaustion develops can help to maximize the potential of T cell mediated responses to better treat infectious diseases.

Increased Hepatic IGFBP-1 Phosphorylation in Glucose Deprivation is Mediated by mTORC1-AMPK Signalling

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Introduction: Fetal liver facilitates maternal-fetal nutrient exchange. Poor fetal glucose delivery due to maternal nutrient restriction or placental insufficiency can result in intrauterine growth restriction (IUGR), which increases perinatal morbidity and mortality. Low glucose availability can trigger a compensatory signaling cascade: glucose deprivation causes energy depletion and activates the energy sensor AMP-activated protein kinase (AMPK), which inhibits mechanistic target of rapamycin complex-1 (mTORC1) by phosphorylating tuberous sclerosis complex-2 (TSC2) at Ser1387, or regulatory-associated protein of mTOR (raptor) at Ser792. Inhibition of mTORC1 decreases cell proliferation. Insulin-like growth factor-1 (IGF-1) promotes fetal cellular growth by binding to its receptor, IGF-1R. IGF-1 bioavailability is regulated by liver-secreted IGF binding protein-1 (IGFBP-1), which sequesters IGF-1 and inhibits binding to IGF-1R. IGFBP-1 phosphorylation at Ser101/Ser119/Ser169 increases its affinity for IGF-1. In IUGR, IGFBP-1 phosphorylation at these serine residues is increased. mTORC1 inhibition increases IGFBP-1 phosphorylation in a nutrient-deprived model of fetal hepatocytes, HepG2 cells. The role of hepatic IGFBP-1 phosphorylation during glucose deprivation remains to be investigated. We tested our hypothesis that glucose deprivation regulates hepatic IGFBP-1 secretion/phosphorylation through the AMPK-mTORC1 pathway.

Methods: HepG2 cells were cultured with and without glucose, and AMPK was activated pharmacologically by AICAR, an analog of AMP. AMPK and TSC2 were inhibited using targeted siRNA silencing. Protein expression was determined using western blot analyses.

Results: Glucose deprivation activated AMPK, as seen by increased AMPK phosphorylation at Thr172. Glucose deprivation also increased IGFBP-1 secretion and phosphorylation at Ser101, Ser119 and Ser169, and inhibited mTORC1 activity, seen by reduced phosphorylation of its functional readouts: p-P70S6K1(Thr389) and p-4E-BP1(Thr70). Interestingly, p-TSC2(Ser1387) was increased in glucose deprivation but p-Raptor(Ser792) was unchanged. Activation of AMPK by AICAR similarly inhibited mTORC1 and increased IGFBP-1 secretion and phosphorylation at Ser101/Ser119/Ser169 in glucose-available conditions. Utilizing AMPK and TSC2 siRNA silencing, we found both AMPK and TSC2 silencing prevented the inhibition of mTORC1 activity

and prevented increased IGFBP-1 secretion/phosphorylation, suggesting they play an important role in mTORC1-mediated IGFBP-1 secretion/phosphorylation.
Conclusion: This study shows AMPK-mTORC1 signaling mediates increased hepatic IGFBP-1 secretion/phosphorylation in glucose deprivation and may be a mechanism regulating IUGR development.

White Matter Lesion Load is Associated with Lower Within- and Greater Between-Network Connectivity across Older Age

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Introduction: White matter hyperintensities (WMH) are among the most prominent structural changes observed in older adulthood. These changes coincide with functional changes to the intrinsic network organization of the aging brain. Yet little is known about how WMH are associated with changes to the whole-brain functional connectome in normal aging.
Methods: We used a lesion prediction algorithm to quantify WMH as well as resting-state multiecho functional magnetic resonance imaging to characterize resting-state functional connectivity in a cross-sectional sample of healthy older adults (N = 105, 60–83 years of age).
Results: In a multivariate analysis, we found that higher lesion load was associated with a global pattern of network dedifferentiation, marked by lower within- and greater between- network connectivity. Network specific changes included greater visual network integration and greater posterior-anterior connectivity. The relationship between WMH and resting-state functional connectivity was negatively associated with fluid IQ as well as Blood Oxygen Level Dependent (BOLD) signal dimensionality. Reduced functional network segregation is a widely observed pattern of age-related change.
Conclusion: Our findings show that these functional changes are associated with the accumulation of WMH in older adulthood.

Development and Validation of a Murrim a Currim Autism Digital Evaluation Tool (CADET) for Screening in Children with Limited Language Skills in Urban Karachi

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Background: Early detection of Autism in children is critical to creating an autism-friendly environment leading to a better prognosis. In a low middle-income country like Pakistan, there is a dearth of trained professionals and a lack of infrastructure to screen Autism in children. This study aimed to develop and validate a technology-mediated culturally relevant diagnostic tool, shifting the task of a detailed assessment of gold standard by a trained professional to a layman by giving an equally robust Screening of Autism in children in low resource settings, enhancing chances of early diagnosis of Autism hence improving prognosis.

Methods: The study was conducted in two phases; during Phase 1 the autism screening tool, by the name of 'Currim Autism Digital Evaluation Tool (CADET)' was developed, In Phase 2 CADET was evaluated on children aged. CADET was developed in 6 steps. In the first step, the tool was conceptualized, based on existing validated tools and discussed with an expert group who selected, and prioritized six items on their relevance for autism diagnosis in children. In the third step, these 6 items were illustrated on the storyboard and animations were developed in consultation with Dr Saleem Sayani Director Technology Innovation Support Center and his group at Aga Khan University CADET was pretested on 5 mothers. Based on the feedback tool was modified and was rolled out for validation. During validation, user feedback was taken to improve the tool further.

Phase 2: In this phase the validation study of the CADET was carried out. The validation study of the tool consisted of content validity, construct validity, and criteria validity. Content validity was part of the phase one as well where experts selected the items and gave their consensus on the content of the six items. Construct validity and criteria validity was assessed by screening and comparing children using CADET in 3 groups who have been earlier diagnosed as autistic, atypically developing, and typically developing children by a standard tool "Childhood Autism Rating Scale)'. A total of 84 children, 28 in each group were randomly selected from the data records of Out Patient Department of Children's Hospital, Aga Khan University Hospital. The construct validity and criteria of CADET were assessed by checking the tool's internal consistency and ROC analysis using Stata version.14 and Medcalc 19.6.

Results: The CADET is a web-based application consisting of 6 items. Each item had two animations showing autistic and normal behavior. The construct validity of CADET was assessed by using Cronbach's α with an estimated value of 0.93. Considering typically developed children as reference, CADET showed sensitivity of 96.4% (95% CI (81.6% - 99.9%) and specificity of 100% (95% CI (87.6% - 100.0%) The mean score difference between an autistic and typically developing child was 5.35 (p-value <0.001).

Conclusion: The tool's initial testing shows it works at par with the Gold standard and can be easily used by a layperson.

Meta-Analysis of Early-Childhood-Caries Cohorts to Identify the Microbial Markers for Disease Classification Using Oral Microbiome Data

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Introduction: Significant caries status in the primary dentition of young children (2-6 yrs old) is commonly referred as early childhood caries (ECC), and it affects half of the children worldwide. Previous studies built models to predict ECC status. However, most of these studies suffer from a small sample size. Here we hypothesize that machine learning (ML) based meta-analysis by combining several oral microbiome studies of ECC can help provide robust results for ECC classification and identification of microbial taxonomic biomarkers.

Methods: Studies were selected from 5 cohorts which included ECC or SECC (severe-ECC) with 16S rRNA oral microbiome data. The raw data was processed in Qiime2 to obtain the taxonomic abundance. For comparison, the relative abundances were normalized with centered log-ratio transformation for each sample and batch effects in studies were corrected by sPLSDA method. Association analysis was performed using DESeq2 along with the Phyloseq package in R. For integrated analysis, ML methods such as Lasso, RandomForest, and XGBoost were used. For the meta-analysis, to check the generalizability of the models, they were trained on multiple datasets and then tested on the holdout dataset (Leave-one-Dataset-out (LODO) validation).

Results: In five oral plaque datasets, the total number of OTUs ranged from 133 to 342 at the species-level with an intersection of 96 species common across all the samples, and 59 to 101 with 50 common genera at genus-level. In association analysis, DESeq2 method identified 15 to 79 differentially abundant species and among them no OTUs were found to be common among all 5 studies. In ML-analysis, Lasso outperformed XGBoost, RandomForest and SVM based methods. Lasso method gave a performance of 0.82 AUC at species level and 0.70 at genus level for combined datasets and the LODO results varied between 0.60 to 0.86 in 5 studies.

Conclusion: This meta-analysis identified the oral microbial taxonomic variations in ECC which arise due to the different cohorts. ML analysis provide good classification for ECC on single as well as combined studies. Furthermore, from LODO analysis, it is evident that ML can provide generalizability of the model for ECC classification among different studies.

Elucidating the Transcriptional Regulation of Nucleolar RNA Polymerase II

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Introduction: Ribosome biogenesis is the most energy demanding process in cells, as it is responsible for generating the universal protein synthesizing machineries, known as ribosomes. Ribosome synthesis is initiated by transcription of the ribosomal DNA (rDNA), which in humans is present as tandem repeats clustered in the nucleoli. The rDNA repeats consist of ribosomal RNA (rRNA) genes with intervening regions referred to as intergenic spacers (IGS). We have previously shown that the mammalian rDNA is cohabited by both RNA polymerase I (Pol I), which synthesizes the precursor rRNA (pre-rRNA), and nucleolar RNA polymerase II (Pol II). Nucleolar RNA Pol II is present at the IGS where it generates three-stranded nucleic acid structures, known as R-loops, that help maintain ribosome biogenesis and overall nucleolar organization. Following this work, we have now identified two putative transcription regulators, that were exclusively identified to be in close proximity to nucleolar Pol II in a BioID experiment, and we hypothesize to regulate nucleolar Pol II.

Methods: Nucleolar enrichment of the two transcription regulators was determined using sucrose gradient nucleolar fractionation and chromatin immunoprecipitation. Transient depletion (KD) of the transcription regulators was achieved in HEK293T using 50nM siRNA for 48 hr. To measure nascent pre-rRNA, cells were incubated with 5'ethynyl-uridine (EU) for 1 hr followed by RNA extractions. Processing of the pre-rRNA was assessed by incubating the cells an additional 2.5 hr with EU-free growth medium. To assess levels of R-loops at the rDNA, DNA-RNA immunoprecipitation (DRIP) was performed on the KD cells.

Results: The two transcription regulators are found to be enriched in nucleolar fractions with one being significantly enriched across the rDNA. Transient depletion of the two factors resulted in significant decrease in IGS ncRNAs, nascent pre-rRNA and mature rRNAs, but elevated levels of steady state pre-rRNAs. Finally, transient depletion of the factors results in reduced levels of R-loops at the rDNA promoter.

Conclusion: Our findings suggest the importance of these two transcription regulators in regulating rRNA synthesis. Future work will uncover the mechanism by which these factors regulate rRNA synthesis and help maintain ribosome biogenesis and nucleolar organization.

Isothermal Nucleic Acid Amplification Coupled with CRISPR-Cas12b for Rapid and Sensitive Detection of SARS-CoV-2 and Nipah Virus

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Introduction: The ongoing SARS-Cov-2 pandemic has outlined a critical gap in rapid, sensitive, and specific diagnostics and for the global capacity to respond to high-impact infectious disease events. Novel molecular diagnostics like SHERLOCK (specific high-sensitivity enzymatic reporter unlocking) and HOLMES (one-hour low-cost multipurpose highly efficient system) leverage an archaic prokaryotic defense mechanism, CRISPR (clustered regularly interspaced short palindromic repeats), to detect programmed pathogens of interest. These nascent diagnostic methods combine the sensitivity and specificity of RT-qPCR and the rapid turn-around-time of lateral flow antigen tests while remaining inexpensive and available for use in point-of-need (PON). Another high-risk respiratory pathogen, Nipah virus, is considered by the World Health Organization, to be a top 10 threat to global health and could potentially be the etiological agent responsible for the next pandemic, thus highlighting the need for CRISPR diagnostics for both pathogens.

Methods: We propose in this study to optimize and adapt the SARS-CoV-2 SHERLOCK STOPCovid.v2 POC assay to be used as Canada's ability to respond to the SARS-CoV-2 pandemic. Conversely, we propose to adapt the STOPCovid.v2 protocol and design for the purpose of detecting Nipah virus. This can be done by modifying the primers and single-guide RNA (sgRNA) used for SARS-COV-2 detection, to align, amplify and detect a conserved region within the nucleoprotein gene region of the NiV genome.

Results: The SHERLOCK RT-LAMP Cas12b protocol detecting SARS-CoV-2 has been adapted to detect SARS-CoV-2 at 100 genome copies per microliter. In addition to an enrichment extraction using Ceres Nanotrap magnetic particles, assay detection limit can reach 2 PFU per microliter. Primer and sgRNA designs have been completed for the detection of all Nipah virus strains, and are currently being optimized to increase the limit of detection and sensitivity.

Conclusions: The SHERLOCK protocol using RT-LAMP Cas12b to detect SARS-CoV-2 has demonstrated sensitive, specific, and rapid functionality. In our hands, the assay has been optimized to detect extremely low viral load while functioning as an inexpensive point-of-care test. The novel RT-LAMP Cas12b assay detecting Nipah virus shows tremendous promise, with functional detection of the viral genome and, upon further optimization, will be useful to detect NiV at the PON.

The Absence of Blk Leads to Increased Inflammation in a Murine Model of Kawasaki Disease

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Introduction: Kawasaki Disease (KD) is the leading cause of acquired heart disease in children in developed countries. Single nucleotide polymorphisms (SNPs) in B-lymphoid tyrosine kinase (BLK) have been linked to increased susceptibility in KD. Common disease-associated SNPs are known to cause a 20-70% decrease in the expression of BLK, however the mechanism that this loss has in the development of inflammatory diseases is unknown. BLK is a non-receptor tyrosine kinase, part of the Src family kinases (SFKs), most studied in the context of B-cells. Here, we examine BLK -/- mice in a model of KD to gain insight into its undetermined role in disease pathogenesis.

Methods: Bone marrow derived macrophages (BMDMs) from BLK+/+, BLK +/- and BLK -/- mice were stimulated with various TLR agonists for 6 hours. Production of IL6 and TNFβ were measured via ELISA and RNA was prepared for mRNA sequencing by Novogene Inc. *In vivo*, KD was induced by intraperitoneal LCWE injection. Coronary artery inflammation was scored by a blinded pathologist. To examine intrinsic versus extrinsic effect of the loss of BLK, bone marrow chimeras (BMCs) were used. Briefly, BLK +/+ hematopoietic stem cells were reconstituted via intravenous injection into irradiated BLK -/- recipient mice and vice versa followed by monitoring of engraftment via flow cytometry.

Results: At steady state, in comparison to wild-type littermate controls, BLK -/- mice have significantly lower leukocyte counts, specifically monocytes within the periphery. Following stimulation with either TLR2 or TLR9 agonists, BLK -/- BMDMs have increased IL6 and TNFβ production. Furthermore, BLK -/- mice develop significantly larger lesions due to increased inflammation when injected with LCWE. BMC experiments demonstrate it is the absence of Blk in stromal cells specifically that is necessary in developing LCWE induced carditis.

Conclusion: Our data supports the importance of Blk in the pathogenesis of KD through the increased production of proinflammatory cytokines and increased severity of LCWE induced carditis in its decreased expression or absence. Our data also highlights an unknown, important role of BLK expression in stromal cells. These observations will provide important insight into the future for treatment of patients with KD.

The Reflexive Control of Knee Stability During Movement

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Introduction: Stimulation of the common peroneal nerve (CPN) has been demonstrated to evoke biphasic excitation of the quadriceps motoneurons. The CPQ reflex pathway is important for reflex adjustment during locomotion .The main goal of this project is to investigate whether the CPQ reflex produces significant changes in the kinematics of the leg and the ground reaction forces when evoked during walking and 2) depends on the coordination of the left and right side and 3) relates to other heteronomous pathways between the ankle and knee and/or back muscles of the lower back during, standing, walking and running.
Methods: Neurologically intact, generally healthy participants (n=12) in the 20-48 years old age range are recruited. Participants are evaluated in 8 different conditions (standing,6 different walking patterns, and running).The walking patterns are listed here as follows:1-Both legs are walking (BLW), 2-Stimulated leg is walking, other leg is standing (SLW), 3-Stimulated leg is standing, other leg is walking (SLS), 4-Both legs are walking with different speeds at three different left-right speed ratios of 1:1.25, 1:1.5 and 1:2 (BWD-1.25; BWD-1.5 and BWD-2). Subjects are asked to walk on the treadmill at 4 km/h, while the CPN stimulation (1.5 - 2.5 $\times$ motor threshold) is applied at an optimal window after heel strike. The forces, the EMG responses and the joint trajectories will be compared in steps with and those without CPN stimulation.
Results: Preliminary results confirm that a single shock to the CPN induces a reflex that changes the force (Y) during the next step; and that there is a direct correlation between CPQ reflex size and force. When the subject put more weight on the stimulated leg, regardless of the left-right coupling, we have observed an increase in the amplitude of CPQ reflex pattern.
Conclusion: Understanding of interactions between reflexive muscle control is important to incorporate to CPQ reflex into balance exercises in clinical settings to improve people's quality of life after neuronal injuries.

Chronic Constipation among Manitobans with Intellectual and Developmental Disabilities, who transitioned from an Institutional Model of Care into Community Homes

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Background: Approximately 40% of persons with intellectual and developmental disabilities (IDD) experience chronic constipation. Poorly treated constipation can provoke negative health outcomes in this population, such as problem behaviours, sepsis, and intestinal obstruction. Transitioning from an institution to a community home has been associated with improved quality of life of those transitioning. However, the health outcomes of community transitions of adults with IDD are not well understood.
Objective: St.Amant is a not-for-profit organization in Winnipeg that provides a wide range of services to persons with IDD and their families. From 2014-2020, sixty-one persons with IDD moved out of a complex care facility, St.Amant's Health and Transition Services, and are now living in community homes. Our research team conducted a longitudinal study to examine the health and quality of life outcomes for those who transitioned from an institutional setting to community homes.
Methods: Individual-level data prior to and after community transitions were collected to track changes over time. Pre-transition data were obtained through medical chart reviews and comprehensive health assessments. Post-transition data were obtained through comprehensive health assessments. Pre-and post-transition data on a number of health indicators for 30 persons with severe IDD were analyzed. Results on chronic constipation are presented in this poster.
Results: There was a significant ($p < .001$) decrease in chronic constipation among persons who transitioned from Health and Transition Services of St.Amant into community homes. Among the study group, 70.4% had chronic constipation when living at Health and Transition Services. This proportion was decreased to 16.7% after their transition. 36.7% of participants stopped experiencing chronic constipation after moving into a community home.
Conclusion: Community living was associated with significant reduction in rate of chronic constipation among our study population. The 2018 Canadian Consensus Guidelines for persons with IDD recommends frequently monitoring constipation in this population to address other health problems that might be causing constipation. It is recommended for community staff to continue using a bowel movement monitoring chart.

The Lipoxygenase Pathway, Potential Role in Aspirin-Induced HIV Prevention

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Background: Globally 1.5 million new HIV infections occurred in 2020, therefore, new prevention methods are needed. Inflammation is a risk factor for HIV acquisition as it attracts HIV target cells to the female genital tract (FGT) where HIV is encountered. Our lab conducted a study to reduce FGT HIV target cells using the safe, affordable, and globally available anti-inflammatory, acetylsalicylic acid (ASA/Aspirin). We found ASA decreased the proportion of FGT HIV target cells (CD4+CDCR5+Tcells) by 35%. However, the mechanism remains unknown.

Goal: To assess if ASA reduces mediators of inflammatory pathways such as the lipoxygenase pathway.

Methods: Women from Nairobi, Kenya took low dose ASA (81mg) daily for 6 weeks. Blood was drawn at baseline and following 6 weeks daily ASA. Plasma was frozen at -80˚C and shipped to Winnipeg, Canada. On thawing, oxylipins in the plasma were stabilized with antioxidants, spiked with an internal standard, extracted on Strata-X-SPE columns, and quantified using liquid chromatography-tandem mass spectroscopy. Oxylipins from 12 possible pathways were assessed.

Results: Of the 6 detected cyclooxygenase metabolites from arachidonic acid all were downregulated, four of which reached statistical significance. We detected at least one metabolite from 9 of the 12 possible oxylipin pathways, 4 pathways had more than one analyte significantly different following 6 weeks ASA. Interestingly, most lipoxygenase metabolites from all 4 pathways were significantly downregulated: 5/13 from arachidonic acid, 2/10 from docosahexaenoic acid, 2/6 linoleic acid, and 2/2 from dihomo-γ-linoleic acid. Finally, the majority of lipoxygenase metabolites affected by ASA are metabolites of 15-lipoxygenase (5/11) or from either 5-lipoxygenase (4/11) with the rest derived from either 12-lipoxygenase (1/11) or generated through non-enzymatic oxidation (1/11).

Conclusion/Discussion: Here, we show that following 6 weeks of ASA treatment, metabolites from both the lipoxygenase and cyclooxygenase pathways were downregulated. While ASA directly inhibits cyclooxygenase function, this is not the case for lipoxygenase. However, inflammation increases lipoxygenase expression and ASA reduced inflammation in our cohort. We speculate that ASA-associated reduction in inflammation, decreased lipoxygenase expression resulting in a secondary decrease in lipoxygenase metabolites.

From Survive to Thrive: Evaluating the role of GraS and novel fatty acid metabolic pathways in promoting *Staphylococcus aureus* adaptation to antimicrobial free fatty acids of human skin

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Staphylococcus aureus is a Gram-positive opportunistic pathogen and significant cause of morbidity and mortality, that also asymptomatically colonizes 30% of humans where it is well adapted to survive on the skin in the presence of innate immune defense mechanisms such as antimicrobial free fatty acids (aFFA). While aFFA function to inhibit the growth of *S. aureus*, they also provide a valuable source of lipids for membrane synthesis and energy production. We hypothesized that *S. aureus* possesses novel aFFA resistance pathways that are activated under conditions found on human skin, and that under these conditions, *S. aureus* can metabolize exogenous fatty acids to fuel growth and virulence expression. Our data show that when grown with cationic antimicrobial peptides or at an acidic pH, conditions encountered on human skin, *S. aureus* becomes extremely resistant to aFFA. This resistance is dependent on the sensor kinase GraS, as well as the downstream effector protein MprF. While MprF is known for synthesizing lysyl-phosphatidylglycerol, this aFFA resistance is independent of this synthase activity, highlighting a novel function for MprF. Once resistant to high levels of aFFA, we see expression of putative ß-oxidation genes, fadXEDBA, occur. Expression is upregulated by exogenous fatty acids and is repressed by glucose. Knocking out known fatty acid metabolic pathways results in heightened fadXEDBA expression, highlighting a role of FadXEDBA in fatty acid metabolism. Furthermore, preliminary evidence shows supplementing media with exogenous fatty acids results in improved growth, viability, and protease expression, in a fadXEDBA-dependent manner. Additionally, under membrane stressing conditions, knocking out fadXEDBA significantly impaired growth, indicating a role in membrane homeostasis. Accordingly, expression of fadXEDBA appears to be regulated by the gene directly upstream of the locus, prsW, which is a membrane protease proposed to modulate the function of a stress response Sigma Factor. Finally, knocking out GraS or FadXEDBA result in reduced virulence in a murine abscess model, indicating both resistance and metabolism of host derived fatty acids are essential during infection. While antimicrobial FFA normally function to inhibit bacterial growth, *S. aureus* has evolved to thrive in this environmental niche through the use of GraS, MprF, and FadXEDBA.

Towards Improved Precision Oncology: Development of a quantitative immuno-MRM assay for the PD 1/PD L1 axis

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Checkpoint inhibitors (CPI), such as anti-PD-1 drugs, have revolutionized lung cancer treatment. Despite the increasing use of CPIs, predictive biomarkers that reliably identify patients who respond to the treatment are missing. To date, PD-L1 immunohistochemistry (IHC) is used for patient screening, but only 40-50% of PD-L1-positive patients respond to CPI treatments, while 10% of PD-L1-IHC-negative patients do benefit. PD-L1 IHC is heterogenous, is affected by post-translational modifications (PTM) such as glycosylation and does not reflect the tumor immune microenvironment. We developed a mass spectrometry-based proteomics workflow enabling "absolute" quantitation of the PD-1/PD-L1 axis in formalin-fixed paraffin-embedded (FFPE) tumors. This assay provides the concentrations of six proteins and distinguishes three PTMs. To determine the concentration of PD-L1, PD-1, PD-L2, NT5E, LCK and ZAP70, we used unique and well detectable proteolytic peptides as surrogates. In a refined protocol, we optimized protein extraction and digestion, peptide immuno-enrichment, chromatography, and multiple reaction monitoring (MRM) parameters to maximize recovery, increase target-specific signal and reduce noise. Plus, we assessed the glycosylation status of PD-L1, PD-L2, and PD-1. The entire workflow was fully validated using 31 NSCLC FFPE tumors. PD-L1 quantitation by immuno-MRM (iMRM) was compared to PD-L1 IHC clone 22C3. On average, 71±29 µg (n = 52) of protein could be extracted from each 1–3 mm3 NSCLC tumor FFPE core. The optimized iMRM method allowed the quantitation of PD-L1 and PD-1 down to 21 amol on-column. Inter- and intra-day repeatability were well below FDA guidelines (coefficients of variation [CV] < 20%) with average CVs of 5.2±4.0% (intra-day) and 4.5±2.6% (inter-day). Sample storage had no significant effect on peptide quantitation. The final multiplexed iMRM assay enabled successful quantitation of the PD-1/PD-L1 axis proteins in 31 NSCLC FFPE tumors. PD-L1 expression ranged from 2 amol/µg to 61 amol/µg of total protein. As expected, iMRM results correlated moderately (R = 0.56, ρ <0.001) with PD-L1 IHC. PD-L1 average glycosylation status was 99.9±0.2%, hence did not explain the discrepancies between IHC and iMRM for these samples. A multiplexed immuno-MRM workflow was developed and validated for the quantitation of the PD-1/PD-L1 axis in NSCLC FFPE.

Enhancing Anti-Cancer Immunotherapy by Disruption of the non-Receptor Tyrosine Kinase Fes

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Introduction: Cancer immunotherapy is an emerging pillar of treatment, but it still faces many barriers due to the immunosuppressive nature of cancer. Cancer immunotherapy relies on the activation of both innate and adaptive anti-cancer immune responses. One way of stimulating these responses, known as immunogenic cell death (ICD), causes the release of various tumour-associated antigens and damage associated molecular patterns (DAMPs). DAMPs function to recruit and activate antigen-presenting cells (APCs) by binding to specific receptors known as pattern recognition receptors (PRRs), which signal the production of proinflammatory cytokines required for adaptive immune cell activation (e.g., Cytotoxic T Lymphocytes [CTLs]). The non-receptor tyrosine kinase Fes, abundantly expressed in antigen-presenting cells (APCs), suppresses innate immune responses by inhibiting downstream components of the PRR signaling cascade. In non-cancer contexts, our lab has shown that negative regulation of APCs by Fes may guard against consequences of overactive innate immunity, such as endotoxic shock. However, we hypothesize that this same inhibitory effect on APC function may also serve as a barrier to successful anti-cancer immunotherapy, by obstructing efficient priming of cancer specific CTLs by APCs.
Methodology: Immunoblotting assays quantify signal transduction cascades regulated by Fes. A syngeneic (immune competent) engraftment model of triple negative breast cancer cells in wildtype and fes-/- mice is used to assesses tumour growth, survival, and immune profiles of the tumour and spleen through flow cytometry.
Results: Fes-/- APCs display stronger PRR signaling compared to wildtype APCs *in vitro* following PRR agonist stimulation. *In vivo*, we show increased tumour control and survival in fes-/- mice compared to wildtype mice, which is further enhanced by stimulating ICD with doxorubicin. CTL activation and PD-1 positivity (a potent inhibitory receptor) is increased in fes-/- mice; and increased further by doxorubicin. Fes-/- mice also display higher degrees of activated and PD-1-positive NK cells; indicating a potential novel role for Fes in NK cell regulation. Finally, when treated with anti-PD-1 antibody, fes-/- mice demonstrate greater tumour control and survival than wildtype mice.
Conclusion: These results provide strong rationale for targeting Fes in the tumour microenvironment to enhance cancer immunotherapy.

Patient and Therapist Perspectives on Motor Imagery Training in Parkinson's Disease: A qualitative descriptive study

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Purpose: To examine therapist and patient perspectives on motor imagery and home-based training programs for individuals in the early stages of Parkinson's Disease.
Methods: This study followed a qualitative descriptive design. We used purposeful sampling to recruit patients with early stage Parkinson's Disease and therapists experienced in treating neurodegenerative diseases. One-to-one interviews were carried out over telephone or video conferencing. Interviews took between 30-75 minutes. They were digitally recorded, transcribed verbatim, and analysed by two researchers following the Framework Method, enabling a combination of deductive and inductive analysis.
Results: A total of 10 patients (3=F) and 6 therapist (3 occupational therapist, 3 physical therapists) participated. Therapists were familiar with motor imagery, and all used attentional strategies to facilitate movement in Parkinson's Disease. Many patients reported generating their own strategies to manage movement symptoms. Both groups cited apathy as a main barrier to participation in home-based programs. Therapists were more likely to identify cognitive decline as a challenge to rehabilitation. While patients and therapists also prioritized different activities for potential training, both groups identified a range of adaptations to facilitate participation.
Conclusions: This research provides evidence that can be used to develop home-based training programs for people with Parkinson's Disease. It highlights the need to consult both patients and therapists during program development. Programs should be tailored to the needs of the individual and take the non-motor features of the disease into consideration.

Role of PARP-1/TRPM2 in Regulation of Modality-Specific Microglial Phagocytosis

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Introduction: Microglia are immune cells in the brain that manage neuroinflammation by clearance of beneficial targets such as cellular debris/pathogens via controlled phagocytosis. In Alzheimer's disease (AD) there is excessive amyloid-beta (Aβ), leading to uncontrolled phagocytosis of aberrant targets such as healthy neurons and synaptosomes. Previous work has shown that microglial nuclear enzyme poly(ADP-ribose) polymerase-1 (PARP-1), may be involved in phagocytic regulation. PARP-1 acts in concert with the Ca2+ permeable cation channel TRPM2 (transient receptor potential melastatin type 2), and is also a pro-inflammatory driver that promotes production of nitric oxide, through precursor enzyme iNOS (inducible nitric oxide synthase). Inhibition of PARP-1 has been shown to suppress release of nitric oxide, and impact modality of aberrant phagocytosis (neuron targets) while not impacting beneficial phagocytosis (Aβ targets). This project aims to further investigate PARP-1, as well as TRPM2 and iNOS, as regulators of modality-specific phagocytosis in context of AD.
Methods: Primary mixed glial cultures are prepared from cortices of newborn (<48 hours) or embryonic (E18) CD1 mice. Microglia are harvested from mixed glia cultures at 8-12 days *in vitro*, and phagocytic assays carried out by incubating cells with cargo of fluorescently labelled amyloid beta. Cells are imaged on EVOS Cell Imaging System and phagocytic activity assessed by counting percentage of cells with cargo uptake.
Results: Phagocytic assays demonstrate dose dependent Aβ uptake within 2.5 and 8 hours, where average percentage of cells with engulfed cargo ranges from 35% (0.1 μM Aβ) to 94.5% (1 μM Aβ). Aβ-stimulated cells (0.3 μM) treated with inhibitors for PARP-1 or iNOS did not prevent phagocytosis of Aβ after 2.5 hours incubation. However, PARP-1 and iNOS inhibitors suppressed Aβ-induced nitric oxide production by 78% and 54% respectively, after 20 hours incubation.
Conclusion: Current preliminary findings show that PARP-1 or iNOS inhibitors do not suppress microglial phagocytosis of Aβ, while nitric oxide production can be suppressed. This suggests that nitric oxide production and phagocytosis of a beneficial target (Aβ), are not regulated in same manner by PARP-1 and iNOS. Further work will assess TRPM2 inhibition on these outcomes, as well as study aberrant phagocytosis targets (synaptosomes).

Working through Stigma: A constructivist grounded theory of delivering health services to 2SLGBTQ populations in Nova Scotia

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This study was designed to generate new theory that works to further understandings of the processes and factors that influence the conditions under which primary care services are delivered to diverse 2SLGBTQ populations in Nova Scotia. This Constructivist Grounded Theory study employed Critical and Intersectional lenses to complicate the notion of identity in a broadly inclusive manner and gain insight into structural level forces that influence access to health services in primary care settings across the province of Nova Scotia. By way of conducting a preliminary literature review, stigma was identified as significant to the health outcomes of 2SLGBTQ populations, which justified its use as a starting point for the investigation. Upon REB approval, a diverse sample population was recruited; variation was maximized across categories of identity (sexual orientation, gender, race, (dis)ability, and citizenship), geographical areas, and when appropriate, professional role. Sampling purposefully from historically underrepresented groups so they were overrepresented in the sample population potentiated the creation of multiple points of comparison for the purpose of analyzing data in such a way that a robust grounded theory would be generated. The sample population (n=30) was comprised of three subgroups: 2SLGBTQ health service users (HSUs) (n=10), 2SLGBTQ health service providers (HSPs) (n=10), and non-2SLGBTQ HSPs (n=10). Semi-structured interviews lasting up to 90 minutes were conducted with each participant using video-conferencing software. Stigma was confirmed as a meaningful construct by participants from the onset of data collection. Conceiving stigma as a structurally embedded phenomenon allowed for an exploration of 2SLGBTQ stigmatization in health care that came by way of investigating how primary care services were being delivered to 2SLGBTQ populations across a provincial health system. Analysis of data started at the onset of its collection by way of constant comparison, and continued through coding methods, memo-writing, diagramming, and writing this dissertation. As such, the level of abstraction was raised. A substantive theory was thus co-created and understandings were furthered on how stigma might be worked through in ways that transform the conditions under which health services are delivered.

Translation, Adaptation and Validation of an Instrument for Measuring Family Resilience in Parents of Preterm Infants

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Introduction: Hospitalization of a preterm infant in the neonatal intensive care unit (NICU), which accounts for 7% of all births in Canada, can induce psychological symptoms for parents that are believed to affect family functioning. In other family health experiences, family resilience is recognized as a protective factor against negative symptoms, which seems promising for promoting better mental health for parents of preterm infants. To adequately assess family resilience, a reliable and valid instrument is imperative. The Family Resilience Assessment Scale (FRAS) has undergone several successful cross-cultural validations but remains unavailable in French and unspecific to the neonatal context. This doctoral research project aims to translate into French, adapt and validate an instrument for measuring family resilience with French-speaking parents of preterm infants.

Methods: A five-phase methodological design is planned: (i) double-back translation into French (ii) adaptation of the items by three independent experts guided with a systematic review of the literature (iii) content validation of the adapted items by a group of 12 nurses, conceptual experts and parents of preterm infants, (iv) computer-based administration of the FRAS, of a psychological distress measure (K-6) and of a qualitative questionnaire to 315 parents of preterm infants from two selected level III neonatal units (v) evaluation of the psychometric properties (internal consistency, construct validity, factor structure and inter-rater reliability).

Expected results: This study will provide researchers and clinicians with evidence of a reliable and valid instrument of family resilience in French and specific for the neonatal context for use in future research across disciplines. As such, it would allow for the acquisition of compelling evidence in both research and clinical settings concerned with family resilience in the NICU.

Conclusion: This study could guide the implementation of family-integrated care practices in NICUs. The findings may also support the development, evaluation and implementation of innovative interventions consistent with the family-centered approach implemented in Quebec neonatal units. At the clinical level, personalized follow-up according to the resilience of families could help prevent the onset of psychological symptoms and minimize the impact on long-term family health.

Impact of COVID-19 Pandemic on the Prescription Trends of Antiseizure Medications: A population-based cross-sectional study

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Introduction: Epilepsy is a chronic disorder affecting 50 million patients worldwide. There is a lack of real-world evidence on the effect of the COVID-19 pandemic on epilepsy patients, including access to antiseizure medications (ASMs).

Methodology: We conducted a population-based cross-sectional study using the provincial-level administrative health databases from Manitoba, Canada. We used interrupted time series analysis with autoregressive models to investigate the change in level and slope pre – and during the pandemic period between June 1, 2016, and March 1, 2021, quarterly. We used the 2nd quarter of 2020 as the intervention point. We examined the changes in all ASMs, old generation ASMs, and new generation ASMs, among incident and prevalent users.

Results: We observed a significant increase in the prevalent users of new generation ASMs with a percentage change of 0.114% (P value = 0.04) and significant decrease in the incident prescriptions of all ASMs with a percentage change of -1.567% (P value = 0.01). We found no significant effect on prevalent use of all and old generation ASMs, nor among incident users of new and old generation ASMs. Significant trend changes were observed among the new generation ASMs prescriptions (β 3 = 0.02, P value = 0.04) among prevalent users, as well as among all (β 3 = -0.01, P value = 0.04) and new generation ASMs (β 3 = 0.02, P value = 0.02) incident users. We observed no significant trend changes in the prevalence use of all and old generation ASMs, nor the incidence use of old generation ASMs.

Conclusion: Our study indicated that the pandemic restrictions were associated with a small but significant immediate increase in prescriptions of new generation ASMs among prevalent users. We observed a decline in the overall ASMs use among incident users. Further studies are needed to evaluate if those changes could be associated with an increase in healthcare use or adverse outcomes in this vulnerable group.

Preliminary Analysis of Cefazolin Serum Concentrations and Protein Binding in Hemodialysis Patients

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Introduction: Cefazolin is a commonly used antibiotic to treat infections in hemodialysis (HD) patients. Currently, pharmacokinetic (PK) data for cefazolin in HD patients is limited, including no information on protein binding. It is unclear whether the common one-dose-fits-all approach to cefazolin dosing achieves therapeutic concentrations, especially given variability in patient characteristics (e.g., weight) and cefazolin PK during HD. Our goal was to characterize and evaluate the PK of cefazolin in HD patients including protein binding and pharmacologically active free concentrations.

Methods: Twenty HD patients receiving cefazolin (2g thrice-weekly post-HD) were enrolled in a clinical PK study at St. Boniface Hospital HD Unit. Three blood samples were collected from each patient, including 2 pre-HD troughs, and 1 post-HD/post-dose peak. Samples were centrifuged to obtain serum and 20 samples were re-centrifuged in Centrifree® filters to obtain ultrafiltrate. Total and free cefazolin concentrations were measured in serum and ultrafiltrate, respectively, using a previously validated UHPLC-MS/MS assay. Based on pharmacodynamic principles, target concentrations were defined as free troughs ≥8 mg/L (4x a susceptibility-breakpoint of 2 mg/L).

Results: Nineteen peak and forty trough samples were collected. Median [interquartile range] total and free cefazolin trough concentrations were 72.2 [38.3, 90.6] mg/L and 11.9 [8.8, 23.1] mg/L, respectively, while total and free peaks were 246.0 [207.1, 261.5] mg/L and 89.7 [73.3, 116.0] mg/L, respectively. Although the average protein binding was 70.7 ± 15.0%, a significant difference was observed between peaks (59.5 ± 12.9%) and troughs (81.8 ± 5.6%) (P = 0.0001). Based on total trough concentrations and 81.8% protein binding, 32.5% (13/40) of cases would have predicted free troughs less than the target of 8 mg/L.

Conclusions: Current cefazolin dosing may result in below-target concentrations in almost one-third of HD patients. These new PK data will be used to investigate optimal dosing for this high-risk and understudied population.

Assessment of Natural Killer Cell Function and Susceptibility to Cell Death

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Background: In Sub-Saharan Africa, the most common form of injectable contraceptive is depot medroxyprogesterone acetate (DMPA); a synthetic formulation of natural progesterone. Unfortunately, both epidemiological and basic science studies have associated DMPA-use with increased risk for HIV acquisition. Previously, our lab also found female Kenyan sex workers using DMPA to exhibit higher levels of HIV target cells in their genital tract. Natural Killer (NK) cells are among the first responders of the immune system and are associated with protective immunity against HIV. In exposure to progesterone, NK cells exhibited reduced activity along with increased susceptibility to caspase-induced cell death. It remains unclear how DMPA affects the function of NK cells. Further, this study aims to optimize a flow cytometry panel to study the functions of blood NK cells.

Methods: To identify NK cells, lymphocytes from healthy Winnipeg blood donors were excluded from non-NK cells by staining for CD3 (T cells), CD14 (monocytes), and CD19 (B cells). NK cells were specifically identified by their expression of CD56 and CD16. NK functions such as degranulation and cytokine production were measured by staining for CD107a (an early marker of degranulation) and IFN- γ in response to stimulation with either PMA/ionomycin (PI) or K562 cells (NK target cells). To assess for NK susceptibility to cell death, cells were also stained with NucView (a fluorescence caspase-substrate) in order to detect Caspase-3 activity.

Results: Stimulation of NK cells with PI resulted in an average 38.58% increase in the percent of NK cells producing IFN-γ. In addition, PI stimulation also induced an average 25% increase in NK cells with high caspase-3 activity. Whereas, with K562 stimulation, it was able to increase the % of CD107a+ and IFN-γ + NK cells by 15% and 7%, respectively. Moving forward, the panel will be subjected to further optimization steps including antibody titration, voltage titration, plate titration, and fresh vs frozen stain analysis.

Conclusion: Using this panel, we can assess NK functions using frozen blood samples collected from women in Nairobi, Kenya to measure how DMPA affects NK cells and its potential implications for HIV susceptibility.

Proof-of-Concept for a Pan-Coronavirus Attachment Inhibitor

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The COVID-19 pandemic marks the third emergence of a highly pathogenic coronavirus (CoV) in the 21st century, and underscores the threat posed by emerging CoVs. The abundance and diversity of novel CoVs in bats highlights the likelihood of future spillover into human populations. Even with early virus identification and accelerated development timelines, vaccine development requires 12-18 months in the best-case scenario, which leave us vulnerable in the interim. Novel countermeasures, such as broadly-acting antivirals that could be administered prophylactically to prevent infection, are required to protect against future emerging CoVs. However, the development of a pan-CoV entry inhibitor with therapeutic potential remains elusive. Most human viruses, including CoVs, initiate attachment to cell surfaces through interactions with complex carbohydrates called glycans. Blocking these primary glycan-dependent interactions is a demonstrated approach to inhibit entry of diverse viruses. We aim to target conserved glycan-dependent interactions to develop pan-CoV entry inhibitors. As proof-of-concept, we show that the green tea polyphenol epigallocatechin gallate (EGCG) inhibits entry of endemic human CoVs (HCoV-229E, HCoV-OC43) and highly pathogenic emergent and pre-emergent CoVs CoVs (SARS-CoV-2, MERS-CoV, bat WIV1-CoV). Our results show that EGCG inhibits CoV attachment by competing with heparan sulfate for virion binding, revealing a conserved role for heparan sulfate proteoglycans (HSPGs) in mediating CoV attachment. We show that virion pre-treatment with heparin, a structural analog of heparan sulfate, inhibits entry and attachment of SARS-CoV-2 and, unexpectedly, HCoV-OC43. To further confirm the role of HSPGs in CoV infection, we used CRISPR/Cas9 to knock-out EXT1, a glycosyltransferase required for the biosynthesis of heparan sulfate, and observed reduced susceptibility to SARS-CoV-2 and HCoV-OC43 infection. We are expanding the scope of these studies to other CoVs, and are using these findings to guide rational design of multivalent glycan-based dendrimers as more potent CoV entry inhibitors. Overall, we have identified HSPGs as conserved CoV attachment factors, and are advancing the development of pan-CoV entry inhibitors to enhance pandemic preparedness.

The Role of the Tight Junction Protein, Claudin-3, in Tissue Fusion during Neural Tube Development

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The embryonic precursor to the central nervous system, the neural tube, begins as a flat sheet of epithelial cells; whose edges, the neural folds, elevate upwards and fuse along the dorsal midline of the embryo. Neural tube defects are the second most common birth defect, occurring when the neural folds do not fuse properly causing the brain and/or spinal cord to develop externally. In chick embryos, depletion of Claudin-3 (Cldn3) causes spinal neural tube defects due to failure in the least understood phase of neural tube development, neural fold fusion. I hypothesize that Cldn3 regulates the localization of proteins essential for cell shape changes, movements, and membrane projections required for neural fold fusion. To better characterize neural fold fusion, I am live-imaging wildtype and Cldn3-depleted embryos. So far I have identified differences in the process of neural fold fusion between the cranial and spinal region. In the spine the neural folds approach each other at distinct points along the embryo with a buttoning motion rather than the established zippering motion identified in the cranial region. I will be repeating the live-imaging experiments on embryos electroporated with fluorescent membrane and cytoskeletal markers to look at cell morphology during spinal neural fold fusion in wildtype and Cldn3-depleted embryos. By scanning electron microscopy, I observed that the apical cell surfaces of cells undergoing neural fold fusion are altered in Cldn3-depleted embryos. Cldn3-depleted embryos lack thread like protrusions and display increased membrane blebbing, a sign of cytoskeletal dysregulation. Immunofluorescence of apical and basal cell markers demonstrated that apical-basal polarity is maintained, but the apical polarity protein, Par-3, had increased apical aggregates, suggesting that proper apical protein localization patterns are disorganized in Cldn3-depleted embryos. In the future, I will be looking at the Cldn3 *in vivo* protein-protein network using the latest technique in proximity labelling, Turbo-ID. I will identify proteins whose localization patterns are altered in Cldn3-depleted embryos and access their functions in neural fold fusion. This research is working towards a better understanding of the mechanisms of neural fold fusion and the role that Cldn3 is playing in neural fold fusion defects.

Trajectories of Frailty and Associated Factors in Older Men

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The health and functional life courses among older adults are highly heterogeneous. Frailty, which refers to instability and risk of loss of function, is a potential explanation of the health diversity of older adults. The frailty phenotype and the Frailty Index are the most frequently used frailty definitions, but the more practical measures of frailty have not been developed. There also is little research done about the trajectory of frailty over long time frames. The objectives of this project are 1) to develop and validate the measures of the frailty index; 2) to identify the distinctive trajectories of frailty for older men, and 3) to examine early- and mid-life risk factors associated with the distinctive trajectories. We will use data from a long-term cohort study of 3983 Royal Canadian Air Force aircrew. The confirmatory factor analyses will be used to develop measures of frailty. The latent class trajectory analysis will be conducted to explore the trajectories of frailty from 1996 to 2019. This project will provide important information for interventions to reverse frailty, the best timing for intervention, and education/training of healthcare professionals, which will result in better care and healthier aging for older people.

Antigenic Variations of the SARS-CoV-2 Delta and Omicron Variants of Concern: Immune Recognition Impacts Viral Virulence?

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Introduction: Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has infected 456 million and killed six million to date. Five descendant lineages are designated variants of concern (VOCs) and threaten global public health. It is critical to understand how viral mutations influence antigen recognition in VOCs, as recognition and binding affinity by HLA are key indicators for host anti-viral immunity. By comparing putative immune epitopes between VOCs and the ancestral reference strain, we may better understand how the immune system recognizes VOC lineages and how this affects disease severity.

Methods: Comparisons were completed individually for the delta and omicron VOCs versus the Wuhan reference strain. For each strain, T cell (class II) epitopes were predicted for the spike protein with the IEDB-AR TepiTool. Epitopes were compared using CAVES, a self-developed tool that determines to what extent epitope sequences match/mismatch, to identify pairs of similar epitopes that covered a VOC mutation locus. For each pair, the HLA alleles predicted as binders and their corresponding percentile ranks (indicates binding affinity) were compared to determine how immune recognition differed between the VOCs and reference strain.

Results: Omicron comparisons: 7/12 epitope pairs showed that majority of shared HLA alleles had better binding to the omicron epitope than the reference strain epitope, while 2/12 pairs showed worse binding. Additionally, 6/12 pairs predicted that the omicron epitope could be bound by more HLA alleles than the reference epitope, whereas one pair predicted less alleles.

Delta comparisons: 4/9 pairs showed better binding to the delta epitope on shared HLA alleles while 4/9 pairs had worse binding. Likewise, 2/9 pairs found that the delta epitope was bound by fewer HLA alleles than the reference epitope, but none reported more alleles.

Conclusion: This demonstrates an important putative trend where omicron epitopes are recognized by more HLA alleles than the reference strain and are bound with higher affinity. Conversely, delta epitopes had similar or worse immune recognition than the reference strain. These changes in immune recognition may contribute to the reduced and increased disease severity seen in the omicron and delta VOCs, respectively. Further work characterizing other VOC lineages is underway.

Mechanism of Glucose Sensing in the Nucleus Tractus Solitarius

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Introduction: Type 2 diabetes is characterized by a disruption in glucose homeostasis, but the underlying mechanisms remain elusive. The brain detects changes in circulating glucose levels at sites such as the nucleus tractus solitarius (NTS) and in turn lowers hepatic glucose production (GP) to mediate glucose homeostasis in chow but not high fat-fed rats. It remains unknown how NTS glucose elicits changes in glucoregulation and the site(s) of disruption. Herein, we set out to dissect the mechanisms of NTS glucose sensing.

Methods: Male Sprague-Dawley rats received NTS cannulation and vascular catheterizations to allow infusions or sampling of blood. Using pancreatic euglycemic basal-insulin clamps combined with the tracer-dilution methodology, we assessed changes in glucose metabolism in response to various NTS treatments.

Results: We first evaluated whether cellular glucose uptake via glucose transporter-1 (GLUT1) is required for NTS glucose infusion to suppress GP. Using lentiviral injection of GLUT1-shRNA, NTS GLUT1 expression was reduced by ~40% and NTS glucose infusion failed to lower GP during the clamps. Next, to assess for a role of cellular glucose metabolism, we infused the glycolysis product, pyruvate, into the NTS and found that it recapitulated the effect of glucose in lowering GP. We then prevented pyruvate formation from glucose by knocking down pyruvate kinase and found that NTS glucose failed to lower GP. These findings demonstrate that upon cellular entry via GLUT1, glucose metabolism into pyruvate is sufficient and necessary for NTS glucose sensing. In high fat-fed rats where NTS glucose/pyruvate infusion failed to suppress GP, NTS GLUT1 expression was found to be reduced by 20%. When we further assessed pyruvate metabolism by stimulating its immediate conversion to acetyl-CoA, we found that GP was suppressed in chow but not in high-fat fed rats. These results suggest that aside from NTS GLUT1 reduction, high-fat feeding induces additional impairment(s) downstream of pyruvate/acetyl-CoA formation to disrupt NTS glucose sensing.

Conclusion: In summary, we report the role of GLUT1 and pyruvate metabolism in NTS glucose sensing. Future studies are needed to dissect the downstream pathway to reveal the site(s) of impairment and unravel potential therapeutic targets for diabetes.

Antibiotic Susceptibility of *Helicobacter pylori* in Canadian Arctic Indigenous Communities

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Introduction: Our community-driven projects address concerns of Canadian Arctic Indigenous communities about *Helicobacter pylori* (Hp) infection, responsible for elevated gastric cancer mortality in the region. A key concern is poor effectiveness of anti-Hp treatment. We aimed to describe antibiotic resistance patterns in Hp isolated from project participants.

Methods: Participants in 7 communities underwent upper gastrointestinal endoscopy with 2 gastric biopsies taken for tissue culture during 2008-2017. We tested Hp isolates for resistance to 7 antibiotics by ETEST® and assessed 4 outcomes: resistance to metronidazole, clarithromycin, 1+ antibiotics, and 2+ antibiotics. We tabulated the proportion positive among isolates tested with 95% confidence intervals (CI) and used logistic regression to assess the relation of age and sex to resistance outcomes.

Results: Of 259 Hp isolates tested, resistance to metronidazole, clarithromycin, 1+ antibiotics, and 2+ antibiotics were (% [CI]): respectively, 35 [29-41], 19 [15-25], 44 [38-50], and 12 [8-17] overall; 38 [30-47], 24 [17-32], 49 [41-58], and 16 [11-23] in 146 isolates from women; and 30 [22-39], 13 [8-21], 36 [27-46], and 6 [3-12] in 113 isolates from men. Odds of resistance to clarithromycin, 1+ antibiotics, 2+ antibiotics, and, to a lesser degree, metronidazole were elevated in women relative to men after age adjustment and increased with age in women but not men.

Conclusion: In Arctic Indigenous communities in Canada, women were more likely than men to harbor antibiotic resistant Hp, and their frequency of resistant Hp infection increased with age.

Differential Expression of Endocannabinoids in Endometriosis and Therapeutic Targeting

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Endometriosis (EMS), a chronic inflammatory gynecological disease, is characterized by the growth of endometrial lining at extrauterine locations including pelvic cavity and ovaries. Distinctive features of EMS lesions include hyperproliferation, vascularization and hyperalgesia. In this context, endocannabinoids (EC) have emerged as a potential therapeutic candidate given their well-established functions of pain modulation, inflammation, and cell survival. However, major knowledge gap exists around whether ECs contribute to EMS pathogenesis or merely a bystander. Using EMS patient and healthy, fertile samples, we assessed circulating and tissue resident ECs, presence of cannabinoid receptor 1 (CB1) and 2 (CB2), and gene transcripts involved in the EC signalling in EMS patient samples and healthy, fertile controls. To understand the relevance of ECs in EMS pathogenesis, we analyzed both circulating and tissue levels of ECs in immunocompetent, syngeneic mouse model of EMS. Our results indicated significantly increased PEA levels in the EMS lesions compared to eutopic endometrium from EMS patients. Transcripts involved in EC signal transduction were differentially expressed in the EMS lesions compared to both eutopic endometrium from EMS patients and endometrium from healthy, fertile controls. Analysis of CB1 and CB2 receptors revealed significantly lower CB2 receptor expression in the EMS lesions (no change in CB1 receptor expression) compared to both endometrium from EMS patients and healthy endometrium. Further, we determined the effects of synthetic cannabinoid, WIN 55212-2 (WIN 55) in the context of therapeutic utility using EMS representative cell lines (*in vitro*) and in syngeneic mouse model (*in vivo*). WIN 55 reduced proliferation and angiogenesis *in vitro*, via MAPK/Akt-mediated apoptosis. These findings were corroborated in a mouse model of EMS, where we found reduced TRPV1 expression in the dorsal root ganglia of EMS mouse model exposed to WIN 55, suggesting reduced signaling of pain stimuli. Together, we provide evidence towards dysregulation of members of the ECS in both EMS patients and mouse model of EMS and provide insights into therapeutic targeting using CB1/CB2 agonist. These findings will be critical in understanding the role of ECs in EMS pathophysiology and their potential implications as therapeutic targets.

Vaccine Effectiveness against Severe COVID-19 Outcomes during Different VOC-Dominated Periods in Manitoba, Canada

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Introduction: SARS-CoV-2, which causes COVID-19, mutates over time. The mutations on the spike protein could disguise the detection of the virus from the immune system and thus make the COVID 19 vaccines less efficient. In this study, we will compare the incidence rates of severe outcomes between the vaccinated and unvaccinated populations, and analyze the vaccine effectiveness as the dominant variant changes over time.

Methods: This study was conducted as part of the provincial COVID surveillance efforts. Individuals received 0 doses or <14 days following the first dose are considered as unvaccinated, >=14 days following the second dose are fully vaccinated, and >= 14 days following one additional dose are vaccinated by 3 doses. Age-standardized person-time rates for severe outcomes were calculated for 3 different time periods: April 1, 2021 – June 30, 2021 (Alpha dominant), August 1, 2021 – November 30, 2021 (Delta dominant), and December 15, 2021 – March 7, 2022 (Omicron dominant).

Results: For the Alpha variant dominated period, compared with fully vaccinated individuals, unvaccinated people were 10.8 (95% CI, 7.3-15.9) times likely to be hospitalized, 170.1 (95% CI, 23.9-1211.3) times likely to be ICU admitted, and 7.1 (95% CI, 3.7-13.5) times likely to die; For the Delta period, the incidence rate ratios (RRs) are 13.4 (95% CI, 11.5-15.6), 27.9 (95% CI, 19.0-41.1), and 13.0 (95% CI, 9.5-17.8), respectively; Finally, for the Omicron dominated period, the RRs decreased to 1.4 (95% CI, 1.3-1.5), 2.6 (95% CI, 2.0-3.4), and 2.1 (95% CI, 1.6-2.8) when compared with fully vaccinated. However, when compared with those vaccinated by 3 doses, these RRs increased to 4.8 (95% CI, 4.2-5.5), 5.3 (95% CI, 3.2-8.7), and 18.3 (95% CI, 11.0-30.4).

Conclusion: Vaccines have provided huge protection against COVID-19 for Manitobans. The vaccine efficacy decreased against the Omicron variant, but receipt of a third dose was highly effective at preventing COVID-19 associated severe outcomes during Omicron dominated period.

Bayesian Tensor Factorization-Drive Breast Cancer Subtyping by Integrating Multi-Omics Data

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Breast cancer is a highly heterogeneous disease. Subtyping the disease and identifying the genomic features driving these subtypes are critical for precision oncology for breast cancer. This study focuses on developing a new computational approach for breast cancer subtyping. We proposed to use Bayesian tensor factorization (BTF) to integrate multi-omics data of breast cancer, which include expression profiles of RNA-sequencing, copy number variation, and DNA methylation measured on 762 breast cancer patients from The Cancer Genome Atlas. We applied a consensus clustering approach to identify breast cancer subtypes using the factorized latent features by BTF. Subtype-specific survival patterns of the breast cancer patients were evaluated using Kaplan-Meier (KM) estimators. The proposed approach was compared with other state-of-the-art approaches for cancer subtyping. The BTF-subtyping analysis identified 17 optimized latent components, which were used to reveal six major breast cancer subtypes. Out of all different approaches, only the proposed approach showed distinct survival patterns ($p < 0.05$). Statistical tests also showed that the identified clusters have statistically significant distributions. Our results showed that the proposed approach is a promising strategy to efficiently use publicly available multi-omics data to identify breast cancer subtypes.

Investigating the Role of Interferon-Lambda 3 in Regulating Human T cell Differentiation

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Introduction: Type III IFNs (IFN-lambdas (λs)) were originally discovered for their potent antiviral activity at mucosal barriers. Upon binding to their unique heterodimeric receptor (IFN-λR1/IL-10RB), IFN-λs induce ISGs (Interferon-Stimulated Genes), which can directly inhibit virus replication and spread. Beyond their role as antiviral cytokines, IFN-λs were also shown to influence immune responses in mouse models of allergic asthma by dampening T helper 2 (Th2)-mediated inflammation. We recently discovered that unlike in mice, human T cells express the IFN-λ receptor and that IFN-λ3 addition regulates Th2 cytokine responses in total peripheral blood mononuclear cells (PBMCs). This project seeks to understand how IFN-λs regulate CD4+ T helper cell activation and function. We hypothesize that IFN-λ3 directly regulates human CD4+ T cells to promote Th1 and inhibit Th2 differentiation.

Methods: Total or naïve CD4+ T cells were isolated from healthy donor PBMCs by magnetic isolation or sorting. CD4+ T cells were cultured with T cell receptor (TCR) activating antibodies (anti-CD3/anti-CD28) +/- IFN-λ3, +/- differentiation cocktails for 1-7 days. RNA was extracted for RT-qPCR to quantify IFNG (Th1 cytokine), IL13 (Th2 cytokine), IL2 and IFNLR1 expression. T cell proliferation, activation markers and quantification of IFN-λR1 protein at the cell surface were measured by flow cytometry.

Results: First, we determined the ideal stimulation conditions by optimizing antibody concentrations and media types and settled on using serum-free ImmunoCult media for future experiments. Thus far, we have found that IFN-λ3 addition significantly reduced IL13, had no effect on IL2, and increased IFNG expression in stimulated purified CD4+ T cell cultures. We also found that anti-CD3/anti-CD28 stimulation significantly upregulated IFNLR1 expression in CD4+ T cells over time in culture.

Conclusion: Our initial results indicate that IFN-λ3 can directly regulate CD4+ T cell activation without the presence of accessory cells. Our long-term goal is to fully understand the therapeutic potential of IFN-λs to modulate immune responses against viruses or during periods of chronic inflammation.

Interaction of Neurobeachin-like 2 (NBEAL2), PI(3,5)P2 and α-granules

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Platelets are blood cells that play a key role in hemostasis, inflammation and wound healing. They are derived from megakaryocytes (MKs) which mostly reside in bone marrow. Many aspects of platelet function are related to the release of proteins from α-granule, a lysosome-related-organelle which contain proteins such as fibrinogen and Von Willebrand factor (VWF). Neurobeachin-like 2 (NBEAL2) is required for the retention of cargo proteins by α-granules. NBEAL2 deficiency in MKs leads to the bleeding disorder Gray Platelet Syndrome (GPS). The mechanism by which NBEAL2 facilitates the maturation and stability of α-granules is unclear. Phosphatidylinositol phosphates (PIPs) comprise less than 1% of cellular lipids, yet they regulate fundamental biological processes including signalling, vesicle trafficking, and membrane dynamics. The specific intracellular distribution of PIPs is key to their role in regulating membrane trafficking and downstream signaling pathways. PI(3,5)P2 is extremely low relative to other PIPs. It is concentrated on late-endosomal membranes and plays an important role in endosome and phagosome maturation. NBEAL2 has a Pleckstrin homology (PH) domain, which is commonly found in PIP binding proteins. We hypothesize that NBEAL2 interacts with the membranes of α-granules via interaction with granuleassociated PIPs. Our in-vitro data shows NBEAL2 and its PH-BEACH subdomain preferentially binds PI(3,5)P2. One GPS-associated missense mutation abrogates PI(3,5)P2 binding. Pharmaceutical inhibition of PI(3,5)P2 synthesis causes a loss of VWF in α-granules which recapitulate the phenotype of NBEAL2 deficient MKs. Our data suggests that the interaction of NBEAL2 and PI(3,5)P2 is critical in α-granule maturation and MK cargo protein trafficking.

Semaphorin-3E – a Novel Factor that Regulates Splenic Natural Killer Cell Homeostasis at Steady-state

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Introduction: Natural killer (NK) cells are innate cells that are critical in anti-tumor and anti-viral immune activities. Putative factors that regulate NK-cell development, homeostasis and functions remained to be defined. Recently, our lab has demonstrated that Semaphorin-3E (Sema-3E), a protein first described in nerve cells, has the novel ability to regulate NK cell migrations through NK-DC crosstalk. In addition, NK cells express cognate receptor for Sema-3E. We therefore hypothesized that Sema-3E has regulatory effect on NK cell development and functions via direct signaling through Plexin-D1 receptor expressed on naïve NK cells.
Methods: Spleen and bone marrow were harvested from either inbred BALB/c or Sema-3E -/- mice. When needed, NK cells were further isolated (purity >90%) using a commercial NK cell isolation kit. They were further activated in IL-2 containing complete medium for 4 days. Their cell numbers were determined by hemocytometer and flow cytometry. NK cells were further phenotyped for NK subsets, NK receptors and/or exhaustion markers in flow cytometry.
Results: I observed the mature NK cell number in the spleen of naïve Sema3E-/- animals was reduced by ~28%, when compared to the control BALB/c mice, whereas bone marrow NK cell number remains the same. I also observed no change in splenic and bone marrow T cell number. Further phenotyping of developing immature NK subsets in bone marrow revealed no difference in the BALB/c and Sema3E-/- mice. Short-term goal is to examine the mechanism(s) (such as metabolism, exhaustion, proliferation, apoptosis) underlying the role of Sema-3E in regulating splenic NK cell homeostasis at steady-state. Future work will examine whether Sema-3E deficiency impacts NK-cell migrations and functions.
Conclusion: Deficiency of Sema3E did not impact NK-cell development in bone marrow, but reduced mature NK cell number in the spleen. My data supported a novel role of Sema-3E in the regulation of splenic natural killer cell homeostasis at steady-state. As Sema-3E is expressed in other immune cells and cancer cells in different tissue microenvironments, my work will inspire novel therapeutic development that supports manipulation of NK cells in these microenvironments in specific disease settings.

Central Oxidative Stress and Early Vocational Outcomes in First Episode Psychosis: A 7-Tesla Magnetic Resonance Spectroscopy study of Glutathione

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Background: Following the first episode of psychosis, many patients develop poor social and occupational outcomes, while others display a pattern of preserved functioning. Evidence from preclinical, genetic and biochemical studies suggest a role for high oxidative stress in poor functional outcomes among patients. The measurement of intracortical glutathione (GSH) using magnetic resonance spectroscopy (MRS) provides an opportunity to investigate the relationship between central antioxidant tone and functional outcomes at the time of first episode psychosis (FEP). Importantly, an increase in GSH is hypothesized to be a protective response to high oxidative stress. Research directly assessing the link between GSH and functional response *in vivo* among FEP patients is needed to elucidate this relationship.
Methods: We scanned 57 patients with FEP and 30 matched healthy controls and estimated GSH concentrations in the dorsal anterior cingulate cortex using 7-Tesla MRS. We minimized the confounding effects of illness chronicity, long-term treatment exposure and metabolic complications by recruiting patients with <2 weeks of lifetime antipsychotic exposure and followed this cohort for 1 year to determine occupational and community functioning. Functional outcomes were measured by determining if the patient was engaged in employment, education or training, as well as with the Social and Occupational Functioning Assessment Scale (SOFAS), after 6-12 months of treatment.
Results: Patients who achieved employment/education or training status (EET) in the first year, had higher baseline GSH than healthy controls. Similarly, higher baseline GSH scores predicted higher SOFAS scores at follow up, after adjusting for various confounds including gender and baseline functioning. Patients who were not in employment, education or training (NEET) did not differ from healthy subjects in their GSH levels.
Discussion: Our observations support a key role for the central antioxidant tone in the functional outcomes of early psychosis. These results implicate GSH as a possible biomarker for determining good versus poor functional response during FEP. Further, these findings suggest that augmenting GSH at baseline may improve functional response in a subset of low-GSH patients, further investigation into this pathway is warranted.

Adenylyl Cyclase Isoform 6 is Inhibited by Hypoxia but Rescued by Forskolin – A Potential Treatment for Hypoxic Pulmonary Hypertension of the Newborn

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Background: Persistent pulmonary hypertension of the newborn (PPHN) is a catastrophic failure of neonatal pulmonary vascular relaxation causing 10-30% mortality. It features hypoxemia and pulmonary vasoconstriction; first-line therapies include inhaled nitric oxide NO). The adenylyl cyclase (AC) signaling pathway is crucial for vascular relaxation, and could be a therapeutic target in PPHN. We previously reported that activity of the dominant pulmonary arterial AC isoform, AC6, is persistently inhibited after hypoxia *in vivo* and *in vitro*. We hypothesized that both hypoxia and NO can promote cysteine thiol nitrosylation of AC6, thereby decreasing its activity; and that the allosteric AC activator Forskolin can rescue this inhibition.

Methods: HEK cells stably overexpressing individual AC isoforms (AC 3, 5, 6, 7, 9) were cultured in a normoxic (21% O2, 5% CO2) or hypoxic (10% O2, 5% CO2) incubator for 72 hours. Selected normoxic cells were treated with nitroso-donor S-nitrosocysteine (CySNO, 250μM) for 30 mins. In cells from all treatment groups, we measured AC activity (ATP dose response by Terbium(III)-norfloxacin fluorescence AC assay; and live cell cAMP assay) and protein S-nitrosylation (Biotin-Switch Assay kit to label nitrosylated thiols). We also quantified Forskolin dose responses of hypoxic and normoxic cells.

Results: Among the five AC isoforms studied, only AC6 activity was significantly inhibited by hypoxia at all ATP doses, and by CSNO treatment, resulting in impaired cAMP generation. Hypoxic inhibition of AC catalytic activity correlated with increased nitrosylation of AC6 protein. The inhibited AC6 could be reactivated by Forskolin, though not to a normoxic level.

Conclusion: We conclude that hypoxia uniquely inhibits activity of AC6, leading to impaired cAMP production. This may be mediated by AC6 nitrosylation. Forskolin partially reactivated AC6 activity and restored cAMP generation. These findings suggest that use of targeted forskolin derivatives to selectively activate AC6 could provide new treatments for hypoxic PPHN.

Examining Integration, Sense of Belonging, Health and Wellbeing, and Physical Activity among Newcomers in Canada

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Introduction: Newcomer women deal with many resettlement challenges that affect their community belonging which can lead to an overall poor health status (physical and mental). Thus, it is important to understand newcomer women's lived experiences to focus on strategies that mitigate these challenges. Physical activity (PA) participation has been associated with benefits for physical health, mental health, community belonging, and social support. The COVID-19 pandemic has individuals' health and behaviours worldwide. The purpose of the proposed research is to explore the role of PA in integration, sense of belonging, and health outcomes among newcomer women in Canada through three studies.

Methods: The first study is a cross-sectional analysis of the Canadian Community Health Survey (2017-2020). Associations between PA and health status (physical and mental) among immigrants will be explored through descriptive statistics, Pearson Chi-square analysis, and logistic regression analysis using SAS software. The second study is a systematic literature review of PA-based interventions among immigrants in immigrant-adopting countries using four scientific databases. Two independent reviewers will screen titles and abstracts using Covidence, then full articles, and extract data into a piloted Excel sheet. Disagreements will be resolved by a third reviewer. The third study uses a community-engaged research approach to understand newcomer women's immigration experiences, community belonging, and their attitudes toward PA participation, with consideration for the COVID-19 pandemic in Kingston, ON. Semi-structured interviews will be conducted using different questions on newcomers' immigration experiences, community belonging, and their attitudes toward PA participation before and during the COVID-19 pandemic.

Results: Findings from the first study will provide insight on gaps in the literature that can inform the content of health promotion and disease prevention programs at a national level and inform policymakers and researchers. Findings from the second study will direct the designing of an effective intervention that promotes PA among newcomer women in Canada. Findings from the third study will be helpful in the development and planning of any PA-based intervention among newcomer women in Kingston.

Conclusion: Overall findings will provide direction for public health programs aimed at improving health status for all newcomer women in Canada.

Role of Endoplasmic Reticulum Stress in Doxorubicin-Induced Cardiomyopathy and its Mitigation by Interleukin-10

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Introduction: The use of doxorubicin (Dox) in cancer patients carries the risk of cardiotoxicity via an increase in oxidative stress, mitochondrial dysfunction and disturbed endoplasmic reticulum (ER) homeostasis in cardiomyocytes. Disturbed ER homeostasis resulting in the accumulation of unfolded and/or misfolded proteins is referred to as ER stress. This study explores which of the ER transmembrane sensors is involved in Dox-induced apoptosis and whether interleukin-10 (IL-10) has any mitigating effect.

Methods: Adult male Sprague Dawley rats' cardiomyocytes were treated with Dox (10μM) in the presence or absence of IL-10 (10ng/ml) for 24hrs. For the combination group of Dox+IL-10, cells were treated with IL-10 for 1hr before the addition of Dox. Treatment groups were compared *by one* way analysis of variance (ANOVA), and Tukey-Kramer's test was performed to identify differences between the groups, $P \leq 0.05$ was considered to be significant.

Results: Dox induced ER-stress via an activation of ER chaperones, protein foldases and upregulated the expression of various genes, including X-box binding protein 1 (XBP1). The splicing of XBP1 mRNA on Dox treatment generated spliced XBP1 and subsequently up-regulated chaperones like glucose regulated protein 94 (GRP94), glucose regulated protein 78 (GRP78), and/or protein disulfide-isomerase (PDI). These Dox-induced changes in upregulation of ER stress proteins were blunted by IL-10. In Dox-exposed cardiomyocytes, IL-10 also promoted expression of protein kinase-like endoplasmic reticulum kinase (PERK) and inositol requiring kinase 1α (IRE1α) as well as inhibited cleavage of activating transcription factor 6 α (ATF6α) which helped in maintaining ER homeostasis. Additionally, IL-10 treatment down-regulated caspase-12 activation as well as phosphorylation of c-JUN NH2-terminal kinase (JNK), thereby promoting cardiomyocyte survival. IL-10 was able to reduce the overexpression of mitochondrial apoptotic proteins caspase-3 as well as Bax which were upregulated upon Dox treatment.

Conclusion: The beneficial effects of IL-10 in modulating ER-stress and ER-initiated apoptosis may prove to be a significant advance in restricting cardiac damage during stress or inflammation. The recognition of Dox-induced cardiotoxicity as well as a dire need for a cardio protective agent to be administered in conjunction with Dox is evident and IL-10 may prove to be such an agent.

Knowledge Translation to Learn and Empower Children and Adolescents in Schools and General Community about Air Pollution and its Health Effects

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Objective: i) To know the needs and suggestions of general community, regarding air pollution and ii) To describe the environmental initiatives of high schools about air pollution, and its health effects.

Materials and Methods: a mixed design-qualitative study with descriptive-interpretive scope and cross-sectional analytical study was conducted in Medellin, Colombia. Through focus groups and interviews, general community, research centers, government institutions and children/ adolescents from 8 high schools were invited. Responses were coded and emerging categories were analyzed with NVivo 12. An indicator was constructed to assess significant environmental experiences in high schools, and knowledge, attitudes, and practices (KAP) regarding air pollution were assessed in children and adolescents.

Results: General community formulated 4 questions that guided the discussion in the dialogue spaces and 492 responses were obtained: 1) Daily effects, 2) Effects and those responsible, 3) Additional proposals and 4) Follow-up and control. 19 categories emerged and the general community agenda included 9 proposals to improve air quality. None of the high schools had air pollution as a central axis within its environmental initiative, but 3 significant environmental experiences were found. In the 1676 students evaluated, it was found that 15.1% had high knowledge, 43.9% had a positive attitude and 6.8% had good practices regarding the reduction of effects caused by air pollution. Infographics, videos, the website https://escuelaenelmapa.com and the general community agenda for healthy air (https://repository.upb.edu.co/handle/20.500.11912/6191) were created.

Conclusion: knowledge translation can promote habits of democratic coexistence and increase the argumentative capacity of the general community. More spaces to promote the community's participation in research processes are required.

Sex-Related Differences in Allergen House Dust Mite-Mediated Changes in the Lung Proteome

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Introduction: There are clear sex-related differences in the prevalence and severity in asthma, with women experiencing higher prevalence and severity in adults. However, sex dimorphism in allergen-mediated protein production in the lungs has not been completely defined. Therefore, in this study we comprehensively examined sex-related differences in allergen mediated protein profile using a murine model of airway inflammation.
Objective: To characterize changes in the lung proteome in response to inhaled allergen house dust mite (HDM) in a mouse model of airway inflammation.
Methods: Male and female BALB/c mice (7-8 weeks) were challenged with intranasal administration of 35uL of whole HDM extract (0.7mg/mL) in saline for 2 weeks. Lung tissues from naïve and HDM-challenged mice (n=5; per group per sex) collected 24h after last HDM challenge were used for quantitative proteomics with LC-MS/MS. Log2-transformed protein levels reported from mass spectrometry results were analyzed using linear models with terms for exposure, sex and their interaction. The interaction terms were then evaluated and summarized using volcano plots.
Results: 2586 proteins were identified with high confidence by LC-MS/MS, of which 163 proteins were significantly different between males and females in response to HDM challenge. These 163 proteins provided insight into pathways that may contribute to underlying mechanisms of sex differences in asthma.
Conclusions: HDM-mediated changes in the lung proteome reveal specific sex-related differences, which may be further targeted to unravel underlying mechanisms that contribute to sex disparity in asthma.

Targeting Metabolism as a Novel Therapeutic Strategy to Suppress Oncogenic c-MYC in group 3 Medulloblastoma Tumors

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Background: Brain tumors are the leading cause of cancer death in children, and medulloblastoma (MB) is the most common pediatric central nervous system malignancy. MB is classified into four molecular subgroups that differ in prognosis: WNT, SHH, group 3, and group 4. Amplification of the c-MYC oncogene is one of the strongest molecular predictors of poor survival in MB patients and is commonly observed in group 3 MB tumors but not in other subgroups. Unfortunately, the functional ubiquity and disordered structure of c-MYC makes it difficult to target for cancer treatment. Therefore, recent efforts have been made to develop out-of-the-box strategies to indirectly target hyperactive c-MYC in cancer. Recently, metabolism has emerged as a major regulator of overall cellular signaling processes through post-translational and epigenetic mechanisms. While c-MYC is a well-known regulator of cell metabolism, the role of metabolism in regulating c-MYC is unknown.
Hypothesis: We hypothesize that an intrinsic feedback mechanism may exist where metabolic activity modulates c-MYC expression or stability that could be exploited for cancer therapy.
Methods and Results: Using 3 distinct well-characterized group 3 MB cell lines (HD-MB03, MB3W1, and D283) as well as a patient-derived xenograft (PDX) group 3 MB cell line(SU_MB002), we have identified a targetable metabolic vulnerability where group 3 MB cells demonstrate exquisite sensitivity towards inhibitors of oxidative phosphorylation (OXPHOS). In-depth molecular characterization by immunoblotting, immunoprecipitation, fluorescent microscopy, half-life analysis, and oxidation assays, unveiled a novel mechanism where targeting OXPHOS leads to rapid oxidation and proteasomal degradation of c-MYC. Importantly, oral administration of IACS-010759 impairs the growth of intracerebellar MB xenograft tumors in mice and significantly prolongs animal survival.
Significance: Altogether, these findings unveil a novel mechanism through which metabolism regulates the post-translational stability of the c-MYC oncoprotein in group 3 MB cells and provides a new precision medicine-based strategy with broad therapeutic implications for targeting virtually all c-MYC-driven cancers.

Characterizing Melanoma Diagnostic Pathways for Patients in Routine Practice using Administrative Health Data in Ontario, Canada: A population-based study

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Introduction: Diagnostic pathway guidelines were introduced to optimize diagnostic processes. However, actual diagnostic pathways experienced by patients with melanoma have not been characterized. We identified the diagnostic pathways experienced by melanoma patients in routine practice and compared patient, disease, and diagnostic interval characteristics across pathways.

Methods: Population-based cross-sectional study of all melanoma patients diagnosed in Ontario from 2007-2019 using linked administrative data. Latent class cluster analysis (LCCA) identified clusters of patients with similar diagnostic experiences. Input variables for LCCA characterized the patient's medical history, first physician specialty seen in primary and specialist care, initial procedures, number of visits and procedures, and healthcare activity on the diagnosis date. We assessed associations between demographic and disease factors and pathway membership using chi-square tests and Pearson residuals and characterized pathways by their interval distributions.

Results: We identified four diagnostic pathways: 'primary care only' (N=6107), 'referred to specialist for immediate action' (N=8987), 'multiple visits and procedures in specialist care' (N=11893), and 'specialist care only' (N=6384). 75% of patients first saw a GP, and 80% saw a melanoma-related specialist. A higher proportion of patients in the 'primary care only' pathway lived in rural areas. In contrast, a higher proportion of patients in the 'referred to specialist for immediate action' and the 'specialist care only' pathways lived in major urban centers. Across pathways, median diagnostic interval varied from 1 day to 67 days, median primary care subinterval varied from 1 day to 30 days, and median specialist care subinterval varied from 1 day to 25 days. Patients in the 'primary care only' pathway experienced the shortest diagnostic intervals, and patients in the 'multiple visits and procedures in specialist care' pathway experienced the longest diagnostic intervals.

Conclusions: Melanoma patients experience varying pathways to a diagnosis that are associated with characteristics of the patient and where they live. Shorter diagnostic intervals in the 'primary care only' pathway demonstrate the important role of primary care. Future research should address reasons for the associations we observed, and whether they are indicating problems with access to high quality care.

Mechanism of Action of Novel Antibacterials Revealed via Chemogenetic Profiling of a Barcoded Transposon Mutant Library

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The *Burkholderia cepacia* complex (Bcc), a group of Gram-negative multi-drug resistant pathogens are notorious for causing persistent lung infections in people with cystic fibrosis, especially Burkholderia cenocepacia. We synthesized chemical analogs of auranofin, an anti-arthritis drug, to develop new antimicrobials against the Bcc. These novel antimicrobials are bactericidal to clinical cystic fibrosis pathogens, can kill metabolically dormant cells, including persister cells, and do not select for resistance. Auranofin is known to inhibit the enzyme thioredoxin reductase, however, additional targets of auranofin as well as the mechanism of action of MS-40 and MS-40S is not known. We hypothesize that the cellular uptake and mechanism of action of the auranofin derivatives involve cell envelope elements and thioredoxin reductase, and the structurally and functionally similar protein, glutathione reductase. Multiple processes in the mechanism of action of these novel compounds were identified through a method called BarSeq. To create this tool, a high high-density barcoded transposon mutant library was built in B. cenocepacia K56-2 containing more than 300,000 uniquely barcoded mutants to create this method. We exposed this library to sub-inhibitory concentrations of the active compounds to selectively kill hypersusceptible mutants, where next generation sequencing and enumeration of the barcodes served as a proxy of mutant abundance. Through this, we found mutants in glutathione and purine synthesis, pyruvate metabolism, and several transporters, some of which have not been associated with antimicrobial uptake before. These compounds will be further explored to identify new druggable targets and may result in a new class of antibiotics.

The Acute Effect of a Laboratory Shame Induction Protocol on Endothelial Function

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Introduction: Shame is a self-conscious emotion that arises when one internalizes social devaluations from others. Increased shame in a laboratory setting is associated with increased cortisol and tumor necrosis factor alpha activity, however, no studies have investigated the acute effects of shame on important indices of cardiovascular disease risk such as endothelial function. The endothelium is a single layer of cells which lines the inside of all arteries and acts as a vasoprotective and vasoactive agent by inhibiting the development of atherosclerosis and adjusting vessel diameter in response to changes in blood flow associated shear stress (flow-mediated dilation). The objective of this study was to examine the impact of a shame induction protocol on endothelial function.

Methods: Fifteen, young (23±2 years), healthy participants (n=7 men, n=8 women) completed both a 20-min written shame induction and control protocol on two different experimental days. Pre- and post-protocol we assessed: 1) Endothelial function via a standard brachial artery reactive hyperemia flow-mediated dilation (RH-FMD) test which was characterized as the peak % and absolute change in diameter following release of 5 min of forearm occlusion, and 2) Perceived shame via the Experiential Shame Scale (ESS).

Results: Shame increased in response to the shame induction protocol (pre: 2.9±0.6 vs. post: 3.7±.5, $p<0.001$) but not the control protocol (pre: 3.0±0.5 vs. post: 2.8±0.5, $p=0.15$). %RH-FMD significantly decreased in response to the shame protocol (pre: 4.8±1.9 vs. post: 3.2±1.6, $p<0.001$) but not the control protocol (4.2±1.8 vs. post: 3.8±1.5, $p=0.45$). Covariation of the shear stress stimulus for RH-FMD did not alter the RH-FMD results. Increased shame was significantly associated with decreased absolute RH-FMD ($r=-0.37$, $p=0.046$).

Conclusion: The shame induction protocol caused a significant increase in shame and reduction in RH-FMD. This suggests that temporary increases in shame cause transient endothelial dysfunction which, if chronically repeated, could manifest as reduced vasoprotection against atherosclerosis.

Echocardiographic Features of a Hypoxic Piglet Model of Persistent Pulmonary Hypertension of the Newborn

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Background: Persistent Pulmonary Hypertension of the Newborn (PPHN) is a rapidly progressive vasculopathy marked by failure of pulmonary vascular relaxation and right ventricular (RV) dysfunction. It is pathologically similar to brisket disease in piglets, and accurately modelled by exposure of newborn piglets to hypoxia for 72 hours. Echocardiographic cardiovascular imaging in piglets is complicated by the oval shape of the thorax, small intercostal spaces and limited acoustic windows. Piglets with PPHN are intolerant of handling and of deep anesthesia, necessitating image capture during light procedural sedation.

Objective: To study pulmonary and systemic hemodynamics by echo in hypoxic and control piglets at 72 hours age, and identify markers of pulmonary hypertension.

Methods: PPHN was induced in newborn piglets by continuous exposure to FiO2 10% x 72 hrs; controls were age-matched 3-day-old piglets. If needed, animals were sedated with ketamine/xylazine before study. Transthoracic echo performed by a neonatal echocardiographer, analyzed offline by another neonatologist. Echo parameters analyzed: Pulmonary VTI [velocity time interval], pulmonary artery diameter [PAD], right ventricle cardiac output [RVCO], pulmonary AT/ET [acceleration time/ejection time], TAPSE [tricuspid annular plane systolic excursion], TR [tricuspid regurgitation].

Results: We studied newborn controls (N=5), 3-day controls (N=4), and 3-day PPHN piglets (N=7). Tricuspid regurgitation jet was significantly greater in hypoxic animals, and TAPSE was shorter in hypoxic compared to 3-day normoxic animals. Pulmonary artery diameter was wider in hypoxic compared to control. Pulmonary AT/ET trended lower in the hypoxic group although it was not significantly different. Most animals exhibited tachycardia with handling. Normoxic animals had higher heart rates; hypoxic animals occasionally became bradycardic during study. The measurements of the hypoxic group are similar to those of the newborn normoxic group, which is representing the transition period from physiological pulmonary constriction in utero.

Conclusion: We conclude that hypoxic PPHN can be diagnosed in piglets by severe TR gradient indicating increased pulmonary vascular resistance. The decreased TAPSE, load-dependent index of RV function, indicates very impaired RV contractility in PPHN. Intolerance of handling resulted in significant tachycardia in all animals, worsening RV dysfunction in the PPHN animals; this impaired utility of some other classic PPHN markers.

HCAR1 Nuclear Location Bias Drives Cancer Malignancy by Multiple Routes

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Introduction: GPCRs are virtually involved in all physiological processes. HCAR1, as a GPCR, is endogenously activated by lactate and has been shown to promote cancer malignancy via higher level of glycolysis due to the Warburg effect. It has a high expression level in many cancers and negatively correlates with patient's prognosis. However, its mechanism of action is ill-understood. On the other hand, nuclear localization of several GPCRs have been described albeit its unusual feature for them. Additionally, it has been shown nuclear GPCRs can perform functions distinct from their cell surface counterparts *in vivo*. Here we demonstrate HCAR1 has a nuclear localization and this localization pattern promotes cancer malignancy by multiple routes.

Methods and Results: We determined HCAR1 nuclear localization pattern by cell fractionation, immunofluorescence confocal imaging and TEM. Site-directed mutagenesis showed ICL3 and phosphorylation of C-terminal domains are required for nuclear localization. We also demonstrated that this localization is ligand-independent and there is a pool of nuclear HCAR1 (N-HCAR1) in the cells. We show N-HCAR1 induces intra-nuclear signaling through Gαi and Gßγ by WB and ELISA leading to increased phosphorylation of nuclear AKT and ERK resulting in increased cancer cell survival and proliferation. Our ChIP-sequencing data shows N-HCAR1 binds to the genes regulating cell migration and we prove N-HCAR1 promotes migration *in cellulo*. We identified N-HCAR1 interactome by Bio-ID coupled with mass spectrometry and found, it interacts with proteins involved in translation and DNA damage repair and our experimental data demonstrates that specifically the N-HCAR1 promotes both of those process *in cellulo*. Additionally, we identified the transcriptomic signature of N-HCAR1 and showed it regulated a larger gene network than its plasmoa membrane counterpart. Concordantly, our *in vivo* tumor xenografts and tail vein injections prove that tumors without N-HCAR1 have smaller size and tumor mass and lower metastatic rate as well.

Conclusion: Here we show an unusual localization of a GPCR in the nucleus and provide evidence that N-HCAR1 specifically promotes various hallmarks of cancer malignancy. The effect of N-HCAR1 is validate *in vivo* in tumor xenografts as well. Understanding these mechanisms can provide targets and cues for therapeutic developments.

Investigating the Role of Lectins in Lymphocyte Activation Gene-3 (LAG3) Function and T-cell activity

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Background: Immune cells, like T-cells, are modulated by several checkpoints to prevent unrestrained immune activation which leads to autoimmunity. Unfortunately, upon chronic antigen exposure, as in cancer or HIV/AIDS, these regulatory "brakes" of immune cells are coopted to escape host immunity. This T-cell exhaustion is provoked by the overexpression of immune-checkpoints (IC), surface negative co-receptors that impede appropriate effector cytokine responses, like IL-2. As LAG3, an IC, is extensively glycosylated, the lectins, Galectin- 3 and liver sinusoidal endothelial cell lectin (LSECtin), have been proposed to bind glycans on LAG3, which may engage this IC's function, and thereby inhibit T-cell activation. Understanding the interaction and contribution of these ligands may broaden the scope of LAG3 activity and its subsequent impact on T-cell function and immune exhaustion. This study seeks to clarify the role of lectins (LSECtin and Galectin-3) in regulating LAG3-mediated T-cell suppression.

Expected Results: A ligand binding assay will confirm the necessity of LAG3 glycosylation and test lectin binding efficiency by comparing % of LAG3+ and LAG3- CD4+ T-cell lines bound to either LSECtin or Gal-3 ligands by flow cytometry. Moreover, IL-2 levels within treated cells will compare the differential inhibitory strengths for ligands. Thus, if lectin binding can induce inhibitory LAG3 activity, we hypothesize greater T-cell inhibition (via lower IL-2 production) in LAG3+ cells than LAG3- cells.

Conclusion: Ligand-binding by lectins may be a previously overlooked mechanism mediating LAG3 activity and T-cell immunosuppression. Understanding the role of lectins in regulating LAG3 engagement can inform the efficacy and optimal use of a lectin or LAG3 antibody blockade in relieving T-cell exhaustion, alongside current immunotherapies. By "removing the brakes" and reversing cellular exhaustion, it is possible to re-invigorate host-immunity and ultimately, provide novel therapeutics for HIV/AIDS and cancer.

Fibro-Adipogenic Progenitors and Bioactive Lipids Interaction, a New Targetable Interaction to Treat Duchenne Muscular Dystrophy?

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Introduction: Skeletal muscle has an incredible regeneration capacity involving activation and fusion of muscle stem cells (MuSC) to form myotubes. However, perturbations within this process can lead to dystrophies such as Duchenne muscular dystrophy (DMD). DMD is due to a mutation in the gene encoding for dystrophin and is characterized by muscle wasting, chronic inflammation, and fibrosis. Fibro-adipogenic progenitors are the main source of extracellular matrix and thus, actively participate to the fibrosis found within dystrophic muscles. Sadly, DMD cruelly lack efficacious treatment as anti-inflammatory glucocorticoids, the gold-standard cure, exert numerous side effects. Recently, bioactive lipids have been emphasized as a promising therapeutical avenue for DMD. Among these molecules some demonstrate an essential role in muscle regeneration in both normal and aging context. Nonetheless, bioactive lipids' role in DMD and particularly on FAPs is still unknown and could represent an interesting cure for this disease.
Results: We have shown that FAPs express bioactive lipid receptors, EP1, EP2, EP3 and EP4. Our *in vitro* assays exerted that these compounds directly affect FAPs behavior by inhibiting their proliferation rate. RNAseq analysis demonstrated that they could act as a pro-apoptotic factor on FAPs and prime these progenitors towards an adipocyte differentiation process. Given these results, we tested these molecules as a potential therapeutical avenue by treating mdx mice, a known model of DMD. Preliminary results showed that treated mice tend to have a greater muscular strength and a better fatigue resistance.
Conclusion and future: Through bioactive lipids' various effects, on proliferation and adipocyte differentiation of FAPs, these actors anchor themselves as a promising therapeutical avenue for DMD. Bioactive lipids' effect on FAPs are not only relevant in DMD but could also been extended to other muscular pathologies.

Measurement Properties of Remotely or Self-Administered Lower Extremity Mobility Performance Measures in Adults: A systematic review

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Introduction: For individuals who face barriers to assessing care there is a need for remote and/or self-administration of physical performance measures assessing mobility to determine current functional status, predict future status, and monitor change. The purpose of this systematic review is to evaluate (i) the test procedures and population suitability of remotely or self-administered lower extremity mobility performance measures in adults, and (ii) the measurement properties of their scores.
Methods: Five databases; MEDLINE, EMBASE, CINAHL, AMED and Cochrane CENTRAL were searched to January 26, 2021. Two individuals independently conducted article screening, data extraction and risk of bias assessments using COSMIN's risk of bias tools. Data was qualitatively summarized, and results were compared against COSMIN's criteria for good measurement properties.
Results: Fourteen studies detailing nineteen outcome measures were included. Many studies displayed "sufficient" measurement properties of scores according to COSMIN's criteria, however most studies risk of bias ratings was adequate or doubtful. Thirteen studies included a comparison between a traditional (in-person) and remote or self-administered assessment; five comparisons rated 'adequate' reported ICCs from 0.73 to 0.93 (good to excellent). Only three studies included adverse event reporting.
Conclusion: Assessing mobility via remote or self-administered physical performance measures in adult populations appears feasible using a variety of methods including simple tools (chair, stopwatch), videoconferencing, and smartphone applications. This may be particularly valuable for self-management of chronic conditions and decreasing barriers to accessing care. However, higher quality research is needed in this area. Future research should utilize the study checklist criteria presented by COSMIN and include transparent reporting on adverse events.

Aligning Dietary Intake to Nutrient Metabolism in Experimental Models of the HNF-1αG319S Variant

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Background: 40% of Indigenous youth with type 2 diabetes (T2D) in Manitoba carry a variant in the HNF-1α gene (HNF-1αG319S). The G319S variant is thought to drive pancreatic β-cell dysfunction; however, youth-onset T2D is a relatively recent phenomenon. We hypothesize the G319S variant impairs β-cell insulin secretion when exposed to modern dietary carbohydrate stress but is protective in the context of traditional off-the-land foods that are rich in fat and protein and low in carbohydrate.

Methods: CRISPR/Cas9 was used to knock-in the G>A.955 substitution into MIN6 β-cells (G319S-MIN6) and C57/BL6 mice. Mice were weaned onto (1) a high-fat, low-carbohydrate (HFLC) diet reflecting off-the-land foods, or (2) a high-fat, high-carbohydrate (HFHC) diet reflecting present-day dietary patterns. β-cell function was assessed by glucose-stimulated insulin secretion (GSIS) and oxygen consumption rates in the presence of glucose and/or palmitate. Whole-body outcomes including body weight, insulin sensitivity, glucose tolerance, and pyruvate tolerance (a surrogate measure for endogenous glucose production) were examined at 12- and 24-weeks-of-age.

Results: Chronic exposure to palmitate impaired GSIS in wild-type MIN6 β-cells as expected; however, G319S-MIN6 cells were protected from palmitate-induced impairment via elevated fatty-acid β-oxidation (1.5-fold). Given this propensity for fatty-acid metabolism, G319S-expressing mice retained both glucose tolerance and GSIS capacity when fed a HFLC diet that otherwise impaired glucose tolerance and GSIS in wild-type mice at 12-weeks-of-age. This protection may be conferred by improved regulation of hepatic endogenous glucose production as G319S-expressing mice showed constrained glucose production during the pyruvate tolerance test. Conversely, a present-day HFHC diet elevated weight gain, accelerated the development of glucose intolerance, and impaired islet GSIS in G319S-expressing mice between 12- and 24-weeks-of-age.

Conclusion: The G319S variant appears to shift β-cell metabolism towards fatty-acid oxidation. To align nutritional intake with this metabolic shift, the consumption of a HFLC diet appears to normalize insulin secretion and glucose tolerance in G319S carriers, although studies to assess long-term effects are underway. Conversely, a present-day HFHC diet accelerates poor metabolic outcomes and impairs β-cell insulin secretion in G319S-expressing mice. These studies may inform nutritional interventions for children with T2D while ultimately supporting community-led efforts to access off-the-land traditional foods.

Global Post-Transcriptional Regulation in Human Neurodevelopment and Rett Syndrome

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Molecular mechanisms that regulate mRNA stability during human neurodevelopment are relatively unknown. Our lab uses human induced pluripotent stem cells (iPSC) to model neurodevelopment *in vitro* by directed differentiation into neural progenitor cells (NPC) and cortical neurons. Then, mRNA regulatory programs are observed during neurodevelopment and compared between wild-type and MECP2-deficient neurons. Deficiency in the global transcriptional regulator MECP2 leads to a neurodevelopmental disorder Rett syndrome (RTT). Recent reports captured misregulation of miRNA biogenesis and protein synthesis in the MECP2-deficient neurons. The prevailing explanation of protein shifts in RTT neurons is misregulation in the transcription rate. However, altered miRNA abundance suggests shifts in mRNA stability. This could explain the surprisingly small magnitudes of mRNA steady-state shifts observed in RTT models. A thorough understanding of the post-transcriptional regulatory layer could improve our understanding of molecular mechanisms underlying RTT. I dissect the post-transcriptional regulatory landscape by simultaneously measuring transcription rate and mRNA half-life. To permit analysis of RNA stability mechanisms, I also sequenced microRNAs in all cell types. To begin, I observed large magnitude shifts in RNA stability during the transition from iPSC to neurons. This RNA stability modulation shapes the landscape of detected gene isoforms through a common across genes mechanism. A concurrent shift in microRNA copy number per cell suggests that a cumulative effect of all microRNAs referred to as microRNA load is a driver of the process. Next, I probed the degree of coupling between transcriptional and mRNA stability programs. I observed a widespread boosting and buffering of transcription rate shifts during neurodevelopment. This crosstalk between the nucleus and cytoplasm machinery acts even stronger upon the MECP2 loss. Reanalysis of mouse Mecp2-deficient neurons supported the existence of global buffering, suggesting that global mRNA stability response is a conserved feature of RTT models. In summary, I identified a unique role of mRNA stability in the generation of transcriptome diversity and in mounting a genome-wide balancing response to perturbation of a global transcription factor.

The role of Odd-Skipped Related 1 in a Mouse Model of Bladder Dysfunction

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Bladder dysfunction is a prevalent childhood disorder that has significant health and social implications. It is incredibly heterogeneous and can show as urinary incontinence, frequent or infrequent urination, urine retention, and urinary tract infections. Unfortunately, the causes of these voiding dysfunctions are largely unknown. To better understand the anatomical and cellular mechanisms underlying bladder dysfunction we use a genetic mouse model with a mutation in the gene Odd-skipped related 1 (OSR1). Osr1 is a transcription factor that regulates cell differentiation and development of several tissues. The bladder consists of three primary layers, the epithelial layer, the collagen rich lamina propria which helps the bladder maintain its form while stretching, and the muscle layer which contracts to expel urine. These three layers act in a coordinated manor to hold and expel urine. We found that Osr1 is expressed in all three cell layers of the bladder throughout its development. Furthermore, bladders of homozygous Osr1 knockout mice had a significant reduction in the muscle layer and had abnormal bladder shapes. Because Osr1 knockout mice die due to heart and kidney defects during the embryonic stage, we analyzed Osr1 heterozygous mutants at the newborn and adult stages. Osr1 heterozygous newborns lacked fibroblast cell populations in the lamina propria of the bladder, resulting in a significant decrease in collagen. The loss of these cell populations was not due to cell death or proliferation suggesting a defect in differentiation. When assessed for bladder function at 4-6 weeks, Osr1 heterozygous mice had lower bladder capacities and voided more frequently compared to their wild-type littermates. Osr1 heterozygous mice also displayed indications of fibrosis and damage in the bladder wall. In conclusion, we show a model by which defects in the musculature and collagen-rich lamina propria of the bladder can increase urinary frequency and cause bladder damage. Furthermore, we have identified a novel role for Osr1 in mediating cell differentiation in the bladder. Using this knowledge cell-based therapies to improve bladder function, as well as biomaterial generation of tissue used to replace damaged bladder tissue could be developed to treat this debilitating disorder.

Characterization of Cytokine Receptor Expression in the Female Genital Tract

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Introduction: Cytokine's expression levels in the female genital tract (FGT) are influenced by external factors like sexually transmitted infections, commensal microbiota, and intravaginal practices. Increased genital cytokines have been associated with risk of HIV acquisition, but few studies have focused on expression of the cytokine receptors through which they signal. This study aims to quantify expression levels of a wide range of cytokine receptors and Toll-like receptors, and correlate their expression to microbiome, barrier integrity markers and behavior associated with sex work.

Methods: We collected Softcup samples from 96 sexually-active adolescent girls and young women from Mombasa, Kenya, a subset of whom engage in transactional sex and/or sex work. RNA was extracted from Softcup pellets to measure genetic expression of cytokine receptors, toll-like receptors, immune cell subsets and DNA for 16s rRNA sequencing respectively. Supernatant were used to measure cytokines and sE-Cadherin. Downstream analysis was carried out using SPSS.

Results: Approximately 66.6% of the participants had diverse microbial populations and only 33.3% of the participants had Lactobacillus dominant groups. Cytokine receptor expression was significantly upregulated among women with lactobacillus dominant microbiome while it was down regulated in women who had a diverse microbiome. To understand the impact of receptor expression on barrier integrity we carried out correlation analysis and found that only four receptors (IFNgR2, IL1Rap, IL10RA and GMCSFRA $p<0.05$) were inversely correlated to cytokine receptors. We observed positive correlations between markers of immune cells (CD4, CD45, CD69 and CD14) and several cytokine receptors, (IL8Rb, IL1R1, IL1R2, IL1RAP, IFNaR1, IFNaR2, IL6R, TNFRSF1a and TNFRSF1b, all $p< 0.05$).

Conclusion: Downregulation of cytokine expression among individuals with lactobacillus dominant populations could be a strategy employed by the pathogens to avoid detection or a strategy by the host to prevent further mucosal damage. When there is evidence of immune cell presence, there is more cytokine receptor expression suggesting the cells may increase the receptors, and/or the receptors may be expressed mainly on immune cells. Importance for HIV is that the effects of cytokines are likely to be more dramatic if receptor expression is high, in order to complete the signaling loop.

Role of microglial PARP-1/TRPM2 Signaling Axis in Promoting Amyloid-Beta Synaptotoxic Effects

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Introduction: Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterised by memory loss and cognitive decline due to accumulation of amyloid-beta oligomers (AβOs). AβOs are known to disrupt synaptic transmission and plasticity in the form of long-term potentiation (LTP). AβOs also trigger chronic microglial pro-inflammatory responses that contribute to impaired synaptic transmission and plasticity. Our past work has identified PARP-1 activity as a central driver for microglial inflammatory functions. Moreover, our recent work suggests that sustained microglial PARP-1 activation requires Ca2+ influx initiated as a result of transient receptor potential melastatin-2 channel (TRPM2) activation, a Ca2+ permeable channel most highly expressed in microglia. Moreover, ADP-ribose, a by-product of PARP-1 activation, is required for TRPM2 activation. Thus, PARP-1/TRPM2 activity is coupled through positive feedback. In my study I aim to establish whether inhibiting PARP-1/TRPM2 pro-inflammatory signaling can prevent synaptotoxic effects of AβOs on LTP.

Methods and Results: To investigate the role of TRPM2 in this signalling axis, I derived hippocampal slices from 4–6 week-old male and female CD1 mice and recorded field excitatory post-synaptic potentials (fEPSPs). Once the baseline was established, LTP was induced by 100Hz high-frequency stimulation. AβO treatment did not affect the baseline amplitude, but inhibited LTP in both sexes. Intriguingly, slices from females required 50% higher AβO concentration than males to induce comparable inhibition of LTP. To establish the contribution of TRPM2 to AβO-mediated inhibition of LTP, I treated slices with clotrimazole, a TRPM2 inhibitor. Clotrimazole treatment abolished the effect of AβOs on LTP in both sexes. Next, I utilized a novel NUDT5 inhibitor that causes ADPR accumulation and thus PARP-1 feedback inhibition. The NUDT5 inhibition also prevented AβO-induced LTP depletion.

Conclusion: My findings highlight the importance of PARP-1/TRPM2 pro-inflammatory signaling in promoting synaptotoxic effects of AβOs. Disrupting the integrity of this cascade using two independent inhibitors abolished inhibition of LTP in both sexes. Future experiments using microglia-specific TRPM2KO mice will allow me to establish whether microglia-targeted disruption of microglial TRPM2 can protect against cognitive decline in a mouse model of AD.

Characterizing Strain Profiles of Creutzfeldt-Jakob disease (CJD) in Canada

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Introduction: Prion diseases are rare invariably fatal neurodegenerative disorders affecting humans and animals. These diseases are associated with a structural change of a naturally occurring prion protein found within the brain to a misfolded pathogenic form. Chronic Wasting Disease (CWD) is a prion disease that has reached epidemic proportions within North America's deer and elk populations. With the rapid spread of this disease, there are concerns over possible transmission to humans who consume CWD infected meat. If CWD were to cross the species barrier to humans it would likely exist as a novel strain of human prion disease (broadly referred to as Creutzfeldt-Jakob disease or CJD). However, it is impossible to know what human CWD would present with or whether it would differ from other CJD cases. Additionally, there are currently no convenient laboratory methods to study these differences experimentally. For these reasons, we have applied novel technologies in CJD surveillance to analyse strain diversity of CJD in Canada.

Methods: Samples from 32 CJD confirmed patients who resided in Canada at their time of death have been categorized into venison consumers and non-consumers. Analysis of the samples include temperature and protease resistance profiles, glycoform ratio analysis, and seeding profiles. Atypical cases identified have been inoculated into deer mice to observe clinical characteristics of disease.

Results: Following analysis, we found CJD cases of the same type have remarkably similar molecular profiles. Because of this, outliers were easily identified. We report two CJD cases with atypical properties. One atypical CJD cases presented with an increased resistance to high temperatures as well as slow seeding profile. The other atypical CJD cases presented with a fast seeding profile. We hypothesize these atypical cases represent novel strains of disease.

Conclusion: By describing Canadian CJD strains we intend to develop a baseline through which atypical cases of CJD could be readily identified in the future, such as those that may arise through exposure to CWD.

miR-526b and miR-655 Regulate Cellular Metabolism in Luminal A Breast Cancer

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Introduction: miR-526b and miR-655 are observed to be upregulated in aggressive breast cancers through activation of the COX-2, EP4 inflammatory pathway. Overexpression of these miRNA in poorly metastatic breast cancer cell lines promotes aggressive phenotypes such as angiogenesis, proliferation, migration, invasion, and metastasis. The impact of these oncogenic miRNA on the typical metabolic dysregulation seen in cancer has yet to be investigated. As these miRNAs promote many aggressive phenotypes and alter the metabolic demand of cells, we hypothesize that the miRNA contribute to increasing in cellular metabolism and energy production.

Methods: miRNA overexpressed cell culture model is used to investigate metabolic changes induced by miRNA. We analyzed cell secretion of miRNA overexpressed cell lines with liquid chromatographic mass spec analysis. We shall use systems biology and bioinformatics tools to identify and validate metabolic markers in the miRNA secretome.

Results: Overexpression of miR-526b and miR-655 induces more ATP production in the MCF7 cell line as observed in ATP concentration assay. The increased metabolic activity is supported as overexpression of miRNA also resulted in a more rapid development of acidic media. ATP concentration was observed to increase further following treatment with PI3K/Akt inhibitor Wortmannin, indicating that the miRNA promotes ATP production in a manner independent of the PI3K/Akt pathway. We identified 139 differentially regulated proteins in the miRNA overexpressed cell secretome. Mitochondrial ATP synthase subunit F1-alpha is upregulated in the secretome of MCF7-mir526b and MCF7-mir655, prompting our metabolic investigation. We further validated the ATP5A1 expression with Western blot and immunofluorescence assay.

Conclusion: Altogether, these results suggest that miR526b and miR655 contribute to metabolic dysfunction typically seen in many cancer cells. However, upregulation of oxidative phosphorylation enzyme subunit ATP5F1A implies it may not contribute to the more traditional Warburg effect. The pathway of action is yet to be developed and requires further investigation.

Brain Neurogenesis in Response to Demyelination and Treatment Strategies in Multiple Sclerosis

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Multiple sclerosis (MS) is a chronic immune mediated-demyelinating disease of the central nervous system that affects millions of individuals globally with one of the world's highest prevalence in Canada. Cognitive impairment is a common characteristic of disease progression in MS that is attributed to neurodegeneration and neurogenesis decline in the hippocampus and cortex. Adult neural precursor cells (NPCs) are the source of new neurons in the brain. To date, the behaviour of NPCs in progressive MS is largely unexplored. Understanding the response of NPCs to demyelination and during the repair process is of paramount importance for development of targeted treatment strategy aimed at attenuating cognitive dysfunction in MS. Here, we have employed a cuprizone-induced demyelination mouse model of progressive MS in parallel with primary *in vitro* platforms to investigate the behaviour of NPCs in acute and chronic stages of demyelination and remyelination in the hippocampus. We induced demyelination in Nestin-Cre transgenic reporter mouse that allows tracking NPCs and their progenies *in vivo*. In chronic cuprizone mice, we observed depression behaviour and memory deficits that was associated with cortical and hippocampal atrophy, neuronal degeneration and reduced neurogenesis by NPCs compared to control mice. Our *in vitro* studies on NPCs isolated from chronic cuprizone mice also confirmed smaller number of NPCs and their reduced capacity for neuronal differentiation in cuprizone mice. To identify treatments with the potential to increase neurogenesis and neuronal survival, we performed an *in vitro* drug screening on mouse and human derived NPCs. We tested several candidate treatments, some among the existing MS disease modifying treatments. Our findings showed the potential of neuregulin-1β1 and Siponimod treatments in promoting neurogenesis of NPCs. We are currently evaluating these treatments in the cuprizone mouse model to determine their therapeutic potential for attenuating neurodegeneration and cognitive impairment and promoting hippocampal neurogenesis *in vivo*. These studies will address critical knowledge gaps in our understanding of the contribution of NPCs to the repair process in progressive demyelinating lesions of MS, and aid in identifying regenerative treatments aimed at promoting neurogenesis.

Evaluating the Impacts Reduced SKP2 Expression has on Chromosome Instability in Colorectal Cancer

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Introduction: Colorectal cancer (CRC) remains the third most commonly diagnosed and second most lethal cancer in Canada. Chromosome instability (CIN), or ongoing changes in chromosome complements, occurs in ~85% of CRCs and is a proposed driver of cancer pathogenesis, however the aberrant genes underlying CIN remain elusive. Preliminary data from the McManus laboratory suggest that reduced expression of the ubiquitylation gene SKP2 induces CIN, however this remains to be firmly established. Accordingly, we seek to investigate the impact reduced SKP2 expression has on CIN in a CRC context.
Methods: To determine the short-term impact diminished SKP2 expression has on CIN, siRNA-based silencing was used to transiently reduce SKP2 abundance in multiple karyotypically stable, non-malignant colonic epithelial cell lines, including A1309. Single-cell quantitative imaging microscopy was utilized to evaluate CIN phenotypes, including changes in nuclear areas (i.e., large-scale DNA changes), micronucleus formation (i.e., extranuclear DNA-containing bodies) and aberrant chromosome complements. To elucidate the long-term impact reduced SKP2 expression has on CIN, CRISPR/Cas9 approaches were employed to generate stable SKP2 knock-out clones in A1309 cells. Finally, to evaluate the evolution of CIN within SKP2 clones, CIN phenotypes were assessed and statistically compared to controls every 2 weeks for ~3 months.
Results: Qualitative analyses of SKP2 silenced cells revealed an increase in nuclear area heterogeneity and overall increases in nuclear areas that were statistically significant relative to silencing controls. Similarly, micronucleus analyses also identified significant (> 2-fold) increases in micronucleus formation relative to controls. Finally, chromosome enumeration revealed striking increases (> 2.5-fold) in the abundance of mitotic chromosome spreads with aberrant karyotypes. Next, one heterozygous and three homozygous SKP2 knockout clones were employed in long-term CIN assays, which revealed that all SKP2 clones exhibit significant and dynamic changes in nuclear area distributions and micronucleus formation (1.1 to 26-fold increases) throughout the ~3-month experiment. Finally, chromosome enumeration data show that SKP2 clones display ongoing changes in chromosome complements (0.5 to 5-fold increases) over time (i.e., CIN).
Conclusions: Collectively, these data identify SKP2 as a novel CIN gene in clinically relevant models and highlight its potential pathogenic implications in CRC development.

Elucidating Tissue-Niche Interactions with Intestinal Macrophages

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Introduction: The intestine is a complex organ system supported by a diverse set of tissue compartments that work together to uphold vital functions such as nutrient absorption and immunological tolerance induction against orally ingested antigens. Macrophages, acting as surveilling cells, closely interact with microbes, tissue niches, and cells of the adaptive immune system, influencing tissue function and immunity. However, characterization of the gut macrophage population revealed substantial heterogeneity within these cells residing within specific microanatomic locations of the intestinal tract. This suggests that regional specialization and maintenance of macrophages by niche cells may establish this heterogeneity. We previously identified that macrophages, residing in the muscularis layer of the intestine, produce bone morphogenetic protein (BMP) 2 and enabled enteric neurons to modulate intestinal motility. In turn, the neurons near these macrophages provided the macrophage survival factor colony stimulating factor (CSF) 1. We recently identified that gut macrophages outside of the muscularis layer are also capable of BMP2 production.
Methods: To determine the role of macrophage-derived BMP2 on either the intestinal macrophage population or tissue cell compartments, we developed a novel conditional knockout mouse model that allows for macrophage-specific abrogation of BMP2 production.
Results: Our analysis (immunofluorescence and flow cytometry) of these mice reveals a critical role of BMP2 in sustaining intestinal macrophage numbers. Strikingly, BMP2-deficiency had little effect on CSF1-independent macrophages, suggesting a possible macrophage-BMP2-tissue cells-CSF1 axis that regulates intestinal macrophage homeostasis and diversification.
Conclusions: Using single cell RNA-sequencing and spatial transcriptome analysis, we plan to identify BMP2-responsive, CSF1-producing cells within the intestinal tract and identify macrophage-regulated niche cells. The results from this project should yield insights into how the innate immune system not only actively responds to pathogens but is also important in nurturing barrier function. Importantly, these findings could reveal similar immune interactions in other organ systems in the body.

Human Platelet Lysate Stimulates *In Vitro* Proliferation of Primary Endometrial Epithelial Cells from Patients with Recurrent Implantation Failure and Improves Their Attachment to Trophoblast Spheroids

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Introduction: Recurrent implantation failure (RIF) occurs in 10% of *in vitro* fertilization cycles. Inadequate endometrial thickness and receptivity are common causes of RIF and studies suggest platelet-rich plasma, and its derivative human platelet lysate (HPL), improves endometrial thickness and implantation in RIF patients. We hypothesized that HPL will stimulate endometrial epithelial cell (EEC) proliferation and improve their attachment to transferred embryos. HPL may standardize future clinical treatment for endometrial origins of RIF or a thin endometrium (TE).

Methods: Endometrial tissue was collected from ten RIF patients at the CReATe Fertility Centre, Toronto, Canada: five with a TE (RIF+TE) and five without a TE (RIF only). Primary EECs were enzymatically isolated, cultured, and treated with serum-free media (SFM) or SFM supplemented with 1% HPL for 48 hours. Cell viability and proliferation were assessed using the metabolic assay PrestoBlue reagent and immunocytochemistry for the nuclear proliferation marker Ki67. Trophoblast spheroids were generated from the HTR-8/SVneo immortalized cell line, fluorescently labeled with calcein-AM, and size-selected (70-100 μM). Spheroids were then seeded on top of SFM/HPL-treated EECs for 1 hour and unattached spheroids were aspirated. Calcein fluorescence and individual spheroids were quantified to calculate the percentage of spheroid attachment.

Results: Treatment with 1% HPL for 48 hours significantly increased EEC viability and proliferation by 1.45-fold ($P<0.001$) for RIF+TE and 1.28-fold ($P<0.01$) for RIF only cultures. HPL-treated EECs also displayed a significant increase in attachment to HTR-8 spheroids. As measured by fluorescence, the percentage of spheroid attachment significantly increased from 47.98% to 64.27% ($P<0.01$) in RIF+TE cultures, and from 48.12% to 85.77% ($P<0.001$) in RIF only cultures. Individual spheroid counts revealed a significant increase from 57.52% to 86.5% ($P<0.01$) in RIF+TE cultures, and from 42.58% to 68.90% ($P<0.01$) in RIF only cultures.

Conclusion: We report functional assessment of HPL treatment to stimulate endometrial proliferation and implantation in RIF patients using a model of embryo attachment. Our study provides the groundwork to improve the clinical treatment of a TE or RIF. We predict that HPL induces a broad transcriptional response towards improved endometrial receptivity and will next focus on characterizing the transcriptomic profile following HPL treatment.

Immunoregulatory Mechanisms in the Female Genital Tract: the Roles of IL-2 and Regulatory T cells

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Introduction: While inflammation is central to the initiation of effective immune responses, if dysregulated, it may compromise the integrity of the mucosal barrier and increase HIV transmission risk. Understanding mechanisms that regulate inflammation at mucosal sites may contribute to the development of novel HIV prevention strategies. In contrast to inflammatory cytokines, cervicovaginal IL-2 was associated with reduced HIV acquisition in South African women enrolled in an HIV prevention trial. Low dose IL-2 has been shown to expand regulatory T cells (Tregs), and we recently showed in women that endocervical Treg to be associated with decreased inflammation. Hence, we hypothesized that low dose IL-2 delivered with and without nanogel conjugation, decreases inflammation in the female genital tract (FGT) by increasing the frequencies and/or functions of Tregs.

Methods: We modelled the immunological effects of IL-2 administration in the humanized bone marrow, liver, and thymus (BLT) mouse. BLT mice (n=10) with >50% reconstitution of human CD3/CD45+ cells (defined by flow cytometry) were used. The mice were synchronized into the same estrous phase by progesterone injection and randomized into 3 groups: PBS (n=3), low dose IL-2 (n=4) and IL-2/Fc nanogel (n=3). The mice were dosed vaginally 8 times with 1000 IU of low dose IL-2, 0.1ug IL-2/Fc NG or PBS. At the end of dosing, mice were euthanized to obtain FGT for processing and flow cytometric analysis. Treg were defined as the proportion of CD4+ T cells that were CD25+CD127low.

Results: Vaginal low dose IL-2 induced higher frequency of Treg compared to intraperitoneal dosing (10.2% and 7.7% respectively). We also observed IL2/Fc NG to induce higher frequency of Tregs (median 28.6%) in the FGT compared to low-dose IL-2 (median 4.88%) and the PBS-treated controls (median 4.74%; ANOVA $p=0.0002$).

Conclusion: These preliminary data reveal an association between vaginally administered IL2/Fc NG and Treg in the FGT, providing proof of principle for expanded experiments to determine how IL-2 impacts the female genital milieu more broadly, including modulation of HIV susceptibility. This study may shed light on strategies for reducing inflammation and HIV risk at a mucosal level.

Activity Patterns of Sympathetic Preganglionic Neurons during Fictive Locomotion in the *in vitro* Neonatal Mouse Spinal Cord

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Introduction: Spinal cord injury (SCI) is a devastating life altering neurological condition, with lost movement and impaired body functions caused by loss of communication between the brain and spinal cord. Electrical stimulation of lumbar spinal cord has emerged as a powerful intervention for people with SCI as clinical trials have shown its ability to improve both movement and restore lost autonomic body functions (e.g., blood pressure regulation, heart rate/ rhythm control). Although promising, neural mechanisms mediating observed recoveries using this intervention remain unknown, and thus limiting the use for the wider general SCI population. We hypothesize that ascending neurons from the lumbar spinal cord may give input to sympathetic neurons that in turn, provide homeostatic support for locomotion and exercise. Our lab has recently shown anatomically that lumbar locomotor-related neurons synapse on sympathetic preganglionic neurons (SPN) throughout the thoracic spinal cord. We thus aimed to characterize the activity patterns of SPNs before and during fictive locomotion in the *in vitro* neonatal mouse spinal cord.
Methods: To test our hypothesis, we have loaded fluorescent calcium dye into SPNs in the intermediolateral nucleus (IML) of thoracic spinal cord (T4-T11) by applying the dye to cut ends of corresponding ventral roots, and recorded fluorescent signals emitted from SPNs before and during fictive locomotion.
Results: Our preliminary data indicate varying numbers of SPNs active at baseline, which may be correlated with spontaneous ventral root discharge. Additional distinct SPNs become active with the appearance of tonic and/or rhythmic locomotor activity.
Conclusion: Our findings suggest that there may be a functionally integrated relationship between SPNs and locomotor circuits. Experiments specifically activating or inhibiting defined neural pathways between lumbar locomotor networks and thoracic SPNs are ongoing.

Pannexin 3 Channels Regulate Architecture, Barrier Function, Keratinocyte Adhesion, and Inflammation in the Skin

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Introduction: Pannexin 3 (PANX3) is a channel-forming glycoprotein critical in cell communication that facilitates the passage of ATP at the cell surface and calcium at the endoplasmic reticulum. PANX3 is present in adult skin and has been shown to function in cutaneous wound healing and keratinocyte differentiation. However, its regulation throughout skin aging and its role in skin homeostasis is not yet understood.
Methods: We characterized the thin and thick skin of both male and female global Panx3 knockout mice (KO) compared to congenic, age-matched wildtype (WT) controls. Protein levels were assessed via immunoblotting, skin architecture was examined using histological analysis and epidermal barrier function was tested using a toluidine blue dye assay. The incidence of dermatitis in our aging mouse cohort was also investigated. To understand the signalling mechanisms behind the changes to the skin upon Panx3 deletion, we performed a Clariom™ S transcriptomic profiling analysis of WT and KO neonatal epidermis. Clariom™ S findings were confirmed by isolating and culturing primary keratinocytes.
Results: We found that PANX3 was absent in newborn dorsal skin but became upregulated at postnatal day 2 and remained at high levels with age, specifically in epidermal keratinocytes. Structurally, Panx3 KO dorsal skin showed sex differences at various ages, but generally had reduced epidermal, dermal and hypodermal areas compared to aged-matched controls. However, no differences were seen in paw skin epidermal areas regardless of genotype or challenge. Panx3 KO neonatal epidermis showed reduced E-cadherin stabilization and Wnt signalling, consistent with the inability of primary keratinocytes to adhere in culture and compromised epidermal barrier function in KO neonates. Lastly, aged KO mice were 3.75 times more likely to develop dermatitis than WT mice, which was reflected in increased KO epidermis inflammatory signalling.
Conclusion: These findings suggest that during skin aging, PANX3 is critical in the maintenance of dorsal skin architecture and keratinocyte cell-cell and cell-matrix adhesion, and its ablation increases the risk of developing dermatitis. By understanding the role that PANX3 plays in maintaining healthy skin, we will be better equipped to identify its dysfunction in skin pathologies such as keratinocytic skin cancers.

Differential Effects of 5-Azacytidine on the Methylation Level and Usage of the First, Internal and Last Exons of Genes

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Introduction: 5-azacytidine (5-azaC) is an analogue of cytidine and a well-studied inhibitor of DNA methyltransferases (DNMT). 5-azaC binds covalently with DNMT thereby inactivating the enzymes and inhibiting DNA methylation. It also interferes with RNA methylation, processing, and translation. However, its effect at the exome level remains unclear.

Methods: Here, we examined the whole-genome bisulfite sequencing (WGBS) data of nuclear genomic DNA (gDNA) and RNA-Seq data of the cytoplasmic RNA from pituitary GH3 cells treated with 5-azaC (50μM), for the 5-azaC effect on the methylation of CG and CH (H: A, T and C), and exon usage of the first, internal and last exons of genes.

Result: The DNA of the first, internal and last exons have different methyl-CG and -CH ratios, which were changed differentially by 5-azaC. The 5-azaC treatment had opposite effects on the relative usage of the first and last exons and mixed effects on the internal exons in DEXseq analysis. The different effects on the exon usage were contributed in many cases by the alternative usage of polyadenylation sites. Moreover, the fold changes of exon DNA methylation are inversely correlated with the fold changes of exon usage by 5-azaC treatment at the extremes of the changes: the highest methylation changes are found in exons with the least fold change of exon usage or vice versa.

Conclusion: Taken together, our observations suggest that the 5-azaC effect on DNA methylation is dependent on the location of exons in genes, and its effect on the relative usage of many exons is by switching the usage of alternative polyadenylation sites.

Defining the Role of Phosphatidylinositol 3-Kinase (Pi3kδ) Pathway in B Cell Regulatory During Parasitic Infection

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Introduction: PI3Kδ protein is important for B-cell responses to model antigens however, it's role in regulatory B-cell functions in the context of infectious immunity is broadly unknown.

Methods: Intraperitoneal (ip) Infection of PI3Kδ loss-of-function mutant mice (PI3KδLOF/GL) and PI3Kδ B-cell specific gain of function mutant mice (PI3KδGOF/B), with 1000 parasites of the B-cell targeting protozoan Trypanosoma congolense (TC13), Flow cytometry, ip treatment of C57BL/6 (WT- control) mice with the PI3Kδ-inhibitor : Idelalisib, Cytokine analysis by Mesoscale ELISA, Griess Assay for Nitric Oxide (NO).

Results: Mice with a genetic loss of PI3Kδ (PI3KδLOF/GL) have a surprising ability to control parasitemia in early infection (7-9 days), despite impaired lymphocyte activation. Drug (Idelalisib) inhibition of PI3Kδ from WT mice similarly produced improved parasite control, consistent with findings in the genetic mutants. Peritoneal fluid and blood analysis for cytokines, as well as immunophenotyping of the peritoneum indicates significant elevations in IFN (favorable in early disease) and reduction in the suppressive cytokine, IL-10 during early infection. Our results suggest that IL-10 producing B1 cells (regulatory B cells) localized to the peritoneum are the key cells responsible for regulating early immune responses. These cells fail to develop in the PI3KδLOF/GL mutants while their IL10 secretory functions are suppressed in WT mice treatment with Idelalisib. Preliminary results indicate that mice with a B cell-specific hyperactivation of PI3Kδ (PI3KδGOF/B) show a reciprocal effect of impaired early control of parasitemia associated with expanded regulatory B-cell populations. Interestingly, the assessment for Nitric oxide (indicative of favorable host responses from macrophages) show elevation of NO in the PI3Kδ inhibited mice and a reciprocal decrease in NO in the PI3Kδ hyperactivated mice, inside the peritoneal cavity during infection.

Conclusion: PI3Kδ impacts both regulatory and innate immune functions, critical for prompt protection in early Trypanosomal infection.

Development and Characterization of a Novel DC-Targeting Universal Influenza Vaccine

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Introduction: Influenza virus disease is a deadly disease with high pandemic potential. Despite the annual influenza vaccination, the flu still poses a serious threat to the public. Thus, there is an advocate to develop a novel universal influenza vaccine. Since targeting dendritic cells (DCs) is highly immunogenic, and Ebola glycoprotein (EboGP) can target DCs. We hypothesize that the fusion of the conserved influenza hemagglutinin stalk regions (HAcs) and the ectodomain of the matrix protein (M2e) (HM2e) or four copies M2e (tM2e) with DC-targeting domains of EboGP (EΔM) will efficiently deliver influenza antigens to DCs and induce influenza virus broader immune responses.

Methods: In this study, we developed potent immunogens by infusing HM2e or tM2e into the EΔM generating EΔM-HM2e or EΔM-tM2e respectively. The generated EΔM-HM2e or EΔM-tM2e were incorporated into virus-like particles (VLPs) to evaluate their DC-targeting ability *in vitro* or recombinant vesicular stomatitis virus (rVSV) to investigate the immune responses induced in mice sera and nasal wash after immunization. The rVSV-EΔM-HM2e or EΔM-tM2e immunized mice were then challenged with lethal H1N1 or H3N2 influenza to observe the percentage survival.

Results: Our results revealed that VLP-EΔM-HM2e or EΔM-tM2e well infected the DCs/macrophages *in vitro*. Furthermore, rVSV-EΔM-tM2e or EΔM-HM2e immunized mice had significantly high levels of humoral and cell-mediated immune responses. Also, in influenza challenged mice, the rVSV-EΔM-tM2e had 100% protection against the H1N1 and H3N2 while EΔM-HM2e had 60% and 100% protection in H1N1 and H3N2 respectively. Currently we are investigating the mechanism(s) of action underlying the rVSV-EΔM-tM2e- and EΔM-HM2e-mediated protective effects against the influenza challenge, which will be presented in the meeting.

Conclusion: The novelty of this study is the use of EΔM fusion protein technology to present the conserved influenza HAcs and M2e to DCs/macrophages and induce robust and broader immune responses against different influenza virus infections.

Genetic Variation within CD101 Regulates Surface Expression of CD101 on Circulating T cell Subsets

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Background: CD101 is a transmembrane glycoprotein that is expressed on several immune cell subsets including lymphoid and myeloid cells. Multiple immunoglobulin-like and cytoplasmic-like variants have been identified within CD101 that were associated with elevated risk of HIV acquisition among heterosexual HIV-serodiscordant couples in Africa. We hypothesized that these variants may regulate expression levels of CD101 on circulating T cells.

Methods: Individuals were genotyped for CD101 variants and categorized into those with immunoglobulin-like, cytoplasmic-like, or both variants (n = 86, 43, and 20 respectively); those with no key variant (n = 112) were considered controls. We used flow cytometry with conventional manual gating to quantify CD101 expression on different T cell phenotypes in the blood of HIV uninfected individuals enrolled in the Partner's HIV pre-exposure prophylaxis (PrEP) cohort in East Africa. Kruskal-Wallis' test was used to compare differences in expression levels of CD101 on immune cells by CD101 variant group.

Results: CD101 variants were associated with altered CD101 expression on multiple T cell subsets. Those with immunoglobulin-like variants had the highest CD101 expression on most T cell subsets compared to controls. Strikingly, having an immunoglobulin-like variant was associated with increased CD101 expression on CD4+ and CD8+ central memory ($p = 0.0011$ and 0.0001) and effector memory ($p = <0.0001$ and <0.0001) T cells while having no functional variants was associated with higher CD101 expression on naïve T cells ($p = 0.0015$ and <0.0001). This pattern of CD101 expression was reflected among the CD4+ and CD8+ T cell population expressing integrin β7, with those with immunoglobulin-like variants having the highest CD101 expression on their β7hi ($p = 0.0010$ and 0.0008) and β7lo ($p = 0.0006$ and <0.0001) T cells. Increased frequencies of CD8+ T cells expressing proinflammatory cytokines such as IFNγ ($p = 0.0289$) and GMCSF ($p = 0.0100$) were observed in individuals with functional variants.

Conclusion: Genetic variation within CD101 gene significantly modified CD101 expression on circulating T cells. These data provide an immunologic link between CD101 genotype and phenotype and may help to better understand how CD101 variation increases HIV risk.

Investigating Crosstalk Between Endoplasmic Reticulum Stress Sensors

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Introduction: The unfolded protein response (UPR) is a pro-survival pathway activated by Endoplasmic Reticulum (ER) stress, the accumulation of unfolded proteins within the ER. Three ER-anchored receptors: IRE1, PERK and ATF6 control UPR activation. In heathy cells, UPR signaling is transient, but cancer cells differ in that they permanently activate the UPR. Prolonged UPR activation, and in particular sustained IRE1 signaling, is linked to the progression of Triple Negative Breast Cancer (TNBC). For this reason, IRE1 inhibitors have emerged as novel TNBC therapeutics. To use IRE1 inhibitors effectively, it is important that we understand the consequences of IRE1 inhibition. In this study, we ask what is the impact of IRE1 inhibitors upon the wider UPR signaling network?

Methods: A panel of tumourigenic (MDAMB231, 4T1) and non-tumourigenic (MCF10a) breast cell lines were subjected to treatment with chemical inducers of ER stress in the presence/absence of IRE1 inhibitors. Inhibitor based strategies were complemented by using cell lines genetically engineered to lack expression of IRE1 or its downstream signaling mediator XBP1. The outcome of attenuated IRE1 signaling upon wider UPR network in ER stressed cells was assessed by immunoblotting and qPCR.

Results: IRE1 inhibitors, while blocking IRE1 signaling, also lowered protein expression and transcript levels of the key UPR mediator PERK in ER stressed cells. This was consistent across all cell lines and IRE1 inhibitors tested. IRE1 mediated regulation of PERK expression was also reproduced in IRE1-KO and XBP1-KO MDA-MB-231 cells treated with ER stressors demonstrating this to be a true effect of reduced IRE1 signaling and not an off target effect of IRE1 inhibitors.

Conclusion: Our results highlight an unreported amplification mechanism by which IRE1 increases PERK expression during ER stress. We propose IRE1 RNase inhibitors, in addition to reducing IRE1-dependent signaling, also reduce PERK signaling. For this reason, IRE1 inhibitors could represent an effective means by which to block multiple arms of the UPR in cancer cells.

The Role of Dihydrolipoyl Dehydrogenase (DLD) in the Immunopathogenesis of *L. major*

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Introduction: *Cutaneous leishmaniasis (CL)* caused by numerous *Leishmania* species including *Leishmania major*, leads to a range of diseases, from self-healing lesions to chronic disfiguring disease. Protection is achieved from IFN-β producing CD4+ T cell activation on *Leishmania* infected macrophages. Dihydrolipoyl dehydrogenase (DLD) is a critical mitochondrial enzyme in eukaryotic cells including *Leishmania* known to modulate metabolic activities. In different pathogenic organisms, DLD is a promising therapeutic target. The role and contribution of DLD in *L. major* immunopathogenesis is currently not known. We hypothesize that DLD is a virulence factor and its deficiency in *L. major* will result in an attenuated disease pathology and altered host immune response.

Methods: To generate DLD deficient *L. major*, an all-in-one plasmid (pLDCN) containing two short oligonucleotide sequences (guide RNA) complementary to the DLD gene in *L. major* is introduced such that upon expression, Cas9 will initiate cleavage. A homology-directed repair mechanism allowed for the introduction of a donor DNA (bleomycin) PCR product into the deleted site. I functionally validated DLD gene deletion in axenic culture, bone marrow-derived macrophages and in mice.

Results: Deficiency of DLD in *L. major* was confirmed by PCR and *in vivo* by the absence of DLD-specific CD4+ T cells from splenocytes of mice infected with DLD deficient parasites using DLD-specific tetramers. Growth kinetics in axenic culture and macrophages show that deficiency of DLD gene products results in reduced proliferation in comparison to wild-type (WT) parasites. Mice infected with DLD deficient parasites in the footpad had no observable lesion and significantly reduced parasite burden compared to WT-infected animals. The frequency of cytokine (IFN-β and TNF)-producing CD4+ T cells in spleens of mice infected with DLD deficient parasites was significantly lower than those from their WT counterparts. Cells from mice infected with DLD deficient parasites produced significantly reduced levels of these cytokines in the culture supernatant following *in vitro* restimulation with soluble *Leishmania Ag*.

Conclusion: These findings suggests that DLD in *L. major* is a critical metabolic enzyme for intracellular survival both in axenic culture and inside macrophages. Since DLD deficient parasites did not induce pathologies in mice but alters host immune response indicates that they could be good vaccine candidates.

Investigating the Effects of miRNA-High Tumour Cell Secretions on Cancer Stem Cell Reprogramming in Senescent Breast Cancer

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Introduction: Senescence is a form of cell cycle arrest and a mechanism of tumour suppression. Various factors trigger senescence, including oxidative stress (OS), DNA damage and mutations, and is regulated by tumour suppressors such as p53 and p21. A subpopulation of senescent cancer cells can exit the senescent state with cancer stem cell (CSC) properties. CSC have unlimited self-renewal capacity and are responsible for cancer relapse. We discovered two miRNAs, miR655 and miR526b, in aggressive breast cancer. Overexpression of these two miRNAs promotes CSC population in breast cancer and treatment with secretions from these cells promote angiogenesis, hypoxic response, and stemness in poorly metastatic cells. Both miRNAs target a tumour suppressor gene CPEB2, a protein that enhances p53 translation. We aim to determine if miR526b- and miR655-high tumour secretions reprogram senescent breast cancer cells to re-enter the proliferative state with enhanced CSC characteristics.

Methods: Microarray data were obtained from Gene Expression Omnibus and analyzed via GEO2R. OS was induced by various concentrations of H2O2. Senescence was determined by quantifying ß-galactosidase activity and p21 and p53 expression. Stemness was assessed via spheroid formation assay and the expression of NOTCH and WNT pathway markers.

Results: Microarray data show that p53 cell cycle regulation pathways are enhanced for OS exposed MCF7 cells. We show that miRNA-high breast cancer cells are less likely to undergo OS-induced senescence and show decreased p53 and p21 expression. We also show that secretions from these cells promote CSC properties of poorly metastatic cells, such as spheroid formation and upregulation of CSC gene markers, NOTCH-1/2 and WNT genes (c-MYC, CCDN1, AXIN2). Further experiments to determine the full objectives of this study are currently pending results, including mass-spec analysis on miRNA-high cell secretions.

Conclusion: It is possible that miRNA-high tumour secretions allow senescent breast cancer cells to re-enter the proliferative state with enhanced CSC characteristics. This project aims to discover new therapeutic targets to track and treat chemotherapy-resistant breast cancer.

Investigation of Novel Anti-Leukemic Compounds Uncovered Through High-Throughput Screening of Human Model Leukemias

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Acute myeloid leukemia (AML) is a genetically heterogeneous disease characterized by the loss of differentiation of the myeloid progenitors and their unregulated proliferation and accumulation in the bone marrow and peripheral blood. AML affects individuals of all ages; while adult AMLs are characterized by recurrent single nucleotide mutations, chromosomal translocations (especially those involving the lysine methyltransferase 2A (KMT2A) gene) are much more frequent in pediatric AML. Moreover, AML represents ~20% of pediatric leukemia but accounts for most of the disease-related mortality in children due to chemo-resistance or relapse. Given the genetic heterogeneity of pediatric AML and the impact of different alterations on patient survival rate and relapse, a single treatment strategy is not feasible for all AML subtypes, underlining the need for new, targeted, therapies. Through a high-throughput chemical screen of ~11k compounds library based on cell viability, we were able to compare the therapeutic effects of these compounds between AML samples from patients, human model leukemias, and cell lines. These data highlighted several specific compounds for AML and KMT2A fusions that could have therapeutic value. From a primary screen, we identified ~130 compounds affecting AML growth, but not that of CD34+ (control) cells. One of the hits identified on the screen is a compound used in traditional Chinese medicine with a reported anti-cancer activity that has been broadly studied in numerous types of cancer. To gain further insight into the biologic mechanisms of this compound in AML, we have conducted dose-response experiments and compared its anti-AML effect in cell lines, human models, and patients. Moreover, we have performed functional experiments to study the anti-AML effect of this compound against several metabolic enzymes. Taken together, our preliminary results suggest that this hit is a multitarget drug that might have more than two targets in AML. To extend our understanding of the genetic dependencies for drug sensitivity for this compound, and others highlighted on the screen, we will perform CRISPR-based chemogenomic screens. This work will allow us to define the mechanisms of action for novel compounds which could potentially be used as novel and personalized treatments in AML and AML subtypes.

Investigating the Role of the Unfolded Protein Response in Brain Regeneration Using a Larval Zebrafish Model

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Introduction: Traumatic brain injury (TBI) results in a permanent loss of neuronal tissue. A potential therapy for humans suffering from TBI could be stimulating endogenous neural stem cells (NSCs) to increase proliferation and differentiation into new neurons. However, effective methods of stimulating human NSCs *in vivo* remain unclear. Unlike human NSCs, the NSCs of the freshwater teleost, the zebrafish (Danio rerio), respond to TBI by rapidly increasing proliferation and neuronal differentiation. The zebrafish's ability to completely regenerate damaged neural tissue makes this species an excellent model to study NSC-mediated neurorepair. The unfolded protein response (UPR) is a vertebrate conserved stress response that is emerging as a key regulator of cellular activity after neurotrauma. UPR activation plays a role in the responses of neurons, oligodendrocytes, and astrocytes to mammalian central nervous system injury. But whether the UPR contributes to NSC regenerative activity post-TBI is unknown. The objective of this study is to investigate the role of the UPR in NSC-mediated regeneration post-TBI using a larval zebrafish model.

Methods: To characterize UPR activity post-TBI, transgenic reporter fish which fluoresce to mark NSC location and UPR activation will be used. Reporter fish will be injured at the larval stage, for their rapid neuroregenerative rate (4 days post-injury). To induce TBI, larvae will be given a mechanical injury in the dorsal forebrain at 3 days post-fertilization (dpf). Larvae will be sacrificed at 24-hour time points post-TBI from 4-7 dpf for immunohistochemistry (IHC), qPCR, or western blotting analysis.

Results: Preliminary results show that the UPR can be activated in larval brain tissue using the ER-stress inducing drug, dithiothreitol. A larval forebrain TBI model was designed, and injury coordinates confirmed by injuring wild-type larvae and performing IHC staining on the fixed tissue post-TBI. These preliminary experiments will allow me to next characterize UPR activation in NSCs post-TBI using IHC, qPCR, and WB assays.

Conclusion: Results from this study will lead to a better understanding of how UPR activation plays a role in brain regeneration.

Phosphorylation Stimulates Folding of the SLX4 SAP Domain and Enhances its Affinity for the Structure-Selective Endonuclease MUS81

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Introduction: The repair of damaged DNA is essential for cell survival. Most DNA repair pathways rely on DNA endonucleases to remove damaged DNA or branched DNA structures that arise as repair intermediates. Human cells use a tri-nuclease complex, called SMX, to remove various branched DNA structures that would otherwise compromise genome stability. SMX is a hexameric complex composed of three structure-selective endonucleases, namely: SLX1-SLX4, MUS81-EME1, and XPF-ERCC1. In early mitosis, SMX assembles in response to SLX4 phosphorylation by CDK1/Cyclin B, which promotes the recruitment of MUS81-EME1 to a sub-complex containing SLX1-SLX4 and XPF-ERCC1. However, constitutive assembly of SMX, during all phases of the cell-cycle, causes chromosome pulverization and fuels genome instability. In this study, we sought to investigate the underlying biochemical mechanisms that govern MUS81 binding to the SLX4 scaffold protein, and to determine the role of phosphorylation on MUS81-SLX4 assembly.

Results: Association between MUS81 and SLX4 is driven by binding of the MUS81 N-terminal helix-hairpin-helix (MUS81-NHhH) domain to the MUS81-binding region on SLX4 (SLX4-MBR), where SLX4-MBR contains a putative SAP domain. Using circular dichroism and nuclear magnetic resonance (NMR) spectroscopy, we observed that recombinantly purified SLX4-MBR adopts a disordered conformational ensemble. However, *in vitro* phosphorylation of SLX4-MBR (or pSLX4-MBR) using CDK1/Cyclin B triggered folding of the SAP domain into a canonical fold of two alpha helices separated by a turn. Using isothermal titration calorimetry, I determined that the binding affinity of pSLX4-MBR to MUS81-NHhH was 100-fold higher (Kd ~ 10 nM) compared to its non-phosphorylated counterpart SLX4-MBR (Kd ~ 1 μM). Using enzymatic nuclease assays, we also observed that pSLX4MBR effectively stimulates MUS81-EME1 catalytic activity and relaxes its substrate specificity.

Conclusion: The SMX complex plays an important role in safeguarding the genome. This work furthers our understanding of the role of phosphorylation in controlling the cell-cycle dependent assembly of SMX and how the MUS81-SLX4 interaction influences substrate specificity. In addition, reports to-date on post-translational modifications and their influence on protein folding have been sparse. With the advent of AlphaFold in protein structure prediction, our results may contribute toward predicting the impact of phosphorylation on protein folding and protein-protein interactions.

The Potential of Oxytocin in Modulating Female Genital Tract Epithelium to Prevent HIV Transmission

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Introduction: Oxytocin, a neuropeptide released during sexual activity and physical touch, was shown to have anti-inflammatory effects on gut and skin epithelium. Human immunodeficiency virus (HIV) takes advantage of inflammation at the female genital tract (FGT) to facilitate its replication and transmission. This study examines if oxytocin could reduce immune activation, improve epithelial wound healing, and provent productive HIV infection at the FGT.
Methods: Vaginal (Vk2), ectocervical (Ect1), and endocervical (End1) cell lines were grown in monolayers. RNA isolated from these cell lines, before and after stimulation with 1 µg poly(I:C)/LyoVec in presence of 10, 100, 1000, or 10 000 pg/mL oxytocin, was evaluated for the effect of oxytocin on inflammatory gene expression using RT-qPCR. Oxytocin's effects on wound healing were assessed using scratch assays with monolayers pre-treated with 10 000 pg/mL oxytocin. Triple-knockout mice, transplanted with stem cells from human bone marrow and fetal liver, and thymus (TKO-hBLT), were used as an in vivo model to assess whether intravaginal administration of oxytocin (1.6 IU/kg) directly impacted immune activation of peripheral blood mononuclear cells (PBMCs) by measuring HLA-DR, CD38, CD69, and CCR5 expression via flow cytometry.
Results: Oxytocin (10 000 pg/mL) treatment reduced the expression of both IL-6 and IL-1β in End1 cells by 50% ($p<0.05$; n=6). This was reversed by poly(I:C)/LyoVec stimulation. Scratch assays showed no effect of oxytocin on wound closure. Data from TKO-hBLT mice showed that intravaginal oxytocin had no effect on the expression of PBMC activation markers (n=4).
Discussion: Oxytocin reduced the expression of inflammatory genes in End1 cells, which implies its ability to modulate immune activation at the vulnerable endocervical site. Although oxytocin had no direct effect on FGT epithelial wound healing, its indirect role in epithelial barrier properties should be investigated. Further investigation of oxytocin's role in regulating immune activation at FGT mucosa is needed.

Reproductive Injustice in Canadian Prisons for Women

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Introduction: Women are the fastest growing population in prison in Canada, with significant consequences for reproductive health. Almost 50% of incarcerated women are Indigenous; the prison system threatens public commitments to working towards reconciliation. Pregnancy while incarcerated is associated with inadequate prenatal care, prematurity, and low birth weight; incarceration also disrupts fertility and impedes parenting. These harms are disproportionately borne by Indigenous people. Over twenty years ago, Correctional Services Canada launched the institutional Mother Child Program to mitigate harms of separating mothers from babies. The program has never been subjected to internal evaluation or independent study. The purpose of this study is to examine pregnant people and new parents' experiences of the federal mother child program.
Methods: This study uses a qualitative case study design and thematic analysis. It is informed by reproductive justice theory. Multiple data sources include quantitative Mother Child Program participation data; public policy documents; and semi-structured interviews with nine mothers who served federal sentences in pregnancy and early postpartum, and 14 advocacy staff of Elizabeth Fry Societies.
Results: Most lived experience respondents could not participate in the Mother Child Program due to eligibility criteria, or they chose alternatives to protect their children. Restricted access is disproportionately borne by Indigenous mothers. Respondents experienced debilitating trauma, denial of health services and punitive responses to poor mental health. They were subject to surveillance and deprecation of their parenting. Through personal agency and peer solidarity, they resisted institutional restrictions on access to care and to their children.
Conclusion: Far from a panacea for mother-baby connection, this study demonstrates the Mother Child Program to be problematic. It is inequitably available, qualifying is uncertain; continuous participation is precarious; and prisons are perceived as unsafe for babies. Further, addressing the disproportionate incarceration of Indigenous women is imperative for reconciliation and health equity.

Can Substance use Disorder Influence the Practice of Physical Activity?

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Introduction: Substance use disorder (SUD) can have many psychological and physical impacts on a patient. Physical activity (PA) is known to have effects on mental health for the general population, but little is known about people with SUD and its effect on PA behavior.
Objective: To compare the level of PA and sedentary time between individuals with symptoms of SUD and those with other mental health disorders (MHD).
Methods: Cross-sectional study using SIMPAQ (simple physical activity questionnaire) data from 23 countries was used. 946 participants with MHD were included in two separate groups (G1: SUD; G2: MHD). The groups were compared using a questionnaire on their PA level, different PA domains, and sedentary time.
Results: SUD (N = 423; 36% women; 39.2 ± 12.2 years) and MHD (N = 523; 51% women; 39.9 ± 13.1 years). The population was mainly from Australia (14.8%), Swiss (15.1%) and Italy (12.1%). The results show that SUD is significantly more active (2.51 ± 1.89, vs. MHD: 2.23 ± 1.83 h/week; p=0.03) for total PA. A difference was also found on moderate to vigorous PA (SUD: 1.36 ± 1.20; vs. MHD: 1.11 ± 0.97 h/week, p=0.004) and walking (SUD: 1.46 ± 1.32 h/day; MHD: 1.10 ± 1.14 h/day, p<0.0001). On sedentary time, no difference was found (SUD: 8.26 ± 3.55; vs MHD: 7.82 ± 3.57 h/day, p=0.062).
Conclusion: SUD seems to be associated with a different level of PA compared to MHD. On the other hand, the presence of symptoms is associated with the same sedentary time.

Differences in Observed Microbiome Profiles Using Metagenomic and Metatranscriptomic Approaches

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Introduction: Metatranscriptomics is an emerging technique for studying microbial communities, including the human gut microbiome. When combined with whole-genome analyses, metatranscriptomics can provide additional insights into the functional activity and metabolic environment of the microbiome. Thus, investigation of functional activity in dysbiotic environments may help to elucidate the etiologies of chronic diseases and to inform potential treatments. Although metatranscriptomics provides valuable complementary information to metagenomics, the methodologies used to collect, store, prepare, sequence, and analyze metatranscriptomic samples are much less developed relative to metagenomic samples. The objectives of this research are to develop a method for fecal sample collection and processing that will allow for accurate metatranscriptomic analysis of microbiome functional activity.
Methods: Briefly, healthy fecal samples are used to test the effect of different storage and extraction conditions on metagenomic and metatranscriptomic profiles. Stool was first collected in a controlled environment simulating home collection protocols used in cohort studies. Prior to freezing and storage, the fresh stool was aliquoted into one of three preservation conditions. Microbial DNA and RNA from the samples was extracted using several commercially available kits, and the comparative effect of both the preservant and the extraction kit on the metagenomic and metatranscriptomic profile of the sample was observed from sequence data. Shotgun sequencing of nucleic acid libraries was performed using Illumina NextSeq short read sequencing technology, producing high coverage sequence data containing millions of reads. Sequence data was filtered, quality assessed, and analyzed using bioinformatic tools MetaPhlAn and HUMAnN for taxonomic and functional profiling, respectively. Measured community profile outcomes include alpha and beta diversity, and differential abundance of microbial transcripts.
Results: Metagenomic analysis revealed significantly altered taxonomic profiles depending on the combination of nucleic acid preservant and extraction technique used. Major differences in the Bacteroidetes:Firmicutes ratio and overall community diversity were observed between experimental techniques. Metatranscriptomic analysis is pending.

Conclusion: Observed microbiome profiles, including taxonomic diversity and other measures of microbiome health, are significantly impacted by the methods used to collect, store, and prepare stool samples. Therefore, future microbiome research must consider the limitations of certain methods for capturing particular outcomes of health and disease.

The Potential of Oxytocin in Modulating Female Genital Tract Epithelium to Prevent HIV Transmission

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Introduction: Oxytocin, a neuropeptide released during sexual activity and physical touch, was shown to have anti-inflammatory effects on gut and skin epithelium. Human immunodeficiency virus (HIV) takes advantage of inflammation at the female genital tract (FGT) to facilitate its replication and transmission. This study examines if oxytocin could reduce immune activation, improve epithelial wound healing, and provent productive HIV infection at the FGT.
Methods: Vaginal (Vk2), ectocervical (Ect1), and endocervical (End1) cell lines were grown in monolayers. RNA isolated from these cell lines, before and after stimulation with 1 µg poly(I:C)/LyoVec in presence of 10, 100, 1000, or 10 000 pg/mL oxytocin, was evaluated for the effect of oxytocin on inflammatory gene expression using RT-qPCR. Oxytocin's effects on wound healing were assessed using scratch assays with monolayers pre-treated with 10 000 pg/mL oxytocin. Triple-knockout mice, transplanted with stem cells from human bone marrow and fetal liver, and thymus (TKO-hBLT), were used as an in vivo model to assess whether intravaginal administration of oxytocin (1.6 IU/kg) directly impacted immune activation of peripheral blood mononuclear cells (PBMCs) by measuring HLA-DR, CD38, CD69, and CCR5 expression via flow cytometry.
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Discussion: Oxytocin reduced the expression of inflammatory genes in End1 cells, which implies its ability to modulate immune activation at the vulnerable endocervical site. Although oxytocin had no direct effect on FGT epithelial wound healing, its indirect role in epithelial barrier properties should be investigated. Further investigation of oxytocin's role in regulating immune activation at FGT mucosa is needed.

Association of Changes in Gait Biomechanics with Clinically Important Improvements in Pain and Subsequent Total Knee Replacement after High Tibial Osteotomy

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Introduction: High tibial osteotomy (HTO) is a limb realignment surgery for patients with knee osteoarthritis (OA) and varus alignment. This study aimed to evaluate cumulative incidence of total knee replacement (TKR) after HTO, proportion of patients with minimal clinically important improvements (MCII) in pain at 10 years, and the association of changes in gait biomechanics with TKR and MCII in pain at 10 years.

Methods: A patient cohort (n=610) who underwent HTO completed 3D gait analysis <3 months pre-surgery and 12 months post. We calculated the knee adduction moment impulse (KAI) and peak knee flexion moment (KFM) through inverse dynamics. TKRs were identified by electronic medical records. Patients completed the Knee injury and Osteoarthritis Outcome Score (KOOS) for pain <3 months pre-surgery and at 10 years. We evaluated cumulative incidence of TKR at 5 and 10 years and the proportion (%) achieving MCII in KOOS pain (10+ points) at 10 years. We investigated the association of surgery-induced changes in gait biomechanics (KAI and KFM) with TKR using a Cox model, and with achieving MCII at 10 years using logistic regression. We adjusted for surgery-induced changes in alignment and gait speed, and baseline radiographic disease, age, sex, and body mass index. Model results are presented as hazards (HR) or odds (OR) ratios [95% confidence intervals].

Results: We identified 98 (16%) TKRs. Cumulative incidence was 5% (3-7%) and 20% (16-25%) at 5 and 10 years. KAI (HR=0.6 [0.4-0.9]) and KFM were associated with TKR (HR=1.2 [1.1-1.3]) while controlling for covariates. Patients with larger reductions in KAI after HTO were at reduced risk of TKR, while larger reductions in KFM increased risk. 54% of patients improved in pain by the MCII. Larger reduction in KAI (OR=8.41 [2.4- 30.1]) was associated with increased odds of a MCII in pain at 10 years, while KFM was not.

Conclusion: Few patients who underwent HTO had a TKR at 5 and 10 years, suggesting HTO may help in secondary prevention. Over half of patients achieved important improvements in pain at 10 years. Changes in knee loading are associated with improvements in pain and risk of TKR after HTO.

A Machine Learning Model Trained on a High-Throughput Antibacterial Screen Increases the Hit Rate of Drug Discovery

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The emergence of multidrug-resistant bacterial infections is one of the major public health threats of modern times. This rise of antibiotic resistance crisis can only be surmounted by discovering new antibiotics, especially new bioactive compound classes with novel mechanisms of action. However, the search for novel antibacterial compounds requires testing millions of natural and synthetic compounds, which is a time-consuming and costly venture. To address this, we aimed to capitalize the ability of machine learning (ML) for predicting molecular properties to identify novel structural classes of antibiotics. We applied a ML-based virtual screening for antibacterial activity and evaluated its predictive power by experimental validation. We first binarized thirty-thousand compounds according to their growth inhibitory activity (hit rate 0.67%) against *Burkholderia cenocepacia*, a member of the *Burkholderia cepacia* complex (Bcc) that are notorious for causing serious infections in the people with cystic fibrosis (CF). We then described their molecular features with a directed-message passing neural network (D-MPNN). We used this dataset to train an ML model that achieved a receiver operating characteristic (ROC) score of 0.823 on the test set. Finally, we predicted antibacterial activity in virtual libraries corresponding to 1,614 compounds from the Food and Drug Administration (FDA)-approved list and 224,205 natural products. A hit rate of 26% and 12%, respectively, was obtained when we tested the top ranked compounds for growth inhibitory activity against *B. cenocepacia*, which represents at least a 12-fold increase from the previous hit rate. More than 51% of the predicted antibacterial natural compounds also inhibited ESKAPE pathogens, a group of bacteria that are considered dangerous due to their high level of multidrug resistance mechanisms. Overall, the developed ML approach can be used for compound prioritization prior to screening, increasing the typical hit rate of high throughput screens.

Molecular Characterization of PIK3CA Oncogene and Rationale for Targeted Drug Therapy

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Cancer is one of the most leading causes of death in the world. It is estimated that about 10 million people died of Cancer in 2018 (according to NIH). A comprehensive study performed with more than 9000 cancer samples spanning different cancer types, showed that PIK3CA, the gene encoding for the enzyme Phosphatidyl-Inositol 3-Kinase α is one of the most frequently mutated and altered pathways in human cancers. The enzyme is activated upon external stimuli like insulin which leads to the recruitment of the protein to the plasma membrane where it generates the lipid signalling molecule PIP3. PIP3 recruits and activates effector proteins that then perform a variety of important cellular tasks such as proliferation, cell growth, motility, and immune response. Due to its involvement in such important processes, the activity of PI3Kα is tightly regulated by the different upstream activation signals. However, the presence of gain of function mutations mimic activation signals and leads to hyperactivity. The goal of our research is to understand, how all oncogenic mutations in PIK3CA operate leading to a hyperactive enzyme. To this end, I have used cutting-edge technologies that includes Hydrogen-Deuterium Exchange Mass Spectrometry (HDX-MS) and other biochemical characterizations. Our extensive biophysical and biochemical analysis provides an overarching theory that provides the molecular basis of how all cancer mutations promote hyperactivity. This information is necessary for development of mutant isoform specific inhibitors that can help circumvent the ontarget toxicities related with inhibition of PI3Kα.

Chloride Intracellular Channel Protein 1 (CLIC1) is a Critical Host Cellular Factor for IAV Replication

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Influenza A Virus (IAV), an intracellular parasite, depends on the cellular molecules to complete the replication cycle in host cells. Chloride intracellular channel protein 1 (CLIC1) is ubiquitous in almost all human cells. IAV causes higher expression of CLIC1 in human lung epithelial cells, but the role of this protein in IAV replication cycle is poorly understood. In this study, CLIC1 knockdown (KD) in human lung epithelial (A549) cells resulted in a significant decrease in progeny IAV in the supernatant, but virus protein expression was unaffected. On the other hand, in CLIC1 KD cells significantly higher amount of viral RNAs were accumulated after infection. Treatment with chloride channel inhibitor also caused a significant reduction of IAV replication. This suggests that CLIC1 is an important host factor in the IAV replication cycle. SomaScan (targeted 1322 proteins) was used to identify proteins dysregulated in wild-type cells, CLIC1 KD cells, after IAV infection and analyzed by IPA (Ingenuity Pathway Analysis) software. We discovered that after IAV infection, the expression of 116 and 150 proteins was significantly altered in wild-type and CLIC1 KD cells, respectively. However, the expression of 29 proteins was altered significantly only in wild-type cells and 63 only in CLIC1 KD cells, but not the other way around. In conclusion, this study suggests that CLIC1 is involved in the maturation step of IAV replication, but further research is needed to fully comprehend the mechanism.

Sex-Specific Associations among Infant Food and Atopic Sensitizations and Infant Neurodevelopment

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Introduction: Food sensitization is a first and strong indicator of immune deviation in the progression to other allergic conditions. Whereas sensitization to food or other allergens, and related inflammation, during critical windows of infant development may adversely affect progress towards neurodevelopmental milestones, this association has not been tested.
Methods: The study examined associations between atopic (any food or aeroallergen) or food sensitization (specific to egg, soybean, peanut, and milk) at age 1 year and neurodevelopment up to 2 years of age in the national CHILD Cohort Study, with a secondary aim exploring whether these associations were sex-specific. Food and atopic sensitization were assessed by skin prick tests in one-year old infants, with neurodevelopment assessed using the cognitive, language, motor, and social-emotional subscales of the Bayley Scales of Infant Development (BSID-III) administered at 1 and 2 years of age.
Results: Atopic sensitization was present among 16.4% of infants, while 13.4% had food sensitizations. Among the neurodevelopmental scores, only socioemotional scores reached statistical significance. Both atopic and food sensitization at 1 year of age were associated with lower social-emotional scores at that age, independent of the infant's ethnicity. These findings were observed among boys, among whom social-emotional scores were lowered by 5 points if atopic sensitization was present (-5.22 [95%CI: -9.96, -0.47], p=0.03) or if food sensitization was present (-4.85 [95%CI: -9.82, 0.11], p=0.06).
Conclusion: Atopic and food sensitization at 1 year did not predict neurodevelopmental outcomes at 2 years of age. However, we found an inverse, cross-sectional association between atopic and food sensitization status, and social-emotional development scores in males but not females. Further research is required to investigate the bidirectional associations that may explain this inverse association.

DUSP4 Deletion Enhances Macrophage Infiltration, Tubular Fibrosis and Progression of Chronic Kidney Disease

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Background: Chronic kidney disease (CKD) affects approximately 14% of the world population as well as 45% of patients with diabetes, which may lead to end-stage renal disease that requires dialysis or transplantation for those affected. Decreased renal function, measured by albuminuria and reduced glomerular filtration rate (eGFR), is widely associated with increased tubular fibrosis (TF). Myofibroblasts are the main source of TF, which can be derived from different cell types including tubules and macrophages. We have recently published that DUSP4 expression, a protein phosphatase known to inhibit MAPK, is reduced in the kidney of diabetic mice and patients. Interestingly, only diabetic mice with DUSP4 deletion presented with TF, a hallmark of CKD. Thus, we proceeded to evaluate the role of DUSP4 in the progression of TF and CKD.
Methods: Mice with (Dusp4-/-) or without (Dusp4+/+) systemic deletion of DUSP4 were administered an adenine-rich diet for 4 weeks or subjected to a unilateral ureter obstruction surgery, two well-known mouse fibrosis models. Immortalized murine proximal tubules (TKPTS) were infected with adenoviral vector of the native form of DUSP4 followed by TGF-beta (10 ng/mL) treatment.
Results: Dusp4+/+ mice administered the adenine diet presented with significantly elevated urinary albumin excretion, which was worsened in Dusp4-/- mice. The adenine diet and UUO surgery led to TF, macrophage infiltration and increased TGF-beta expression in Dusp4+/+ mice, characteristics that were exacerbated in Dusp4-/- mice. Co-localization of macrophage and myofibroblast markers suggest that macrophages contribute to TF. Furthermore, TKPTS exposed to TGF-beta underwent phenotypic changes with increased presence of stress fibers within the cell, characteristic of differentiation into myofibroblasts. These changes were associated with increased TGF-beta signaling pathway activation (p-Smad2/3, Smad4, p-JNK and p-ERK) as well as tubular epithelial-to-mesenchymal transition markers alpha-SMA and Snail. Interestingly, DUSP4 overexpression decreased TGF-beta-induced phenotypic changes, stress fiber production and reduced JNK phosphorylation, Smad4 protein expression and attenuated EMT markers alpha-SMA and Snail protein expressions.
Conclusion: DUSP4 seems to play a role in attenuating TGF-beta-induced tubular EMT and the loss of DUSP4 expression enhances macrophage infiltration contributing to tubular fibrosis and aiding in the faster progression of CKD characteristics.

Utility of a Saponin-Based Host Depletion Method for Metagenomics-Based Pathogen Detection in Foods

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Introduction: A hybrid approach involving direct sequencing (metagenomics) of primary selective enrichment cultures may expedite pathogen detection in foods and improve public health response. However, several obstacles must be overcome before such an approach can be implemented in the practical setting, including the high host:pathogen ratio, which can impede the detection of low contamination levels. A saponin-based host DNA depletion method has proven effective for removing up to 99.9% host DNA in clinical specimens but has not been evaluated in foods. In this pilot study, we aimed to assess the impact of a saponin-based host DNA depletion method on pathogen detection in foods.

Methods: Pathogens and foods known to be implicated in outbreaks: *Shigella sonnei* (carrots), *Escherichia coli* O157:H7 (lettuce, ground beef), and *Listeria monocytogenes* (ready-to-eat; RTE ham) were artificially contaminated with outbreak and non-outbreak strains (*S. flexneri*, *E. coli* O26:H11, *E. coli* O103:H25, and *L. innocua*) at three levels categorized as high (103 CFU/mL), medium (102 CFU/mL) and low (101 CFU/mL). Genomic DNA was extracted from overnight-enriched cultures and sequenced on the Illumina NextSeq. Low-quality reads were filtered using fastp, followed by host removal with bowtie2. Taxonomic classification was performed using Kraken 2 and a custom-built reference database. Pathogen reads were extracted and investigated using ECTyper (*E. coli* and Shigella) or RefSeq Masher (Listeria spp.) for strain confirmation.

Results: Host depletion in RTE ham samples decreased host reads by a maximum of 77% (min=2%), and increased pathogen reads at the genus and species level by a minimum of 21-fold (max=321-fold) and 18-fold (max=430-fold), respectively. Saponin-based host depletion led to improved detection of L. *monocytogenes* and the non-outbreak strain *L. innocua* at all spike-in levels compared to unprocessed samples. Strain confirmation was achieved using RefSeq Masher for all host depleted samples except *L. innocua* at 101 CFU/mL. Host DNA depletion did not improve the detection of *S. sonnei*, *E. coli* O157:H7, and corresponding non-outbreak strains. All results were concordant with ECTyper.

Conclusions: Saponin-based host DNA depletion can improve pathogen detection in foods with high host content (e.g. RTE ham) but may not be suitable for all pathogen-food combinations and requires further validation.

Diminished USP4 Expression Induces Chromosome Instability that May Drive Colorectal Cancer Development

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Introduction: Despite efforts to improve colorectal cancer (CRC) screening, approximately 50% of CRCs are diagnosed at late stages (III or IV). Accordingly, acquiring a deeper understanding of the etiological events driving CRC development may reveal novel biomarkers to aid in early detection. Chromosome instability (CIN), or ongoing changes in chromosomal complements, is a driver of CRC development, yet the causal genes (i.e., CIN genes) remain largely unknown. Emerging data implicate aberrant ubiquitin regulation as a driver of CIN. Thus, this study seeks to comprehensively assess all ubiquitylation and deubiquitylation genes for their roles in maintaining chromosome stability.

Methods: To investigate the impact reduced expression of ubiquitylation (582) and deubiquitylation (94) genes have on CIN, an siRNA-based screen was coupled with quantitative imaging microscopy to evaluate CIN phenotypes, including nuclear areas and micronucleus formation in two karyotypically stable colonic cell lines (HCT116; 1CT). Subsequent direct tests were conducted to validate 10 putative CIN genes identified from the screen in two non-malignant colonic epithelial cell lines (1CT and A1309). These genes were prioritized based on the magnitude and conservation of phenotypes across experimental conditions. Finally, to evaluate the temporal dynamics of CIN, USP4-knockout clones were generated using CRISPR/Cas9 approaches, validated with DNA sequencing and utilized to assess CIN phenotypes and enumerate chromosome over a 3-month timeframe.

Results: The siRNA-based screen identified 269 (215 ubiquitylation and 54 deubiquitylation) genes in ≥3 experimental conditions. Data from The Cancer Genome Atlas revealed 42 of these genes exhibit copy number losses in ≥10% of CRC cases that also correlate with poor patient survival. Six ubiquitylation (DZIP3, FBXL19, FBXOL19, FBXW11, SKP2, SOCS4) and four deubiquitylation (MYSM1, USP4, USP33, USP53) genes were prioritized for further investigation. Transient approaches validated USP4 as a novel CIN gene in colonic epithelial contexts, while heterozygous (USP4+/--1 and -2) and homozygous (USP4-/--A and -B) knockout clones exhibited dynamic changes in nuclear areas, micronucleus formation and chromosome complements over time (i.e., CIN).

Conclusion: Collectively, this work provides novel insight into the impact aberrant ubiquitin regulation has on CIN and identifies USP4 as a novel CIN gene with pathogenic implications for CRC.

Serum Estradiol Levels and Cardiovascular Risk Associated with Gender-Affirming Hormone use in Transgender Women: A Systematic Review and Meta-Analysis

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Introduction: Estrogen in the form of gender-affirming hormone therapy (GAHT) is associated with higher cardiovascular (CV) risk in transgender women (male at birth with female gender identity). However, it is not known whether these risks are associated with serum estradiol concentration. Our objective was to determine the association between serum estradiol levels and CV-related mortality, adverse CV events and surrogate markers of CV risk in transgender women.

Methods: Three databases (MEDLINE, EMBASE, Web of Science) were searched from inception until present day for studies reporting on 1) transgender women and gender-diverse individuals; 2) estrogen GAHT independently or in conjunction with other GAHT; 3) reported serum estradiol levels; 4) reported CV outcomes (mortality, events, surrogate measures) and 5) are were original studies (control trial, cohort or cross-sectional study designs).

Results: Of the 7,213 articles identified, 22 articles (14 cohort, 11 cross-sectional, 1 randomized controlled trial) ranging from 1999 to 2021 representing 4,040 transgender women aged 16 to 59 years old using oral, sublingual, transdermal, intramuscular or unclear routes of GAHT prescriptions were included. The majority (64%) were Caucasian, with high rates of depression and anxiety (18%), and smoking (18%) reported. No articles reported on CV-related mortality and only one reported on adverse events. Articles reported on blood pressure, lipid levels, body mass index, insulin resistance, fasting glucose and insulin levels ranging from 0 to 60 months post-GAHT commencement. Serum estradiol levels varied widely with prescription type, dose, route of administration and gender-identity goals. Meta-analyses and assessment of heterogeneity of surrogate markers of CV risk stratified by serum estradiol quintiles are currently underway.

Conclusions: The majority of presented articles represent fair to poor-quality data. Through quantifying the association between serum estradiol levels and markers of CV risk, these results will inform the use of estrogen GAHT in transgender women and gender-diverse individuals.

Development of Novel Biomarker Drugs Targeting SAT1 for Detection and Therapeutic Monitoring of Glioblastoma

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Introduction: Glioblastoma multiforme (GB) is the most common adult primary brain tumor with poor prognosis due to delayed detection, restricted treatment options and high rate of tumor relapse even after surgical resection, radiation, and chemotherapy. Previous studies in lung and breast cancer have demonstrated that spermidine/spermine acetyltransferase 1 (SAT1) activity is increased and that formation of the acetylated metabolite of amantadine, a Health Canada approved drug, can be used as a biomarker drug for SAT1 activity and cancer detection. As evidence suggests SAT1 is important in GB and expression is increased in brain tumor compared to normal human brain, we examined the extent to which amantadine and related compounds could be used as biomarkers for monitoring GB progression.

Methods: For *in vitro* investigation we used the U251 human GB cell line. The acetylation of Amantadine or Rimantadine (100uM) in U251 was examined under normal culture conditions and following induction with N(1),N(11)-diethylnorspermine (DENSPM) (10uM) using LC/MS detection methods. Expression of SAT1 at the mRNA and protein level were also determined. Additional metabolism studies were performed using recombinant human acetyl transferase enzymes to determine specificity and kinetic properties related to acetylation of amantadine and rimantadine. In addition, the permeability of both amantadine, rimantadine and the acetylated metabolites were examined in brain micro vessel endothelial cells using a microfluidic culture model.

Result: Acetylation of Amantadine and Rimantadine was detected in media samples from U251 cells. The acetylated product was detected within 15-minutes of drug exposure and reached a maximum within one hour. The formation of Ac- Rimantadine was greater than that observed with Ac-Amantadine. The levels of both acetylated metabolites were increased following SAT1 induction. Studies with recombinant SAT1 and n-acetyl transferases (NATs) suggested the acetylation occurred through an SAT1 pathway. Permeability of both the drug and acetylated metabolites was observed in the microfluidic culture model suggesting transit of drug and metabolite occurs across the blood-brain barrier.

Conclusion: Findings suggests that Amantadine can be used for GBM monitoring however, Rimantadine may have better potential as a brain tumor biomarker. Further *in vivo* studies in mouse brain tumor models are warranted.

Evaluation of 3CL-Main Proteases from Variants of SARS-CoV-2 and their Inhibition by Salvia Miltiorrhiza-Derived Tanshinones

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Objectives: SARS-CoV-2 produces 16 non-structural proteins (NSPs) in form of a polyprotein which underwent numerous mutations in the known viral variants. From these, homo-dimeric 3CL-Mpro cleaves at 11 sites and produces 11 NSPs. Because of its essential importance in the viral life-cycle, studying of 3CL-Mpro from various variants of SARS-CoV-2 can be beneficial to understand their pathogenicity. To date, three dominant 3CL-Mpro mutations in the beta, lambda and omicron variants are known. The understanding of their role in viral pathogenicity will improve our understanding of variant evolution and will better define them as antiviral targets for the development of low molecular weight active site- or dimerization site-directed inhibitors.

Methods: Recombinant SARS-COV-2 3CL-Mpro variants were expressed in *E. coli* and purified using two chromatography steps and were characterized using fluorescence-based substrate and protein degradation assays. Thirty-one Salvia miltiorrhiza-derived tanshinones were evaluated for their inhibitory activity and IC50 values were determined for the most potent compounds. Molecular docking was used to predict potential binding sites.

Results: Variants of 3CL-Mpro of SARS-CoV-2 were characterized and its kinetic parameters were determined. Variants revealed altered activities indicating an altered efficacy in replicon processing and thus likely affecting the pathogenicity of the parent viral variants. Out of 31 screened medicinal plant-derived tanshinones, two have shown promising IC50 values in the low micromolar range. Molecular docking predicted distal binding from the active site for these inhibitors in the vicinty of the dimerization site.

Conclusions: Characterizing mutations present in 3CL-Mpro variants may shed light on the pathogenicity of SARS-CoV-2 variants. Moreover, tanshinones have the potential to inhibit 3CLMpro of SARS-CoV-2 and to act as anti-virals. They may provide a platform for future medicinal chemistry efforts for potent and variant -specific inhibitors.

Development of Vesicular Stomatitis Virus Expressing Lujo Virus Transmembrane Glycoprotein

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Introduction: Lujo virus was first reported in Zambia and South Africa in 2008, with mortality of 80% after four out of the five cases died. Lujo virus is an arenavirus of the genus; mammarenavirus. Arenaviridae family possess ambisense genome made up of bi-segmented RNA, described as large and small (3.4kb). Being a novel virus, there are less than 25 published studies up to date. Symptoms of Lujo virus include fever, respiratory distress, thrombocytopenia, diarrhoea, impaired renal function, facial edema and elevated liver enzymes. Mammalian reservoir remains unknown. There is currently no approved vaccine or therapeutics for Lujo hemorrhagic fever disease.

Hypothesis: Vesicular Stomatitis Virus expressing Lujo virus transmembrane glycoprotein will mount a strong immune protection against Lujo virus infection in guinea pigs.

Design: Lujo Glycoprotein was PCR amplified while VSV vector was linearized with restriction endonucleases. Insert and vector were ligated using DNA ligase. Ligation product was transformed in *E. coli*, plated, and screened for clones by PCR colony, genome sequencing, and western blot for characterization of protein expression. Rescue of recombinant virus will be done by co-transfecting BHK cells with plasmid expressing Lujo glycoprotein and support plasmids encoding VSV nucleoprotein, phosphoprotein and RNA polymerase. Blind passage will be done in Vero E6 cells at 48 hours post -transfection. Cells will be monitored for syncytia formation and or green fluorescent protein. Virus titer will be determined by plaque assay. Western blot will be used to determine Lujo glycoprotein expression. The second objective will be to test immunogenicity of recombinant virus in mice. Finally, protective efficacy of recombinant virus will be tested in Guinea pigs in BSL 4. Samples will be collected from Guinea pigs followed by euthanasia to measure viral titers.

Endotoxin Stimulates Glucocorticoid Production in the Neonatal Mouse Brain

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Introduction: Glucocorticoids (GCs) are steroid "stress" hormones and critical modulators of the nervous system. The brain receives GCs from the adrenal glands via blood. The brain also produces GCs locally (i.e., neurosteroids). The importance of neurosteroids is especially prominent during early life. During post-natal day 2-12, mice exhibit a stress hyporesponsive period (SHRP). There is an analogous period in humans from approximately 6 months to 6 years old. During the SHRP, the adrenals are quiescent and respond minimally to stressors, yet baseline brain GC levels are upregulated from blood. However, it is not known how stressors in early life affect GC levels in specific brain regions.

Methods: We assessed blood and brain corticosterone (main active GC in mice) levels in neonatal (post-natal day 5) C57BL/6J mice 4 hours after administration of lipopolysaccharide (LPS, 50µg/kg) (endotoxin, immunological stressor) or vehicle control (n=12/group, sexes balanced). We examined blood and microdissected brain regions (prefrontal cortex, hippocampus, hypothalamus, amygdala) using Palkovits punch technique. Using a withinsubject design, corticosterone was measured via liquid chromatography-tandem mass spectrometry (LC-MS/MS) and transcripts of key steroidogenic enzymes (Cyp11b1, Hsd11b1, Hsd11b2) were measured via RT-qPCR.

Results: Corticosterone levels increased in the blood and all brain regions in response to LPS. At baseline, corticosterone levels were ~2-fold greater in the amygdala, hippocampus, and hypothalamus and the same in the prefrontal cortex, compared to blood. After LPS, corticosterone levels were ~2-fold greater in the amygdala, the same in the hippocampus and hypothalamus, and ~2-fold lower in the prefrontal cortex, compared to blood. Transcripts of key steroidogenic enzymes were present in all brain regions examined. Hsd11b2 (transcript of enzyme that inactivates corticosterone) levels were significantly increased after LPS in the prefrontal cortex, consistent with the significant local downregulation of corticosterone compared to blood.

Conclusions: These results suggest that specific brain regions actively modulate local GC levels independently of each other, rather than simply being passive recipients of blood corticosterone. Further, these data suggest that LPS rapidly stimulates local corticosterone production in the neonatal mouse brain. Local GC production may provide a mechanism by which bacterial infections can exert region-specific effects on brain development.

Taming the Cytokine Storm by Modulating the NF-κβ Butterfly Effect: Repurposing Montelukast for the attenuation and prophylaxis of COVID-19 symptoms

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The two main complications of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infections are the development of acute respiratory distress syndrome (ARDS), as well as the development of complications from a hyperinflammatory cytokine profile (a cytokine storm). NF-κβ (nuclear factor kappa-light-chain-enhancer of activated B cells) is a protein complex involved in cytokine production and inflammation, with attenuation of NF-κβ associated with a reduction in cytokine production. Montelukast, an FDA and Health Canada approved asthma drug, has been shown to inhibit the signalling of NF-κβ, and other proinflammatory mediators, such as interleukin- 6,8,10, TNF-α, MCP-1. Herein, we outline the scientific rational behind our hypothesis that repurposing Montelukast to target suppression of NF-κβ activation in COVID-19 positive patients will result in a corresponding reduction of proinflammatory mediators, thereby attenuating cytokine production, and dampening of the cytokine storm. Additionally, we postulate that a reduction in proinflammatory mediators by Montelukast will result in a mitigation of severe COVID-19 symptoms and serve as a therapeutic for SARS-CoV-2 infections. In this manner, we propose to target attenuation of COVID-19 symptoms, rather than the virus, as an effective therapeutic strategy to improve COVID- 19 related clinical outcomes. In support of our hypothesis, we explore the latest clinical reports on COVID-19 and cytokine storm syndrome, as well as the clinical role of NF-κβ in COVID-19 pathogenesis, including results from the literature on Montelukast and its ability to modulate NF-κβ, reduce cytokine production, and mitigate RNA virus infections. Currently, as part of a larger team approach, we are preparing to initiate a clinical trial protocol for the study of the efficacy of montelukast in patients who are newly identified as COVID-19 positive, The COvid-19 Symptom MOntelukast Trial (COSMO), NCT04389411. Also, we would share some anecdotal evidence from patients who got benefit by adding Montelukast in their drug regimen.

Urbanization, Air Pollution and Water Exposure: Do they play a role in the development of amyotrophic lateral sclerosis?

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Despite decades of research, causes of amyotrophic lateral sclerosis (ALS) remain unclear. Recent data pointed to a plausible environmental factor in ALS etiology, therefore the aim was to synthesis and appraise literature on the potential impacts of the surrounding environment, including urbanization, air and water pollution, on ALS. We conducted a series (n = 3) of systematic reviews, using a combination of keywords/MeSH terminology and citation analysis, to identify epidemiological studies assessing relationships between urbanization, air pollution and exposure to water with the development of ALS. The combined search strategy led to the inclusion of 44 articles pertaining to at least one exposure of interest. Of the 25 included urbanization studies, four of nine studies on living in rural areas and three of seven studies on living in more highly urbanized/dense areas found positive associations to ALS. There were also three of five studies for exposure to electromagnetic fields and/or proximity to powerlines that found positive associations to ALS. More work is needed on mapping local climate zones. Three case-control studies for each of diesel exhaust and nitrogen dioxide found positive associations with the development of ALS, with the latter showing a dose-response in one study. Three studies for each of high selenium content in drinking water and proximity to lakes prone to cyanobacterial blooms also found positive associations to ALS. Whereas markers of air and water pollution appear as potential risk factors for ALS, results are mixed for the role of urbanization. Thus, ongoing epidemiological work by our research team seeks to further explore how these environmental factors, in particular local climate zones, air pollutants and proximity to water bodies, may play a role in the development of ALS in a population with suspected disease clusters.

Differential Infectivity of Ferret Cell Lines to Marburg and Ebola virus

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The genus Marburgvirus (family Filoviridae) contains a single species, namely Marburg marburgvirus, consisting of two viruses, Marburg virus (MARV) and Ravn virus (RAVV). These negative-sense, single-stranded, non-segmented RNA viruses are endemic across sub-Saharan Africa and have been responsible for sporadic outbreaks of Marburg virus disease with case fatality rates as high as 90%. Recent investigations using a ferret model demonstrated that ebolaviruses, including Ebola virus (EBOV), Bundibugyo virus and Sudan virus, are capable of causing lethal disease in ferrets, while infection with MARV or RAVV does not result in disease or detectable viremia. These findings suggest that while ebolaviruses and marburgviruses are phylogenetically related and cause similar disease in humans and non-human primates, their pathogenesis in ferrets may differ. Hypothesized to be the result of a block in glycoprotein (GP)-mediated viral entry, an *in vitro* study was performed to evaluate the permissibility of ferret cell lines derived from heart, lung and spleen along with Vero E6 cells to GP-mediated viral entry using a recombinant vesicular stomatitis virus (VSV) expressing green-fluorescence protein (GFP) and either MARV-GP or EBOV-GP. This study revealed that both MARV-GP and EBOV-GP were capable of mediating entry into ferret cells with similar efficiencies, suggesting that the lack of disease following MARV infection in ferrets is likely not the result in a block in GP-mediated viral entry. In a follow up study, these same cell lines were infected with authentic MARV or EBOV to evaluate viral entry and replication. While MARV was capable of replicating within ferret lung and heart cells as evidenced by both viral RNA and infectious virus, these levels were lower than those observed in Vero E6 cells. Interestingly, ferret spleen cells did not support replication of MARV. In contrast, EBOV was capable of replicating in both ferret and Vero E6 cells to similar levels. Together these data indicate that unlike EBOV, MARV is only capable of low-levels of replication within ferret cells suggesting that the lack of susceptibility by ferrets to lethal infection with MARV but not EBOV is the result of mechanistic differences between the innate immune evasion tactics of the two viruses.

Linkage of HIV Escape Mutations to a Novel Host Genomic Locus Associated With Control of HIV Replication

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HIV set point viral load (spVL) is a predictor of HIV disease progression and transmission, thus restriction of spVL is key to ending the AIDS pandemic. There is variability in HIV spVL among individuals, with host genetics contributing to this variability. Some individuals have genetic variants, particularly in the HLA region, that limit HIV replication. However, HIV can develop escape mutations to evade host pressure, counteracting their effect. A recent GWAS of >3,800 HIV+ individuals of African ancestry, performed by our group, identified a novel locus on chromosome 1 that is significantly associated with control of HIV replication. A variant within this region, rs59784663(G), is associated with an average ~0.3 log10 reduction in HIV spVL (p=2.0x10-9) and is downstream from the protein coding gene chromodomain helicase DNA binding protein 1 like (CHD1L). Despite the spVL decreasing effect of rs59784663, some individuals with this allele still have high spVLs. Given the high mutation rate and short generation time of HIV, we hypothesized that selective mutation of the viral genome allows HIV to escape restriction by CHD1L. We tested this hypothesis by conducting a viral genome-to-host genome analysis using both human and viral genomic data from 97 South African individuals. Human genetic variants in the CHD1L region and HLA were tested for association with amino acid (AA) variants in the HIV proteome. Analyses found a significant association between the CHD1L variant rs59784663 and AA codon 248 of HIV reverse transcriptase (p=9.9x10-3). In addition, there were significant associations between HLA B*81 (p=1.5x10-5) and HLA C*18 (p=1.4x10-3) with AA codon 4 and HLAB*58 with AA codon 196 (p=1.0x10-3) in HIV reverse transcriptase. Ongoing work will seek to replicate these associations in tow other African populations. This study further investigates host-HIV interaction in an understudied population, thereby increasing our understanding of viral evolution and host control of HIV.

Investigating the Effect of the HNF-1aG319S Variant on Liver and Pancreas Function under Different Physiological States

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Introduction: Genetic testing in Anishininew communities in central Canada led to the discovery of the HNF-1aG319S variant, which may contribute to youth-onset type 2 diabetes. HNF-1a is a transcription factor that controls glucose and lipid metabolism in the liver, and maintenance of pancreatic beta cell identity and function. Currently, it is unclear how the G319S variant influences these pathways. Given the metabolic demand associated with traditional lifestyle practices in central Canada, the G319S variant may instead confer an advantage to prolonged fasting. Here, we examine the impact of prolonged fasting in G319S expressing mice compared to control mice fed a standard chow diet.

Methods: CRISPR/Cas9 was used to knock in the G319S variant in C57BL/6 mice, creating male and female wildtype (G/G), heterozygous (G/S), and homozygous (S/S) mice. At 3 months, mice were sacrificed either under ad libitum condition or after 24 hours fasting. Liver tissues were collected for gene expression and assessment of triglyceride contents. Islets were isolated to assess insulin secretion capacity, insulin content, and for electron micrography (EM) used to investigate morphological differences.

Results: A statistically significant reduction in liver triglycerides was observed in G/S (p=0.0237) and S/S (p=0.0185) mice compared to G/G. In addition, increased expression of genes involved in cholesterol synthesis and ketogenesis was observed, including HMGCR in G/S (p=0.0140) and S/S (P=0.0073) mice, as well as increased expression of genes involved in gluconeogenesis, including G6PT-1 in S/S mice (p=0.0290). Once fasted, a decrease in blood glucose was observed in G/S (P=<0.0001), and S/S (P=0.0385) mice compared to G/G, and a trend towards increased blood ketones was also seen. In pancreatic islets, a reduction in insulin content was seen in G/S (p=0.0175) and S/S (p=0.0065) mice compared to G/G. EM images showed an increase percentage of immature insulin granules in male S/S (p=0.0157) compared to G/G.

Conclusion: Our findings indicate that the G319S variant alters fatty acid metabolism in the liver as there is a shift toward ketogenesis and gluconeogenesis, and a propensity toward insulin depletion in the islets, which may indicate that the G319S variant provides a metabolic advantage during extended periods of fasting.

Comparing Sequencing Accuracy between Illumina and Nanopore Sequencing Platforms when Generating SARS-CoV-2 Whole Genomes

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Introduction: Oxford Nanopore Technologies (ONT) offers portable long-read sequencing devices with a simple library preparation protocol that can generate sequence data within hours. These attributes make it a suitable platform for time-sensitive sequencing. Initially, Nanopore sequencing technology had a higher error rate than Illumina, a short-read sequencing technology; therefore, its use was limited. Recent technological advances in reagent and flow cell stability plus base-calling algorithms have all contributed to increasing sequencing accuracy. In 2020, several countries1,2 and our lab at the National Microbiology Laboratory adopted the ONT platform for genomic surveillance of SARS-CoV-2 during the COVID-19 pandemic. Whole-genome sequencing data from clinical SARS-CoV-2 specimens is widely used to help detect variants of concern which are identified based on mutations present in the genome. Therefore, obtaining accurate sequencing data is critical. In this study, we compared the sequencing accuracy of Nanopore and Illumina platforms using SARS-CoV-2.

Methods: A total of 11 cultured samples spanning diverse SARS-CoV-2 lineages were sequenced on both Illumina MiSeq and ONT GridION sequencers using three primer schemes with amplicon size 400 bp, 1.2 kb, and 2.0 kb. Sequence reads were aligned against the Wuhan-Hu-1 reference genome to generate consensus sequences. Sequences for each sample were compared between sequencers and primer schemes to identify the presence and absence of high-quality single nucleotide variants (SNVs) for evaluating the accuracy of consensus genomes across the sequencing technologies.

Results: We found that consensus sequences generated from both Nanopore and Illumina platforms with three different primer schemes all resulted in identical lineage assignments for the same samples. At the nucleotide level, Nanopore and Illumina data introduced ~1-2 SNVs at random locations in several samples across the entire 29kb genome (0.003-0.007%).

Conclusion: Nanopore has much shorter turnaround time than Illumina platform making it advantageous for sequencing time-sensitive samples. Based on our SNV evaluation, we found a small bias in consensus sequence generation that is dependent on primer schemes and sequencing platforms. However, this bias did not affect lineage assignment of SARS-CoV-2 samples further supporting the continued use of different primer schemes and sequencing platforms for SARS-CoV-2 genomic surveillance.

Assessing the Cross Neutralization of Mumps Antibody between Genotypes

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Introduction: Mumps (MuV) is a paramyxovirus belonging to the Rubalavirus family, with a lipid envelope and a non-segmented, negative strand RNA genome of 15.3 kb. Infection with the virus generally affects the salivary glands resulting in symptoms including fever, headache, and Parotitis which usually resolves in 7 to 10 days but complications can occur which could result in deafness, meningitis, and inflammation of testis and ovaries. The virus encodes for seven different proteins including the two glycoprotein HN and F protein responsible for attachment and fusion of the viral envelope to the target cell membrane. The glycoproteins are important target for the immune system that produces neutralizing antibodies, which are believed to be protective against infection. Prevention of mumps is through two doses of the Measles-mumps-rubella (MMR) vaccine with the first dose administered at 12-15 months of age and seconds dose at 4-6 years of age and has been proven to be safe and effect. Several mumps outbreak have been reported with 24 mumps outbreak across nine provinces across Canada between January 1, 2016 and July 31, 2018 with majority of the cases in adolescents and adults between the ages of 15 to 39 years. The outbreaks observed were due to genotype G whereas the vaccine strain is of genotype A (Jeryl-lynn strain). Mumps infection in vaccinated individual could be due to waning immunity or antigenic differences between the vaccine strain and the current circulating strain. These differences could result in reduced recognition of vaccine induced antibodies to the genotype G mumps virus leaving the population susceptible to mumps infection. Therefore, it is important to understand whether the vaccine induced antibodies are protective against genotype G infection.

Methods: Stable cell lines expressing the F and the HN protein will be created using lentivirus transduction in Vero cells. Immunofluorescence paired with FACS will be used to assess the success of transduction. Cross reactivity of antibodies between genotype will be assessed by Immunofluorescence analysis using patient sera from unvaccinated individual, unvaccinated individual with history of genotype G as well as vaccinated individual with genotype G infection and vaccinated individual without genotype G infection.

Neuron-Specific Thioredoxin-1 Deficiency Causes Neurodegeneration and Widespread Glial Dysregulation in Mice

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Introduction: Neuronal laminopathy (NLP) is a newly described component of cellular pathology in Alzheimer's disease (AD). NLP is viewed as damage to nuclear lamina, a mesh-like protein network located at the interface of nuclear envelope and chromatin. NLP is commonly identified in neurons from autopsy samples from patients with neurodegenerative diseases including AD. Decreased levels of antioxidants including Thioredoxin-1 (Trx-1) have been reported in AD, and our recent work showed that downregulation of Trx-1 results in caspase-6 activation and induction of NLP. The protective role of Trx-1 has been linked to its reducing capacity for rejuvenation of oxidized proteins.

Methods: Using Cre-lox gene knock-out system, we examined induction of NLP and associated changes in a mouse model that harbors neuron-specific Trx-1 depletion. The lifespan of these animals is reduced to 10-12 weeks, and they exhibit signs of motor and as cognitive deficits. We evaluated pathological changes in Trx-nKO mice brain tissue by western blotting, immunohistochemistry, and ELISA.

Results: Immunohistochemistry and western blotting confirmed that significant downregulation of Trx-1 is directly associated with NLP induction in somatosensory cortex from 8-week-old mice. Incidence of neurodegenerative changes was observed by enhanced Tau phosphorylation with a simultaneous increase in amyloid beta levels. Trx-1 deficient mice also exhibit elevated levels of phosphorylated TDP-43 (Tar DNA binding protein 43), a commonly referred marker of neurodegeneration. These changes were associated with apparent decreases synaptogenesis as detected by PSD95, a marker of the postsynaptic membrane. Lower neurogenesis capacity was also detected in cultures of Dentate Gyrus-derived neuronal stem cells. Trx-1 downregulation in neurons perturbed glial homeostasis, as detected by increased astrocytosis and microglial activation. Trx-1 deficient mice also displayed robust demyelination as identified by changes in oligodendroglial shape and decreased levels of myelin basic protein and myelin-associated glycoprotein in cortex and corpus callosum.

Conclusions: These results suggest that induction of oxidative stress is an upstream in the induction of neuronal and glial changes and development of pathologic events including Tauopathy, or Amyloid-beta toxicity, that are commonly considered as the cause of neuronal loss. Our work also highlights the importance of NLP as a potential therapeutic target.

Mass Spectrometric Analyses of Proteomic Dysregulation by Zika Virus in Astrocytic cells

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Introduction: Owing to the outbreaks of Zika Virus (ZIKV) infection in Brazil in 2015 and numerous other cases reported in Europe, Oceania and Latin America, ZIKV was classified as one of the reemerging viruses. In 2016, WHO declared it a Public Health Emergency of International concern. As of July 2019, 87 countries show evidence of its mosquito-borne transmission with 31, 587 cases reported in Americas in 2018. ZIKV is a flavivirus containing a positive single-stranded RNA genome and its pathogenicity includes both the central and the peripheral nervous systems conditions namely microcephaly, Guillain-Barré syndrome, encephalopathy, (meningo)encephalitis and myelitis. Currently no vaccines and reliable patient diagnoses exist and numerous ongoing studies seek to identify therapeutic anti-viral drugs or dispensable host processes that could be targeted for ZIKV treatment.

Methods: Complementing my previously done proteomic study using multiplexed aptamerbased SOMAScan platform, we studied the impact of ZIKV infection on the human glioblastoma astrocytoma cells (U251) proteome using mass spectrometry. Initial infections were performed at MOI of 3 and samples were harvested and analyzed at 48hrs post infection. Plaque assay to determine viral titres and western blotting to measure protein synthesis were performed post siRNA mediated knockdown (KD) of 52 proteins to observe their impact on viral replication and protein synthesis. Two-tailed t-test to determine statistical significance and pathway analyses were performed using Ingenuity Pathway Analysis (IPA) software.

Results: After mass spectrometry, we identified close to 100 targets that were significantly dysregulated by ZIKV. Cellular networks, biofunctions and upstream molecules pertaining to antimicrobial response, cell signaling and infectious diseases, cell survival and energy production were identified as differentially impacted by ZIKV. Post SiRNA mediated KD of 52 of those targets, 3-6 have been chosen for in-depth analyses, looking at the impact of KD on viral titres and protein synthesis.

Conclusion: ZIKV dysregulates astrocytic cellular proteins, pathways and functions and knocking down those cellular proteins result in inhibition of ZIKV replication inside the astrocytic cells.

Assessing Impulsivity Following Non-Lethal Suicidal Behaviour

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Introduction: Understanding fluctuations in time-dependent factors associated with suicidal behaviours may be especially important for preventing future death by suicide. Impulsivity, a construct that includes several trait-level components and state-level cognitive processes, ("facets") has been implicated in the transition from suicidal thinking to suicidal behaviours. Relatedly, emerging research suggest increases in impulsivity closer to the time of a suicide attempt. However, in this research, impulsivity remains predominantly operationalized as a homogeneous construct, despite its significant heterogeneity. Further, most studies restrict investigations of suicidal behaviour to suicide attempts. By excluding aborted and interrupted attempts – suicidal behaviours that share overlapping clinical characteristics with actual attempts – studies to date may not fully capture how impulsivity contributes to suicide. Thus, the present study tested whether impulsivity facets were differentially associated with recency of suicidal behaviours (i.e., aborted, interrupted, and non-lethal suicide attempts) among young adults.

Methods: We recruited 86 young adults (Mage = 20.5, SDage =1.76; 71 female) using social media platforms (e.g., Facebook). Participants first screened for eligibility criteria (i.e., endorsed past year suicidal thoughts and/or behaviours) and then were scheduled for a single remote (i.e., Zoom) assessment. Under the supervision of study staff, participants completed questionnaires and behavioural tasks comprehensively measuring impulsivity facets and suicidal behaviours.

Results: In a series of ordinal regressions controlling for clinical (e.g., psychiatric symptoms) and demographic characteristics, we found that prepotent response inhibition (i.e., the ability to inhibit automatic responses), but not other state or trait impulsivity facets (ps > .06), was significantly associated with recency of prior suicidal behaviours (Wald $\chi 2(1) = 6.29$, $p = .01$). Specifically, contrary to expectations, higher inhibition was associated with greater odds of endorsing more recent versus more distal suicidal behaviours. Critically, 83.9% endorsed at least one lifetime aborted attempt, which differ from both interrupted and actual suicide attempts as they require people to actively stop themselves from an attempt before any active steps are taken.

Conclusion: These results advance our knowledge of what forms of impulsivity are most relevant to short-term escalations in suicide risk. Ultimately, findings have implications for developing better pre-and post-vention strategies for suicidal young adults.

Low-Cost Screening of Sickle Cell Disease for Remote and Rural Settings

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Sickle cell disease (SCD) is an inherited blood disorder associated with acute illness and organ damage. In high resource settings, early screening and treatment options greatly improve the quality of life of people with SCD. In low resource settings, however, the mortality rate for children with the disease is high (50-90%). Low-cost and accurate screening techniques are essential for reducing the burden of the disease, especially in remote and rural settings, where the prevalence of the disease is high. Some standard screening tests include the "sickling test", where blood (mixed with a reagent) is observed under a microscope to assess whether the shape of red blood cells changes (positive result) or not (negative result), and the "HbS solubility test", where blood is mixed with a reagent to result in a clear (negative result) or turbid solution (positive result). However, these tests do not distinguish between patients with SCD and with the asymptomatic sickle cell trait (SCT) condition.

We plan to evaluate the performance of several low-cost point-of-care techniques to detect SCD and SCT in Vancouver, Canada and Nepalgunj, Nepal. We will use a low-cost microscope for the sickling test and use image analysis and machine learning to classify the different cases. Other low-cost tests, such as the Gazelle Hb Variant test, HemoTypeSC, SickleSCAN and HbS solubility test, will also be evaluated. All the low-cost tests will be compared with high performance liquid chromatography (HPLC) as the gold standard or reference test. β-thalassaemia is another inherited blood disorder, associated with deficiency or abnormality in the synthesis of hemoglobin and the transport of oxygen. Unlike Vancouver, places such as Nepal have high prevalence of both β-thalassaemia and SCD. Parents with asymptomatic trait conditions showing limited/no symptoms can unknowingly pass on variant genes to their children resulting in severe compound heterozygous cases. Therefore, in countries such as Nepal, screening communities for both diseases is critical. The study in Vancouver will focus on screening for SCD and SCT in around 90 participants. The study in Nepal will screen for SCD, SCT and β-thalassemia in around 120 locally recruited participants.

Gardnerella Subgroup Contributions to the Cervicovaginal Microbiome and Associated Immune Responses

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Introduction: Vaginal microbiomes associated with bacterial vaginosis (BV) have been linked to increased HIV risk – possibly by altering the mucosal immune milieu. Though Gardnerella species have been implicated in the pathogenesis of BV, the impact that different Gardnerella subgroups have on mucosal immunity remains unclear. To fill in these gaps in knowledge, we used chaperonin60 microbial profiling to resolve four different Gardnerella subgroups and additional bacterial species in cervicovaginal secretion samples, to examine microbial community associations with mucosal immune markers in a longitudinal cohort of reproductive age Kenyan women.

Methods: Cervical cytobrush and cervicovaginal secretion samples were collected across multiple visits from participants (N=41) enrolled in the 48-week KAVI-VZV-001 clinical trial. Cervicovaginal secretions were used for microbial profiling using the chaperonin60 universal target and cytokine quantification. Cytobrush samples were processed immediately to measure total HIV target T-cells and activated subsets, as well as expression of immune activation markers on T-cells. Linear mixed models were used for statistical analysis.

Results: Non-Lactobacillus dominant microbiomes were associated with increases in proinflammatory cytokines. Divergent associations emerged with respect to the chemokine IP-10, whereby polymicrobial communities and those dominated by Gardnerella subgroup A were associated with reduced levels of this chemokine ($P<0.0001$). Similar patterns emerged with related chemokines though those did not reach statistical significance. We did not identify any significant associations between microbial communities and total adaptive T-cells counts and related activated subsets.

Significance: this study adds to our understanding of Gardnerella contributions to mucosal immunity and the cervicovaginal microbiome.

Insight into Pelagophytes: Novel algal genomes and strain level genome variation in the harmful algal bloom causing species *Aureococcus anophagefferens*

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The Pelagophyceae are marine stramenopile algae that include *Aureoumbra lagunensis* and *Aureococcus anophagefferens*, two microbial species notorious for causing harmful algal blooms. Despite their ecological significance, relatively few genomic studies of pelagophytes have been carried out. To improve understanding of the biology and evolution of pelagophyte algae, we produced new high-quality reference genomes using Oxford Nanopore long-read sequencing technology for *A. lagunensis* (CCMP1510) (43 Mbp), *Pelagomonas calceolate* (CCMP1756) (34 Mbp), and re-sequenced *A. anophagefferens* (CCMP1984) (53 Mbp). This includes a fully resolved telomere-to-telomere genome assembly for *P. calceolata*, with 6 chromosomes ranging from 4 to 6 Mbp in size. Furthermore, to investigate intra-species variation we produced high-quality draft genomes for four additional *A. anophagefferens* strains (CCMP1707, CCMP1708, CCMP1850, and CCMP3368). The pan-genome refers to the sum of genes across all strains of a given species, only a subset of which reside in the genome of any given strain. The pan-genome concept readily applies to prokaryotes, where lateral gene transfer (LGT) can lead to enormous intra-species gene-content variability. However, the extent to which LGT-driven pan-genomes exist in eukaryotes is uncertain. Our comparative genomic investigation indicates strain level variation in gene content in *A. anophagefferens* (pan-genome), including genes predicted to be related to bloom conditions, providing insight into both bloom dynamics and how microbial eukaryotes adapt and diversify.

High Level of Short-Chain Fatty Acids has Direct Effects on the Barrier Function of Cervicovaginal Epithelial Cell Lines

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Short-chain fatty acids (SCFAs) are microbial-derived metabolites that enter the blood circulation. SCFAs are reported to maintain the gut mucosal barrier and regulate the inflammation at gut mucosa. In contrast, higher vaginal level of SCFAs was associated with bacterial vaginosis (BV). It remained unclear whether increased SCFAs levels were the result or the cause of infection or BV. As the cervicovaginal epithelial barrier function and vaginal mucosal inflammation are critical in cellular susceptibility to HIV or HPV infection, this study focused on deciphering the relationship between vaginal SCFAs and inflammatory cytokine/chemokine levels in cervicovaginal fluid (CVF) and defining the direct effects of SCFAs on cervicovaginal epithelial barrier integrity. The CVF samples from healthy (n=27) and HPV-infected non-menopausal women with low-grade squamous intraepithelial lesions (LSIL) (n=14) were analyzed for SCFAs and 18 inflammatory cytokine/chemokine. HPV DNA in the vaginal swab and CVF were assessed using PCR. Levels of vaginal SCFAs were: acetate (0.16-2.8mM), butyrate (0.35-41.9µM), propionate (0.23-67.0µM), beta-hydroxybutyrate (0.1-43.4µM), and valerate (0.02-0.6µM). The SCFAs level in LSIL-HPV CVF samples was not different from the healthy controls. We further found that levels of vaginal SCFAs were significantly higher than plasma SCFAs (p-values < 0.01). Furthermore, the LSIL HPV CVF samples had no significantly different levels of proinflammatory cytokine/chemokine from that of control CVFs. Treatment of polarized cervicovaginal epithelial cell lines to 5mM of sodium butyrate or sodium propionate for 48 hours resulted in increased epithelial permeability, with reduced transepithelial electrical resistance across the epithelium. In summary, LSIL-HPV patients do not have increased SCFAs level, nor increased inflammatory cytokine/chemokine cervical vaginal sites, when compared to the HPV negative participants. This finding is surprising, as HPV-infection was thought to elicit inflammation as it is in BV. In addition, *in vitro* exposure to high level of exogenous SCFAs directly increased the epithelial permeability and perhaps, weakened the barrier function of the polarized cervico-vaginal epithelial cell lines examined in this study. Work is ongoing to validate this finding in non-transformed cells and to determine the molecular mechanisms.

Investigating Microglial Responses to Infection-Induced Inflammation as a Mechanism Underlying Cognitive Decline and Neurodegeneration in an Aged Mouse Model

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Introduction: Aging presents a risk factor for the onset of cognitive decline, dementia, or spontaneous neurodegeneration-related disorders. As regulators of brain homeostasis and synaptic plasticity, microglia, brain-resident immune cells, participate in core cognitive processes of learning and memory. Aging-impaired energetic utilization and increasing demand on brain maintenance leads to failures in intracellular machinery underlying loss of microglial flexibility and the emergence of neurotoxic microglia contributing to a low-grade inflammation. I hypothesize that infection-induced exacerbation of inflammation further compromises brain homeostasis, notably via dysregulated microglia-mediated clearance of synapses and misfolded proteins, resulting in cognitive decline and eventually neurodegeneration. Moreover, the impact of aging gut microbiome, an important microglial regulator, is yet to be established in this context.

Methods: To explore the connection between infection, synaptic loss and pathological neurodegeneration, an acute inflammatory response will be induced by an exposure to a viral mimic polyinosinic-polycytidylic acid (poly(I:C)) in 18-20-month-old C57BL6/J mice. Battery of behavioural tests will be conducted in an early- and late post-sickness period to assess the adaptability, anxiety-like behaviour, learning and memory. Besides neurodegeneration markers, samples from the hippocampus, seat of learning and memory, will be used to investigate microglial density, morphology, ultrastructure, and synaptic interactions using confocal and state-of-art electron microscopy. Isolated microglia will be analysed for proteomic and metabolic signatures with advanced mass spectrometry analyses. Plasma and feces were collected and processed for ELISA, metabolomics, and metagenomics, to analyze inflammatory/metabolic markers and the intestinal microbiome features, respectively. Moreover, exploring the recently implied clinical potential of ketogenic diet for treatment of cognitive decline and dementia, mice will be exposed to this high-fat, low-carb regimen prior to the poly(I:C) challenge. Resulting ketosis provides alternative substrates for an impaired cellular metabolism. By preventing mitochondrial dysfunction and maladaptive inflammatory response during the aging process, ketones are expected to restore the microglial metabolic flexibility necessary for their optimal immune and physiological functions.

Conclusions: This project aims to provide novel insights into microglial metabolism manipulation and re-programming. With the increasing senior population, identification of mechanisms underlying cognitive aging and the outcomes of preventative and intervention strategies holds utmost clinical implication for brain health.

Is mRNA Translation Compromised in SIFD?

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tRNA-nucleotidyltransferase 1 (TRNT1) is an essential enzyme responsible for the addition of 3′ CCA ends to all tRNA, a crucial process of tRNA maturation and amino acid attachment. Hypomorphic mutations within TRNT1 result in a rare and severe mitochondrial disease known as SIFD (sideroblastic anemia, B-cell immunodeficiency, periodic fevers, and developmental delay). SIFD patients also experience a wide range of multi-system impairments. The molecular mechanism(s) by which these mutations cause such a broad range of symptoms are not fully understood. It is believed, however, that in addition to affecting the canonical functions of tRNAs, the disease-causing mutations impact non-canonical functions, adding to the complexity of SIFD. The purpose of my research is to understand how hypomorphic mutations in TRNT1 cause SIFD. Preliminary data suggests that patient-derived fibroblasts show significant reduction in cytoplasmic pools for certain tRNAs, and altered levels of certain proteins, compared to healthy cells. I hypothesize that reduction in tRNA pools impairs translation of specific proteins, either through ribosomal stalling or translational infidelity, potentially impacting protein function. To investigate the molecular mechanism(s) of SIFD, the yeast model of SIFD that utilizes temperature sensitive allele of CCA1 (yeast homolog of TRNT1) is being used. Comparing healthy yeast to CCA1 deficient yeast, I observed reduction in tRNA abundances as in patient cells. Using yeast specific tRNA data, a bioinformatic screen of the yeast genome was conducted to identify genes which require large amounts of the reduced tRNAs to be translated. The abundance of these potentially impacted genes and their proteins will be investigated by western blotting and polysome profiling. To assess impacts on protein function, a B-galactosidase assay was conducted, showing that when compared to healthy yeast the CCA1 deficient yeast had a significant ($P<0.001$) reduction in the function of the B-gal protein. In addition, CCA1 deficient yeast showed a severely impacted ability to grow on galactose as an alternate carbon source, showing a potential impact on the ability to adapt to cellular stresses. By using the yeast model of SIFD we can better inform future experiments within patient cells, and potentially work towards the development of treatment options.

Genomic Characteristics of Rett Syndrome Modifier Genes

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Introduction: Genetic modifiers are non-primary disease-causing genes that alter the severity of genetic diseases. Previous studies have demonstrated the importance of genetic modifiers in neurological disease and their potential as therapeutic targets. Rett syndrome (RTT) is a rare neurodevelopmental disorder typically caused by mutations in the X-linked MECP2 gene. While skewed X inactivation has been implicated in the variable severity of RTT, genetic modifiers may also play a role. Recently, a large RTT modifier screen in Mecp2/Y mice assessed phenotype improvement following mutagenesis. This analysis identified 31 genes that improved the RTT phenotype.

Methods: To determine which of these genes are most tractable as human drug targets, the human phenotypes associated with these 31 genes were assessed using the Open Targets Genetics (OTG) database. This resource aggregates results from unbiased genome-wide association studies (GWAS) and performs machine-learning based fine-mapping of significant association signals using the Locus2Gene (L2G) model. GWAS information was extracted from the OTG v6 database for each of these 31 genes. The results were filtered programmatically for signals which were most likely attributable to these genes (i.e., L2G score of 0.5 or greater).

Results: Of the genes analyzed, fine-mapped signals were detected for 16 genes. This represented a total of 215 human trait associations. For these 16 genes, the average number of associations was 13, ranging from one to 69. Over one-third of the trait associations were found in APOA5, which is involved in lipid metabolism. Furthermore, relevant traits included cognitive performance, educational attainment, and cortical surface area.

Conclusion: We have identified human-relevant information for RTT genetic modifiers which we can use to prioritize future functional genomics work. This will allow for the modifier effects to be confirmed and for the elucidation of the underlying biological mechanisms.

Membrane Composition and Anionic Phospholipid Abundance Affect Activity of Single Component Cationic Antimicrobial Selective Efflux Pumps in *Escherichia coli*

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Introduction: Multidrug resistant (MDR) bacteria are a concerning threat to human health globally and the problem is worsening by the constant overuse of cationic antiseptic compounds in clinical, industrial, and household applications. Exposure to sub-inhibitory concentrations of antiseptics can lead to membrane adaptations, reducing the efficacy of other clinically relevant antimicrobials, such as antibiotics. Antiseptic resistance in bacteria is predominantly conferred by efflux pump proteins, comprising several different families with varying substrate selectivity. Here, we examine cationic substrate specific efflux pumps and the role anionic phospholipid abundance plays in bacterial membranes and the folding and activity of these transporters. The role phospholipid-protein interactions play on the function of cation-selective efflux pumps is poorly understood, and knowledge gained in this research help generate novel targets for efflux pump inhibitors and guide antimicrobial stewardship initiatives.

Methods: Efflux pumps EmrE(SMR family), MdtK(MATE family), MdfA(MFS family), and AceI(PACE family) have been cloned into the plasmid vector pMS119EH and transformed into *E. coli* K-12 mutant strains containing different phospholipid biosynthesis pathway gene deletions, to test the effect of phospholipid alteration in the membrane on efflux topology/function. Phospholipid content of *E. coli* strains is being confirmed by Peptidisc® isolation of annular lipids and TLC techniques. The efflux activity of each pump type in the various strains will be tested against varying concentrations of commonly used cationic antimicrobials including quaternary ammonium compounds, bisbiguanides, and relevant antibiotics; utilizing high-throughput assays to determine if efflux topology/function are affected by varying membrane phospholipid compositions.

Expected results: It is expected that anionic phospholipids will have the largest impact on cationic substrate efflux transmembrane topology, reducing efflux pump activity. Reductions in expression of zwitterionic phosphatidylethanolamine(PE) likely increases anionic phospholipid abundance, altering the ability of cationic drugs to permeate these altered membranes.

Expected conclusions: low-abundance anionic phospholipids have a large impact on the insertion, folding, and activity of cationic efflux proteins, and provide promising new drug targets.

Transcriptional Profiling Reveals Cell Type Specific Responses to Prion Infection in Microdissected CA1 and Thalamus Brain Regions of Prion Infected Mice

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Introduction: Prions are misfolded proteins that are infectious and cause a group of fatal neurodegenerative disorders called prion diseases. Accumulation of misfolded prion proteins (PrPSc) within the brain is associated pathological changes that culminate in rapidly progressive neurocognitive decline. Transcriptional profiling has been widely used to describe pathological changes of neural tissues during prion infection, although the response of neurons is subtle and may be masked by a strong transcriptional response of reactive glial cells. Here, we characterized prion associated transcriptional changes within murine tissues isolated from two brain regions abundant with degenerating neurons.

Methods: Mice were intraperitoneally inoculated with either RML scrapie brain homogenate (RML) or non-infectious brain homogenate (Mock) and sacrificed at timepoints ranging from 70- to 150-days post infection (dpi). Neural tissues from the hippocampal-CA1 and thalamus regions were microdissected for RNA extraction followed by Illumina library preparation and sequencing. Sequencing reads were mapped to the mouse reference genome and known transcripts were counted for subsequent normalization and differential expression analysis. Prion altered transcripts were assigned to one of six broadly defined brain cell types prior to functional enrichment analysis.

Results: Transcripts were found to be differentially abundant in clear association with RML infection in the CA1 at 150 dpi (532 transcripts) and in the thalamus at 130 dpi (153 transcripts) and 150 dpi (1799 transcripts). The majority of transcripts with increased abundance were affiliated with microglia, while those with decreased abundance were predominantly affiliated with neurons. There was relatively high overlap between prion altered microglia affiliated transcripts in the CA1 and thalamus, which were related to phagocytosis, synaptic pruning and cytokine production. Prion altered transcripts affiliated with neurons showed almost no overlap between the CA1 and thalamus, yet were enriched in biological processes related to synaptic dysfunction in both regions.

Conclusion: This study identified a strong transcriptional signature in prion disease associated with reactive gliosis, while the response of degenerating neurons was less prominent, distinct between brain regions and may be related to synaptic pathology. Future studies aimed towards uncovering mechanisms of neuronal dysfunction are necessary to identify potential therapeutic strategies for prion disease.

Distinct Conformational Strains of Alpha-Synuclein Aggregates can Form in Identical Experimental Conditions

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Introduction: Aggregates of alpha-synuclein protein are a common pathological hallmark of neurodegenerative diseases like Parkinson's disease, Multiple System Atrophy, and Dementia with Lewy Bodies. As these aggregates can form different structures (or conformational strains), we believe these differences underlie the symptom variability and distinct disease progression rates seen in patients.
Methods: We made alpha-synuclein fibrils from purified recombinant protein by continuous shaking in 20 mM Tris pH 7.4 and 100 mM NaCl. After characterization by limited proteolysis, we injected these aggregates into transgenic mice and monitored them for neurological symptoms such as hind limb paralysis and loss of grip strength. At terminal disease, we collected their brains and analyzed the pathological deposition of alpha-synuclein using immunohistochemistry, limited proteolysis, and chemical denaturation. We also searched for *in vivo* conformational diversity by analyzing another transgenic mouse model that naturally develops alpha-synuclein pathology and neurological disease without fibril injection, using the above-mentioned techniques.
Results: We categorized 30 fibril preparations into two distinct types by their limited proteolysis patterns. These two fibril types caused different symptom onset times when injected into transgenic mice, and the collected mouse brains harbored alpha-synuclein aggregates with different limited proteolysis patterns and susceptibilities to chemical denaturation. Meanwhile, alpha-synuclein aggregates formed in the 23 spontaneously sick mice had three distinct types of limited proteolysis patterns.
Conclusion: The conformational diversity found within recombinant fibrils and spontaneously sick mouse brain extracts demonstrates that the alpha-synuclein protein can aggregate into multiple conformational strains in the same chemical environment. By characterizing the structures of different alpha-synuclein aggregates and the neurological disease phenotypes they produce in mice, we can develop more accurate models to study disease mechanisms and test potential therapeutics.

The Evaluation of the Efficacy of an Immunotargeted Vaccine against SARS-CoV-2 and its Potential for Mucosal Administration

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Introduction: Adjuvants are a major issue in vaccine approval and hesitancy due to adverse effects of adjuvants such as reactogenicity. The development of a safe, effective, non-adjuvanted vaccine is a significant achievement not only against SARS-CoV-2 but for vaccine development research for future applications. The COVID-19 pandemic is still a serious threat to global public health and economies. There is evidently the need for vaccines that not only prevent disease but also prevent transmission to reduce the global disease burden. Approved parenteral vaccines elicit strong systemic immunity but poor immunity at the respiratory mucosa. The development of a non-adjuvanted, mucosally administered vaccine that induces a strong mucosal immune response should prevent not only disease but transmission as well. Further benefits include no reactogenicity, reduction in sharps waste, pain-free vaccine delivery and self-administration thus reducing the burden on health-care professionals.
Methods: Four groups of ferrets were primed and boosted intramuscularly with two different immunotargeted vaccine (ITV-1 and ITV-2) candidates, a subunit vaccine (HEK-RBD) adjuvanted with alum as an adjuvanted 'control' and an unvaccinated group. All groups were challenged with SARS-CoV-2 virus intranasally. Serum and nasal washes were collected weekly to test for the presence of virus neutralizing antibodies and viral titres post-challenge. Two groups of rabbits were then intranasally primed and boosted with two different doses of ITV-1 and serum and nasal washes were collected weekly to evaluate serum IgG and mucosal IgA production.
Results: Both ITVs were found to induce serum neutralizing antibodies in ferrets after one immunization and maintained significantly higher virus neutralizing antibodies and lower viral titres than the HEK-RBD and unvaccinated controls throughout the duration of the experiment. ITV-1 performed better than ITV-2 in all parameters. Preliminary results of the rabbit intranasal immunizations showed low antibody titres in two of the rabbits in the high-dose group at Day 49.
Conclusion: The unadjuvanted, immunotargeted approach to vaccine design elicits a significantly more effective neutralizing antibody response against SARS-CoV-2 in ferrets than an alum-adjuvanted subunit vaccine. Higher doses for intranasal immunization may be needed to successfully develop a mucosal vaccine which induces both IgA and IgG antibodies in rabbits.

Investigating the Influence of Vaccine Formulation on Original Antigenic Sin Responses in a Longitudinal Cohort of Children

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Introduction: Influenza virus infection results in 3-5 million serious illnesses, with children representing a large proportion of those affected. Several seasonal influenza vaccines are available, including inactivated influenza vaccines (IIVs), live-attenuated influenza vaccines (LAIVs), and adjuvanted inactivated influenza vaccines (AIIVs). These vaccines elicit antibodies against the variable hemagglutinin (HA) head domain. Vaccines that stimulate broadly-neutralizing antibodies (bnAbs) are in development. Seasonal vaccine effectiveness (VE) varies greatly from year-to-year, and this variability has, in-part, been proposed to be a consequence of 'Original Antigenic Sin' (OAS). OAS describes a phenomenon wherein the first exposure to influenza virus shapes subsequent immune responses to vaccinations or infections. Antibody titers against the first strain an individual is exposed to are boosted through repeated exposure to antigenically related strains. In the context of seasonal vaccination, OAS boosts antibodies against conserved, but nonneutralizing epitopes. Thus, OAS may be detrimental during seasonal vaccination but may prove to be beneficial when targeting conserved epitopes such as the HA stalk. While the serological consequences of OAS have been well-established, no studies have directly examined the impact of OAS antibodies on VE or the impact of vaccine formulation on OAS. This question is especially important in the context of childhood vaccinations, since children are a high-risk group for serious influenza virus infections and are often naïve to influenza virus prior to vaccination.

Methods: Two cluster-randomized control trials longitudinally followed Hutterite children in three consecutive influenza seasons. The first compared LAIV and IIV between 2012-2015, the second AIIV and IIV between 2016-2019. A subset of pre- and post- vaccination serum samples were tested by hemagglutination inhibition and antibody dependent cellular cytotoxicity assays to measure vaccine strain specific and bnAbs, respectively.

Results: OAS-like responses were observed following successive vaccination in both trials, with IIV causing the most profound OAS. AIIV overcame OAS, particularly in response to H3 vaccine components, and induced the strongest bnAb response in children. Interestingly, bnAbs were induced following the first vaccine exposure, regardless of formulation, in children with no prior immune history to influenza.

Conclusion: These findings will inform the selection of seasonal influenza vaccines, and the design of next generation influenza vaccines that are most suitable for use in children.

Beyond the Binary: A Mixed Methods Study to Improve how Gender Diversity is Measured in Cancer Research

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Introduction: Gender plays a significant role in influencing one's cancer trajectory from risk to end-of-life care and survivorship. Despite growing recognition of gender's importance and efforts to develop novel approaches for evaluating its impact on health, there has been little progress towards developing measures that can capture gender diverse people (e.g. transgender and nonbinary). The inability for gender measures to recognize how gender extends beyond a binary of men and women results in imprecise and inaccurate estimates of the effect of gender on cancer outcomes. This is problematic for gender diverse people whose experiences with oppression lead to higher risks of worse cancer-related outcomes compared to non-gender diverse people. Addressing this problem requires methods that can accurately capture and identify gender diversity in cancer research.

Methods: This community-engaged multiphase mixed methods study will be comprised of two distinct phases. The first phase will entail an environmental scan where I will conduct a systematic review of current practices in measuring gender oncology-specific literature. These results will be merged with findings from interviews with system-level cancer data administrators (n=10), to provide a comprehensive overview of current and potential capacity for including gender diversity in existing cancer data infrastructure. The second phase of the study will focus on identifying barriers to including gender diversity in cancer research and priorities for inclusion. This will be accomplished by conducting a needs assessment among cancer researchers across Canada and holding focus groups (n=8) with gender diverse people. Results from each phase will be synthesized into a comprehensive framework that will guide researchers to incorporate gender diversity into their projects.

Significance: One of most significant barriers to improving gender diverse people's cancer health outcomes is a lack of data. This novel and innovative study will create an inclusive and supportive space for gender diverse people to set priorities and describe how they should be included in cancer research. Ultimately, this project will address the gap that has prevented researchers, policymakers, and administrators from measuring the impact gender diversity has on health and guide efforts to make cancer care more equitable for gender diverse people.

Downregulation of Thioredoxin-1 is Sufficient to Induce Neuronal Laminopathy and Alteration in DNA Methylation Reported in Neurodegenerative Diseases

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Introduction: Alzheimer's Disease (AD) is the most common form of Dementia. Although, the importance of progressive neuronal loss has been identified as the major cause for the disease, the etiology of the disease remains to be confirmed. Excessive oxidative stress, formation of intracellular neurofibrillary tangles, and accumulation of extracellular Amyloid beta plaques have been traditionally targeted as the cause of neuronal loss in many experimental and clinical trials; however, there is currently no effective treatment available for AD. Thioredoxin-1 (Trx1) is a small redox protein responsible for regulating the redox status of cellular proteins. Lowered Trx1 level has been reported in the brain autopsy from AD patients, however, the cause and the consequence remain mostly unknown. Previous reports from our lab have shown that pharmacologic inhibition of Trx system impairs autophagy. We also have identified that loss of Trx1 is an important upstream event in induction of neuronal nuclear lamina (NL) invagination. NL is a protein lattice at the interface of inner nuclear envelope layer and chromatin. Laminopathy is a newly identified mechanism in pathophysiology of AD and has been linked to epigenetic changes.

Hypothesis: In this study, we hypothesize that Trx1 downregulation is the underlying cause for epigenetic modifications reported in AD.

Methods: We used small interfering RNA to downregulate Trx1 protein in human neuroblastoma cells (SH-SY5Y). Structural and molecular nuclear events were confirmed using immunocytochemistry, western blotting, and real-time PCR.

Results: Genetic downregulation of Trx1 in SH-SY5Y cells was sufficient for induction of caspase 6 and NL damage. Moreover, we detected increased levels of endogenous retroviruses that were associated with nuclear laminopathy, DNA damage and activation of cGAS-STING pathway. A significant increase in DNA methylating enzymes DNMT1 and DNMT3b was detected that were accompanied by changes in autophagy related genes. NL damage was partially rescued, and cell viability was improved by administration of Thioredoxin mimetic peptide (CB3; NAc-Cys-Pro-Cys-amide).

Conclusion: This study provides a mechanistic link between Trx1 downregulation and induction of changes in DNA methylation which might contribute to the pathophysiology of AD. Identification of genes with altered DNA methylation will help to design novel therapeutic target.

Investigating the Transcriptomic Profile and Cytokine Profile of Tuberculosis Disease in Persons Living with Human Immunodeficiency Virus in Colombia Populations

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Introduction: In 2020, tuberculosis (TB) was the leading cause of death among persons living with HIV (PLHIV) worldwide. The World Health Organization (WHO) post-2015 End TB Strategy aims to end the TB global epidemic, with developing new ways to detect TB disease as a crucial step. Currently, methods for detecting TB involve looking at symptoms in PLHIV, which makes detecting TB challenging, with up to 77.6% of TB going undetected due to no signs of TB symptoms in PLHIV. This could lead to increases in spread of TB disease and mortality.

Rationale: This study aims to investigate the blood RNA profile and cytokine profile of TB in PLHIV with hopes of improving TB detection. Most RNA and cytokine studies have heterogenous results and lack of reproducibility. The rationale of this project is to look for protein and gene biomarkers, and to further investigate the interactions of HIV and TB co-infections.

Methods: This study will investigate 2 groups of cohorts in Colombia: microbiologically confirmed pulmonary TB, alone or in the context of HIV documented co-infection. The cohort's gene expression profiles will be explored using RNA sequencing. Peripheral blood mononuclear cells from participants will be isolated for total RNA extraction using commercial RNeasy® Plus Mini Kit. The cDNA library preparation will be performed and subjected to sequencing by Macrogen inc. Quality evaluation and gene quantification will be conducted to analyze RNA-seq data and differential gene expression will be assessed to find significant genes. The potential genes of interest will include but are not limited to KCNMA1, TNF, SERPING1, ETV7, SEPTIN4, GBP6 and BATF2. Cytokines that strongly correlates with unique genes from the cohorts and the signaling pathways involved will be investigated using pathway analysis. Finally, the cytokine/chemokine expression levels will be measured using multiplex immunoassays to describe the inflammatory response to TB in PLHIV.

Significance: This study could provide insights to new potential biomarkers that could lead to the development of novel diagnostic tools for accurate detection of TB in PLHIV. This could help in controlling the spread of TB disease and reach WHO's goal of ending the TB epidemic worldwide.

Clostridioides difficile on Retail Meat Samples: A possible source of human clinical infections?

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Introduction: *Clostridioides difficile*, is a spore forming anaerobic bacteria most commonly known as a human nosocomial pathogen. However, *C. difficile* spores can be found dispersed throughout the environment and can asymptomatically colonize and/or infect animals. Previous studies have shown that *C. difficile* spores can be isolated from commercially available beef, veal, pork, vegetables and seafood. However, a definitive link has yet to been made between food contamination and hospitalized cases. This study aims to isolate *C. difficile* from retail meat samples and compare them to human clinical isolates.

Methods: Frozen retail pork, beef and veal samples were obtained from the Canadian Integrated Program for Antimicrobial Resistance Surveillance (CIPARS) program. Defrosted samples were massaged in PBS. The rinse was plated on *C. difficile* moxalactam norfloxacin (CDMN) agar and added to CDMN enrichment broth before being incubated anaerobically. Post incubation, the enrichment broth was subject to an ethanol shock treatment, centrifuged, spread on brucella blood agar and incubated. Growth on agar plates were inspected visually with suspected *C. difficile* colonies confirmed by multiplex PCR. Toxigenic positive isolates were molecularly characterized by ribotyping and pulsed-field gel electrophoresis (PFGE). Antibiotic susceptibility was determined by E-test strips.

Results: Overall, 10 of 644 meat samples (1.55%) tested positive for toxigenic *C. difficile*. Of the 644 samples: no isolates were obtained from 28 beef samples, 4 isolates were obtained from 116 veal samples and 6 isolates were isolated from 500 pork samples. All 10 isolates were toxin A and toxin B positive. 2 isolates were binary toxin positive. Molecular typing by ribotyping and PFGE revealed strain types commonly found in human clinical isolates (e.g. NAP1 (RT027), NAP4 (RT NS195), and NAP11 (RT106)). The 10 isolates from meat samples were all susceptible to vancomycin, metronidazole, tigecycline, rifampin, and clindamycin, whereas the one NAP1 isolate was resistant to moxifloxacin.

Conclusion: A low percentage of retail meats are contaminated by *C. difficile* spores. All ribotypes and NAP types of isolated *C. difficile* from retail meat have been identified in human clinical isolates. Future work includes the use of WGS to genetically compare retail meat isolates from human clinical isolates.

Testing the Feasibility of an Emotion Induction/Regulation Task in an Online Study

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Introduction: In affective science, "emotion regulation" (ER) is an umbrella term for the various strategies that are used to modify one's emotions. In recent years, many affective scientists have started collecting data online to lower public health risks and recruit more diverse samples. Although several emotion induction and regulation paradigms are developed for in-lab use, few are validated for online data collection. The aim of the present study is to validate an emotion induction/regulation paradigm for use in online research.

Methods: 150 young adults (aged 18-29) will be randomly assigned to one of three ER conditions: mindfulness, reappraisal, or suppression. First, participants receive instructions for their ER strategy and practice their strategy with an RA. Participants then view 8 "repulsive" videos (~30s/ea). During the first 4 videos, they are told to let their emotions arise while watching the videos. During the final 4 videos, they are instructed to watch the videos while using their assigned emotion regulation strategy. After the videos, participants are instructed to continue regulating their emotions for 1 minute. Throughout the task, participants rate their disgust feelings on a scale from 0 ("Neutral, no disgust") to 100 ("Extreme disgust").

Results: Data collection is ongoing and will be completed by the time of the conference. We tested our preliminary data ($n = 85$) with a repeated measures ANOVA, which showed a main effect of time predicting negative affect, $F(1, 3) = 77.7$, $p < .001$. Follow-up paired-samples t-tests showed that participants experienced a significant increase in negative affect from baseline to passively viewing the videos, $t(84) = -13.2$, $p < .001$. Participants also experienced a significant decrease in negative affect from passively viewing the videos to watching the videos while regulating their emotions, $t(84) = 4.7$, $p < .001$, and then from watching the videos while regulating to post-induction emotion regulation, $t(84) = 6.7$, $p < .001$.

Conclusions: Findings from preliminary analyses suggest that our emotion induction/regulation task is effective in inducing and down-regulating negative affect in an online study. We faced several challenges in implementing this study that may inform future online research in this area.

Is Flaxseed Equivalent and/or Synergistic with ACE Inhibition in the Treatment of Chemotherapy Mediated Cardiotoxicity?

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Introduction: Breast cancer is a major public health concern in Canada. Although the current combination of surgery, radiation, and chemotherapy may lead to a cure in the breast cancer setting, the administration of the anti-cancer drugs Doxorubicin and Trastuzumab (DOX+TRZ) is associated with an increased risk of developing heart failure. Little is known on whether flaxseed (FLX) is equivalent to angiotensin converting enzyme inhibition (ACEi) in the prevention of DOX+TRZ mediated cardiotoxicity.

Objective: The specific aim is to evaluate whether FLX is comparable and/or incremental to standard pharmacological therapy using the ACEi perindopril (PER) in the treatment of DOX+TRZ mediated cardiotoxicity.

Methods: In a chronic *in vivo* murine model of chemotherapy mediated cardiotoxicity, DOX+TRZ (8mg/kg and 3mg/kg, respectively) were administered weekly for a total of 3 weeks. Following this, the mice were randomized to daily treatment with FLX (10%), PER (3mg/kg), or FLX+PER (10% and 3mg/kg, respectively) via oral gavage for an additional 3 weeks. Serial echocardiography was performed weekly. At the end of week 6, the mice were euthanized, and histological and biochemical analyses were performed on cardiac tissue.

Results: In mice treated with DOX+TRZ, the left ventricular ejection fraction (LVEF) decreased from 72±4% at baseline to 30±2% at week 6. Treatment with either FLX, PER, or FLX+PER improved LVEF to 52±4%, 54±4%, and 55±3%, respectively ($P<0.05$). Histological analyses confirmed significant disruption of myofibrils, vacuolization, and loss of sarcomere integrity in the DOX+TRZ treated mice. Treatment with FLX, PER, or FLX+PER, however, improved myofibril integrity at week 6 in mice receiving DOX+TRZ.

Conclusion: In a chronic *in vivo* murine model of DOX+TRZ induced cardiotoxicity, although FLX was equivalent to PER in the treatment of adverse LV remodeling, the combination of FLX and PER was not synergistic.

Rational Design of a Next-Generation Anti-Malaria Immunogen Guided by Structural Characterization of Antibody Responses against Major Circumsporozoite Protein Antigen

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Malaria is a global health concern and increasing resistance to antimalarial therapeutics and insecticides has amplified the need for a potent vaccine. A major anti-malaria vaccine target is circumsporozoite protein (CSP), the most abundant surface protein expressed by malaria-causing Plasmodium parasites during the infectious life stage. CSP contains a unique central domain that is structurally disordered and largely composed of repeating NANP motifs. Importantly, antibodies targeting this region have been shown to neutralize Plasmodium infection. However, although RTS,S/AS01, the only WHO-recommended malaria vaccine to date, consists of epitopes from the CSP central region, this vaccine provided only 30-50% waning protection in phase III clinical trials. Previous studies suggest that inclusion of the highly similar NPDP and NVDP epitopes originating from the CSP junctional region immediately upstream of the central domain may improve vaccine efficacy by eliciting potent antibodies that are cross-reactive for both regions. Another emerging strategy to induce higher titres of protective antibodies is through the presentation of a conformationally stabilized antigen. To investigate both approaches, I performed structure-guided vaccine design. Recently, I contributed to the development of a high-resolution molecular understanding of CSP recognition by protective antibodies. Based on these novel findings, I designed next-generation nanocage immunogens presenting sites of vulnerability from both the junctional and central regions of CSP. Following biophysical assessment and validation, the top immunogen designs were evaluated in immunization studies using mouse models. Subsequent examination of the resulting antibody responses revealed improved immunogenicity amongst the engineered immunogens. In particular, structural and functional characterization of elicited monoclonal antibodies will provide valuable insights into the molecular basis of cross-reactivity and antibody features associated with protective efficacy, which, in return, can be used to instruct future rounds of immunogen design. Ultimately, through the iterative process of rational structure-based vaccine design, my studies provide a deeper understanding of the antibody response against CSP, which will guide the design of improved next-generation anti-malaria immunogens. More importantly, my work will also explore fundamental principles of immunogen design, with implications for the development of vaccines against all infectious diseases.

Sirtuin 3 Alters the Cardiac Lipidome, Mitochondrial Protein Acetylation and Mitigates Cardiac Dysfunction in the Mouse

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Introduction: Doxorubicin (DOX) is a chemotherapeutic used in the treatment of various cancers but has dose-dependent cardiotoxic side effects. Previously we showed that DOX decreases expression of the mitochondrial lysine deacetylase, SIRT3 in the mouse heart.

Objective: We hypothesize that DOX impairs cardiac function and energy production via reduced SIRT3 expression and dysregulated mitochondrial protein acetylation. We further hypothesize that increased SIRT3 expression could attenuate DOX-induced cardiac dysfunction by altering acetylation of enzymes involved in lipid remodeling and metabolic processes.

Methods: Mice expressing cardiac restricted full length M1-SIRT3 (mitochondrial localized), and short M3-SIRT3 (lacking localization signal) received saline or DOX injections of 8 mg/kg body weight for 4 weeks and were compared to non-transgenic (Non-Tg) littermates. Transthoracic echocardiography was performed on all mice (n=10). Total cardiac lipids were isolated from DOX treated cardiac tissue by chloroform:methanol extraction and global lipid analysis was performed by QTRAP LC-MS/MS (n=6). Cardiac mitochondria were isolated, and an anti-acetylated lysine antibody was used to enrich for tryptic digested peptides containing Acetyl-K and analyzed by QTRAP LC-MS/MS (n=6).

Results: DOX reduced left ventricular posterior wall thickness and ejection fraction dysfunction in Non-Tg mice, while increased expression of M1-SIRT3 and M3-SIRT3 transgenes preserved these cardiac parameters ($P<0.05$) following DOX treatment. Triglycerides and phospholipids (PE, PI) were decreased in DOX treated hearts while phosphatidylserine, sphingomyelin (SM) and ganglioside (GM3) lipid species were increased ($p<0.05$). A negative correlation between decreased cardiac output and increased GM3 24:1 ($R=-0.62$, $P<0.05$), PS 38:4 levels ($R=-0.81$, $P<0.005$) and SM 35:1 ($R=-0.65$, $P<0.05$) was identified. DOX increased the acetylation of 36 peptides ($p<0.05$) involved in metabolic processes (eg. IDH2, HADHA, $P<0.05$) while M1-SIRT3 expression reduced peptide acetylation.

Conclusion: Increased SIRT3 expression in the heart rescued DOX-induced cardiac dysfunction. DOX-induced cardiac dysfunction involved alterations in cardiac lipids and acetylated proteins that could be partially restored by increased SIRT3 expression in the heart.

Gene Expression Trends in HIV+ T Cells and Monocytes Associated with Decreased HIV Set-Point Viral Load

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HIV set-point viral load (spVL) is strongly associated with HIV disease progression, with a portion of the variance attributable to host genetics. The majority of large-scale HIV host genetic studies have focused on individuals of European ancestry. However, by focusing primarily on European ancestry, a large proportion of global genetic diversity is neglected. To address this, our lab conducted a large genome-wide association study of HIV spVL in individuals of African ancestry and identified a novel region on chromosome 1 that is strongly associated with control of HIV replication. The top variant in the region, rs59784663 ($p= 6.4E-10$, $\beta = -0.3$), and variants in linkage disequilibrium overlap three coding genes: CHD1L, PRKAB2, and FMO5. Fine-mapping of the region shows that the GWAS signal is enriched for functional elements around CHD1L, involved in DNA repair and chromatin remodeling, and implicates it as the likely causal gene. However, the mechanism linking these genetic variants with CHD1L regulation has not been fully elucidated. To address this, we sorted cryopreserved PBMCs with different genotypes (N=24) at the top associated SNP into CD4+ T and monocyte fractions. CD4+ T cells were split into unstimulated and stimulated (CD3+CD28+ beads for 8 hours) fractions before RNA collection, while monocytes remained unstimulated. Gene expression differences in CD4+ T cells and monocytes were measured using RT-qPCR assays for CHD1L, PRKAB2, and FMO5. No significant differences in gene expression were observed, but there is a trend that CHD1L expression was decreased in individuals carrying the variant allele at rs59784663 in stimulated CD4+ T cells ($p < 0.15$) and monocytes ($p < 0.15$). We also tested whether genetic variation in the chromosome 1 region has genome-wide effects in CD4+ T cells. Preliminary RNA sequencing data from the CD4+ T cell fractions (N=16) identified significant downregulation of ZNF443, involved in transcriptional regulation, in stimulated CD4+ T cells carrying the variant allele at rs59784663 ($p < 0.001$). No significant differences were seen for CHD1L, PRKAB2, or FMO5. Ongoing work will increase the sample size of individuals carrying the reference and alternate alleles to increase the power of detecting significant differences.

In vivo Analysis of Aspirin on the Vaginal Immune Landscape

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Introduction: It is known that the efficacy of HIV-preventing pre-exposure prophylaxis (PrEP) is significantly lowered by inflammation such as by bacterial vaginosis (BV). PrEP strategies must include ways to mitigate genital inflammation to remain effective. Aspirin (ASA) is an anti-inflammatory that is widely available, affordable, and accepted. Our recent human cohort study found that ASA directly affects mucosal immunity by reducing the number of HIV target cells (CD4+CCR5+ T cells) present in the FGT. *Mobiluncus mulieris* is a BV-associated anaerobic bacteria that we have demonstrated can disrupt the protective epithelial barrier in the female genital tract (FGT) of mice, increase HIV penetrance, and activates and recruits T cells. These readouts may be improved by ASA.

Methods: C57BL/6 mice were challenged with *Mobiluncus mulieris* to study the impact of ASA on inflammation and immune cells in the FGT. Female mice were given ASA daily and confirmed by high-performance liquid chromatography (HPLC). Immunohistochemistry visualized T cell localization, Lucifer Yellow dye evaluated FGT epithelial barrier integrity, and flow cytometry enabled immune phenotyping. Humanized BLT mice were used to assess whether ASA treatment attenuates HIV infection by challenging intravaginally with HIV for 8 consecutive weeks while dosing ASA daily.

Results: ASA levels were validated and confirmed dose dependent by HPLC. Bacterial challenge with *Mobiluncus mulieris* elevated CD4+ T cell counts in the FGT by 3.6 fold and increased the absolute cell counts of subsets expressing the HIV co-receptor CCR5 by 3.1 fold and activation marker CXCR6 by 3.7 fold. Phenotypes of cells in the spleen were not altered similarly, suggesting tissue-specific effects. In the humanized BLT model of HIV infection's *Mobiluncus mulieris* challenge group, ASA delayed systemic viremia from 21 days to 35 days as determined by qPCR.

Significance and Impact: This study investigates whether ASA can reduce FGT inflammation and improve vaginal epithelial barrier function *in vivo*. This study aims to directly evaluate the mechanism of how ASA can possibly affect T cell activation and co-receptor expression in the FGT. This will be the first *in vivo* mouse model to show how anti-inflammatory drugs, like ASA, can help impede HIV acquisition and transmission.

Effect of Chromogranin A on Colonic Epithelial Cells in Experimental Model of Ulcerative Colitis

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Introduction: Chromogranin A (CHGA), a pro-peptide secreted by mucosal intestinal epithelial cells, is highly expressed in colonic tissues of patients with ulcerative colitis (UC), an acute and incurable inflammatory disorder of the colon. Increased CHGA level is shown to correlate with UC disease activity and severity. Furthermore, complete deletion of CHGA results in reduced pro-inflammatory markers known to disrupt the colonic mucosal integrity. However, the effect of CHGA on colonic epithelial barrier function and gut mucosal healing process remain unknown. Here, we characterized the impact of lack of CHGA on colonic epithelial cells function and gut mucosal regeneration potential in animal models of acute colitis induced by dextran sulfate sodium (DSS).

Methods: Colonic tissues were isolated from C57BL/6 (wild-type) and C57BL/6 CHGA knockout (CHGA-/-) mice models treated with 5% DSS or untreated (control). Genetic markers associated with colonic epithelial cells' antimicrobial activities, WAP 4-disulfide core domain 2 (WFDC2) and cathelicidin-related antimicrobial peptides (CRAMP), epithelial barrier function bacterially-modulated proteins mucin 2 (muc2) and resistin-like molecule β (RELMβ), Tuft cells-derived anti-inflammatory cytokine interleukin-25, and fast-cycling stem cells (Lgr5+), reserve stem cells (HOPX+) and fetal-like stem cells (Ly6a+) were evaluated using qRT-PCR.

Results: The lack of CHGA significantly modulated goblet and Tuft cells functions in DSS-mediated colitis. The absence of CHGA significantly increased goblet antimicrobial-associated genes WFDC2 (152.1-250.1, p=0.04) and CRAMP (267.0-350.1, p=0.02). This coincided with a significant decrease of muc2 (318.4-112.1, p=0.002), RELMβ (3.14-0.47, p=0.003), lesser mucosal infiltration by granulocytes, and elevated IL-25 in CHGA-/ colitic mice. DSS-mediated colitis significantly down-regulated Lgr5+ expression and increased Ly6a+ in both colitic wild-type and CHGA-/- mice ($p<0.0001$). Moreover, the lack of CHGA was associated with preserved HOPX expression in colitic mice.

Conclusion: Lack of CHGA increases goblet and tuft cells' antimicrobial and anti-inflammatory functions, promotes gut mucosal regenerative potential, enhances the colonic mucosal integrity, and protects from colitis damage.

Clinical Features of Central Sensitization and Prediction of Pain-related Quality of Life after Endometriosis Surgery

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Introduction: Despite surgery being an effective treatment for endometriosis pain, 20-38% of patients experience no relief, and 50% undergo reoperation within 5-years. Identifying predictors for surgical success is crucial yet challenged by a lack of standardized clinical variables and the complexity of endometriosis pain. Central sensitization (CS) is integral in the endometriosis pain continuum, even after lesions are removed. Therefore patients with CS are less likely to benefit from surgery resulting in poorer pain-related quality of life (QoL). This study investigated preoperative features of CS as predictors of pain-related QoL after surgery in a cohort of clinically phenotyped participants from our centre for pelvic pain/ endometriosis.

Methods: The study utilized prospective registry data. Participants (≤ 50-years) had suspected/ diagnosed endometriosis, completed the pain subscale of the Endometriosis Health Profile (EHP-30) questionnaire (for QoL) preoperatively and at follow-up (1-2 years), and underwent endometriosis surgery (fertility-sparing versus hysterectomy). Outcomes were defined as: (i) follow-up EHP-30 scores (0-100%); and (ii) poor outcome (EHP-30 scores ≥60%) based on the 75th centile cutoff in North American studies. Features of CS included were: abdominal wall trigger point (AWTP), painful bladder syndrome (PBS), pelvic floor myalgia, irritable bowel syndrome (IBS), General Anxiety Disorder (GAD-7) scores, pain catastrophizing scale (PCS) scores, and Patient Health Questionnaire (PHQ-9) scores for depression. Controlling for the type of surgery and preoperative QoL, the relationship between each feature and the outcomes were assessed using linear and logistic regression respective to the outcome definition.

Results: Included were 433 participants. Linear regression showed that AWTP (p=0.007), PBS (p=0.038), and pelvic floor myalgia (p=0.028) correlated with higher follow-up EHP-30 scores. Also, PHQ-9 (p<0.001), GAD-7 (p<0.001) and PCS (p=0.009) scores positively correlated with follow-up EHP-30 scores. Logistic regression showed that higher preoperative GAD-7 (p=0.019) and PCS (p=0.027) scores correlated with a greater likelihood of a poor outcome after surgery.

Conclusion: This study identified clinical features of CS that predict poorer QoL after endometriosis surgery which may lead to a future clinical prediction model for pain outcomes after endometriosis surgery.

Breastfeeding in the Pandemic Study (BIPS): A Qualitative Analysis of Breastfeeding Experiences among Mothers from Canada and United Kingdom

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Introduction: The COVID-19 pandemic has changed the way breastfeeding support is offered, both in hospital and in the community. Previous research shows that the COVID-19 pandemic has had both negative and positive impacts on breastfeeding experiences. However, no research has looked specifically at first time mothers' experiences of breastfeeding during the pandemic or compared experiences of mothers living in different countries.

Methods: This is a qualitative, descriptive study, including 10 semi-structured virtual interviews with first-time mothers who gave birth during the COVID-19 pandemic and are living in Canada or the United Kingdom. Data were collected between March 2021 and May 2021. All interviews were coded inductively using thematic analysis.

Results: Mothers experienced both challenges and benefits related to breastfeeding in the pandemic; however, challenges were discussed more often. Our results centered on the overarching theme of 'all on mother', which described the all-encompassing responsibility of caring for baby without adequate professional or social support. Five further themes were identified, including: 1) accessing and advocating for health care, 2) stress and mental health, 3) becoming a mother in isolation, 4) social support, and 5) breastfeeding baby. Mothers reported that lack of health care and social support created challenges in their breastfeeding journey. Many mothers reported receiving virtual breastfeeding support, which was largely experienced as unhelpful, with some exceptions. In addition, some mothers reported that the pandemic amplified the vulnerability and difficulties of being a new mother. Finally, some mothers reported fewer distractions from visitors and more one-on-one time with their infant, which helped them to establish breastfeeding and a strong mother-infant bond. Similar themes emerged for mothers from either country.

Conclusion: This study highlights the breastfeeding experiences of mothers during the pandemic in Canada and the UK. In both countries, new mothers need consistent, reliable health care and social support when breastfeeding. This study supports the need to protect professional and social breastfeeding support in the midst of a global emergency and beyond to ensure positive breastfeeding experiences for both mother and baby.

Assessment of Stem Cell Response and Repair using *in vitro* Radiation-Induced Salivary Gland Injury Model

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Background: The American Cancer Society reported 54,010 new cancer cases of the oral cavity and pharynx in 2021. Radiotherapy for cancer inherently causes damage to healthy tissues (such as salivary glands) within the radiation zone. Damage to saliva secreting cells leads to a decrease in salivary flow, which causes oral dryness. It increases the chances of oral and systemic infections, dental caries, pain, swallowing difficulties, and malnutrition leading to considerable morbidity lasting several years. With no gold standard treatment available, stem cell therapies are being explored.

Study objectives: The main objective is to study the radioprotective and regenerative effect of mesenchymal stem cell (MSC) secretome in *in-vitro* cell culture and organotypic slice culture model to determine the active proteins.

Methods: Primary salivary cells are cultured after enzymatic digestion of human salivary gland biopsies. Additionally, 100–150-micron thick slices are sectioned from salivary gland samples using a vibratome. The cells and tissue slices are radiated after an appropriate culture period, followed by treatment with the MSC-derived conditioned media (used here as the MSC secretome). DNA damage marker gammaH2AX immunofluorescence for recovery from radiation-induced damage, superoxide dismutase enzyme activity to assess the oxidative stress, and viability studies are conducted to assess the action of MSC secretome. Moreover, salivary gland-specific markers and recovery proteins are assessed through qPCR and western blots.

Outcome: Slice cultures remained viable for over a week. 5 to 7.5Gy radiation reduced the viability of the tissues and cells to 40-60 % within 24 hours. An increase in live cell number and intensity was observed in MSC conditioned media treated slices. Future actions: The outcomes thus assessed will be used further to narrow down the active components of the MSC-derived proteins. It will ensure minimal side effects of stem cell therapeutics and accelerate its clinical translation.

Analytical and Clinical Validation of 3′ RACE RT-qPCR Assay for Detection and Quantification of Hepatitis B Virus (HBV) Serum RNA

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Background: Over 257 million people worldwide are living with chronic HBV (CHB) infection who require monitoring of viral activity and disease progression. Serum HBV RNA is a promising new biomarker in CHB management and has been found to be a surrogate marker of intrahepatic cccDNA transcriptional activity; however, no standardized method for HBV serum RNA quantification has been established.

Objectives: In this study, we have developed and performed analytical and clinical validation of a 3′ RACE-RT-qPCR assay for quantification of HBV serum RNA.

Study Design: The 3′ RACE RT-qPCR method was developed using a synthetic pgRNA standard and published primers. The analytical limit of detection (LoD) and quantification, linearity, and inter- and intra-assay repeatability and reproducibility was conducted using a panel of HBV DNA positive serum specimens and synthetic pgRNA. Clinical specificity and sensitivity analyses involved non-HBV viral hepatitis samples and specimens from various patient populations. An intra- and inter-laboratory ring trial involving three laboratories was completed.

Results: The 3′ RACE-RT-qPCR assay dynamic range was determined to be 25 to 10^8 copies/µL of synthetic RNA. Theoretical and measured HBV serum RNA quantities from a serially diluted sample showed excellent linearity (R^2=0.9995). The inter- and intra-assay repeatability were 98.32% and 98.01%, respectively. Clinical specificity was 100%. In treated patient samples, the clinical sensitivity, based on the expected detection of pre-genomic RNA (pgRNA) in relation to other HBV biomarkers, was 90%. Ring trial results among two laboratories demonstrated high agreement (κ=0.867) whereas one laboratory demonstrated low agreement (κ =0.016) with the other laboratory results, likely due to differences in template isolation.

Conclusions: Our methodology for the quantification of HBV serum RNA is specific and repeatable and employs a biologically relevant RNA standard. Standardization of the methodology is required to facilitate the comparison of studies and better understand the clinical role of this novel biomarker in HBV management.

Neutralizing Antibody for Oxidized Phosphatidylcholine Prevents Airway Inflammation and Small Airway Dysfunction in Allergen Challenged Mice

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Rationale: There is critical need to develop therapeutic options for asthmatics with poorly controlled disease. In human subjects and mice, we reported that allergen challenge induces accumulation of pro-inflammatory oxidized phosphatidylcholine (OxPC) moieties in parallel with the emergence of airway hyperresponsiveness (AHR). We hypothesized that exogenous E06, a natural IgM antibody that neutralizes pro-inflammatory properties of OxPC, can ameliorate allergen-induced airway inflammation and AHR in adult mice.

Methods: House dust mite (HDM)-challenged BALB/c mice (female, 6-8 weeks) were randomized into 3-treatment groups (N=4 per group): vehicle (PBS); [1 µg] and [10 µg] E06. We used age-matched allergen-naïve mice as controls. PBS or E06 were instilled intranasally (i.n.) 1 hr before each HDM challenge (25 µg/mouse, i.n., daily for 2 weeks (5 days/week)). Forty-eight hours after the last treatment, pulmonary function in response to inhaled methacholine was assessed using a flexiVENT small animal ventilator. We also performed manual total cells counts and immunophenotype analysis of inflammatory cells (eosinophils, neutrophils, alveolar and interstitial macrophages, CD3+, CD4+, CD8+, and B cells) in bronchoalveolar-lavage (BALF) using an Attune NxT flow cytometer, and Flow-jo software to identify each cell sub-type. Data were analyzed by one-way ANOVA with Dunnett's post-hoc test.

Results: HDM challenge induced significant accumulation (11.4 fold) of total immune cells in BALF (Table-1), and this correlated with a significant increase in AHR (ie. 60% and 100% higher methacholine-induced central airway and peripheral lung tissue resistance, respectively). Prophylactic treatment with low-dose E06 [1-µg] lend to trend for reduced total cell number, but did have a significant selective reducing effect on the number of neutrophils and CD3+ lymphocytes cells, decreasing them by 58% and 26%, respectively, compared to HDM challenged (vehicle) controls. High-dose E06 [10-µg] significantly reduced accumulation of total inflammatory cells in BALF by 43%,.associated with a significant reduction in neutrophil count by 79%, alveolar macrophage number by 80%, and lymphocyte number (CD3 (32%), CD4 (32%), CD8 (43%)) compared to vehicle (Table-1). The impact of E06 [10 µg] on HDM-induced lung inflammation correlated with a 17% reduction in methacholine-induced peripheral lung resistance, however, central airway resistance was unaffected by E06 treatment.

Conclusion: Prophylactic treatment of mice with intranasal E06, an OxPC-neutralizing antibody, can abrogate allergen induced airway neutrophilia, lymphocyte infiltration and alveolar macrophage number, an effect that correlates with suppressed small airway responsiveness. This implicates OxPCs a regulators of small airway dysfunction and indicates that OxPC may be a treatable target for asthma.

Profiling BMPR2-Derived Endothelial Circular RNAs in Pulmonary Arterial Hypertension

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Background: Circular RNAs (circRNAs) are a novel class of RNAs that act through a variety of mechanisms from protein and miRNA sponging, to the translation of protein products. circRNAs are known regulators of critical biological processes, including angiogenesis and cell proliferation, yet the role they play in many diseases, such as pulmonary arterial hypertension (PAH), a disease strongly associated with mutations in the type II bone morphogenetic protein receptor (BMPR2), has not been well defined. Here, we mapped the circRNA expression profile of human pulmonary artery endothelial cells (HPAECs), with and without BMPR2 loss, and explored the contribution of these novel transcripts to endothelial dysfunction, a hallmark of PAH.
Methods and Results: Using deep RNA-sequencing of BMPR2-silenced or control HPAECs, we screened 92,369 annotated circRNAs across the human genome to identify 425 circRNAs that are abundantly expressed in HPAECs, including 2 of the 26 annotated circRNAs from within the BMPR2 locus (hsa_circ_0005078 and hsa_circ_0003218). hsa_circ_0005078, derived from exon 12 of the BMPR2 gene, was the most significantly differentially expressed circRNA with BMPR2 silencing. qPCR analysis of blood outgrowth endothelial cells from PAH patients and controls revealed that while both known linear forms of BMPR2 were reduced in mutation-bearing PAH patients, neither BMPR2-derived circRNA was affected, suggesting these transcripts serve alternative functions and are not subject to the same nonsense mediated decay mechanisms as their linear counterparts. Functionally, siRNA-mediated silencing of hsa_circ_0005078 enhanced the proliferative rate of HPAECs by 2.02-fold, while loss of hsa_circ_0003218 increased the susceptibility of HPAECs to an apoptotic stimulus by 2.87-fold. Interestingly, co-silencing of hsa_circ_0005078 and linear BMPR2 mRNA eliminated the proliferative effect achieved by silencing hsa_circ_0005078 alone. We continue to investigate the functional relationship between linear and circular BMPR2 transcripts and the pathways through which these BMPR2-derived circRNAs exert their influence.
Conclusions: We present the first profile of circRNA expression in HPAECs, including two circRNAs derived from the BMPR2 locus. hsa_circ_0005078 and hsa_circ_0003218 are novel essential regulators of endothelial proliferation and apoptosis, two hallmarks of the endothelium in PAH.

Comparison of Three Forms of Non-Invasive Spinal Cord Stimulation on Motor Performance and Corticospinal and Spinal Excitability in Persons with Spinal Cord Injury or Intact Controls

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Introduction: Spinal cord injury (SCI) is a severe central nervous system injury causing motor dysfunction and functional impairments. A potential means to improve motor function after the injury is facilitation of remaining neural networks. Electrical stimulation of the cord could modulate multiple levels of the nervous system and facilitate motor function. Three main types of non-invasive stimulation have been used to improve motor function, but they have never been directly compared: transspinal direct current stimulation (ts-DCS), trans-spinal electrical cutaneous stimulation (ts-ECS), and trans-spinal magnetic transcutaneous stimulation (ts-MCS). Thus we aimed to evaluate these methods in the same participant by comparing their effect(s) on facilitation of voluntary and reflexevoked motor performance.
Methods: Surface EMG electrodes were placed bilaterally on muscles of the upper and lower limbs. Baseline measurements were taken during reflex activation of the Biceps brachii muscle to assess spinal excitability and reflex-evoked muscle activity. Transcranial magnetic stimulation evoked potentials (MEP) assessed descending corticospinal motor excitability. After spinal stimulation (either ts-DC or ts-ECS or ts-MS or sham) was applied to the lower thoracic spinal cord for 15 minutes, outcome measures were re-assessed. After stimulation, participants performed a modified Wingate peak test on an arm ergometer. Total distance, duration, power output, heart rate, and ratings of perceived exertion (RPE) were recorded.
Results: Preliminary data from participants tested to date showed an increase in excitability after ts-ECS for spinal-evoked and brain-evoked descending corticospinal responses of the upper and lower limbs. Exercise responses are being analyzed.
Conclusion: Previous spinal stimulation studies have shown increased lower limb spinal reflex excitability with lower thoracic spinal stimulation. Here, we show that thoracic spinal stimulation can also facilitate upper limb spinal reflex-evoked muscle excitability, suggesting an ascending pathway from thoracic spinal regions to neurons controlling movement of the upper limbs. This pathway may be a future therapeutic stimulation target to improve upper limb function after spinal cord injury.

Do Socioeconomic Measures Improve Prediction of Cardiovascular Disease Hospitalization?

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Introduction: Risk prediction models for cardiovascular disease (CVD) hospitalization have improved prediction accuracy when individual-level measures of socioeconomic status (SES) are considered. It is unclear how validated, area-level SES measures, available at the population level, might improve prediction of CVD hospitalization. Our objectives are to (1) test the incremental predictive value of area-level SES on time to CVD hospitalization, and (2) compare the incremental predictive performance of different areal-level SES measures on time to CVD hospitalization.

Methods: This study is a retrospective cohort design using Manitoba administrative health records from fiscal years 2014/2015 to 2019/2020 and area-level SES measures derived from 2016 Statistics Canada Census data. The study cohort will comprise individuals 40 years of age or older as of April 1st, 2016 who will be followed until CVD hospitalization, death, migration out of province or end of study period (March 2020). Covariates will include age and sex, comorbid health conditions, prior healthcare use, prescribed medications, and one or more area level SES measures. Cox proportional hazards models will be used to assess model discrimination and calibration.

Results: The addition of an area-level SES measure is expected to improve prediction of CVD hospitalization, and the greatest discrimination and calibration improvement is expected for the Canadian Index of Multiple Deprivation (CIMD) or the Socioeconomic Factor Index (SEFI) - Version 2 (SEFI-2). The CIMD includes the most unique and updated concepts of marginalization and inequality (situational vulnerability, ethnocultural composition, residential stability, and economic dependency). Additionally, the CIMD is produced for different levels of geographic classification and has been adapted to the context of the prairie region. The SEFI-2 may produce the greatest discrimination and calibration improvement because it is based on the knowledge and expertise of SES characteristics most relevant to the Manitoba population, including income, education, single parent households, and unemployment rate.

Conclusion: This study will provide further insight into the socioeconomic factors that impact Manitobans' cardiovascular health and identify populations that are at greatest risk. The results will be of value to epidemiologists, who conduct CVD surveillance and outcomes in at-risk populations, and policy makers that develop disease prevention programs.

Family Physicians' Perspectives on the Impact of COVID-19 on Preventative Care in Primary Care: Findings from a qualitative study

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Introduction: Family physicians provide comprehensive care while navigating the challenge of competing demands throughout their daily practice. Existing challenges were heightened during the COVID-19 pandemic when family physicians were required to modify their practice to comply with changing policy and public health measures. Specifically, preventative care, such as routine screening for disease detection, was often delayed due to their designation as non-urgent interventions. To describe family physicians' experience of preventative care during the COVID-19 pandemic and their perspectives on the impacts of COVID-19 on disease detection, prognosis, and workflow in primary care practice.

Methods: We conducted semi-structured, qualitative interviews with 68 family physicians across four provinces in Canada (i.e., Newfoundland and Labrador, Nova Scotia, Ontario, British Columbia) between October 2020 and June 2021 as part of a larger multiple case study. These interviews broadly explored the roles and responsibilities of family physicians in the COVID-19 pandemic. Interviews were coded thematically. Codes from the larger study were analyzed further using an iterative, phased process of thematic analysis.

Results: We identified four major themes: (1) moral distress caused by challenges with primary care management; (2) availability and accessibility of preventative testing/screening; (3) patient fear, hesitation, and uncertainty; and (4) managing the backlog as normal operations resumed. Physicians voiced concerns with managing patient needs for routine screening when faced with limitations surrounding in-person visits and testing availability. Patients were also postponing their own primary care appointments, which increased physician workload as pandemic restrictions were lifted. Change in the availability and delivery of routine preventative care led to a perceived increased demand for acute/emergency services; and family physicians used various approaches to ensure routine care for all populations resumed following the pandemic closures.

Conclusion: Family physicians reduced their provision of upstream care during the COVID-19 pandemic. The presence of missed or delayed preventative care interventions has the potential to influence individual and population health during the recovery stage of the pandemic. Family physicians contribute direct insight to primary care delivery that can contribute to pandemic planning to ensure preventative care interventions are sustained during future public health emergencies.

Co-producing Collaboration across Primary Care Workplaces

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Introduction: Recent policy changes in primary care are asking healthcare professionals to collaborate. Although counter-intuitive, collaboration can be explored through boundaries. Changing boundaries in this study included: partnerships, roles, and physical spaces. Boundary objects are used to bridge across boundaries and in this study included: personal protective equipment, funding applications, and virtual technology.
Objectives: 1. To explore when collaboration is happening, who and what are involved in these collaborations, and what sort of action is taken. 2. To demonstrate how collaboration in primary care workplaces is constantly being created, recreated, negotiated and renegotiated through exploration of boundaries.
Research Question: How can the awareness of the co-production of boundary objects and boundaries shift the collaboration that occurs in primary care workplaces?
Methods: Multi-faceted methods were used to compare primary care collaborations across three time periods amplified by COVID-19 (January-August 2020) within interior British Columbia. First, 15 virtual semi-structured interviews with clinical, administrative, and executive informants were conducted to capture their collaborative experience. Second, discourse analysis of the Public Health Act, Public Health Orders and modelling. Phase three included member checking with the informants to discuss themes.
Results: This paper demonstrates the continual changing nature of collaboration as boundaries and boundary objects shifted. The findings highlight that different boundary and boundary object were co-produced across the three time periods. Examples of these changes include: (Pre-Wave 1) swallowing assessments, health service planning, and hallways; (Wave 1) PPE, cleaning, and crisis; (Post Wave 1) telehealth, funding, and communication.
Discussion: One limitation is that those who agreed to be informants may view collaboration positively. A delimitation was primary care services provided by the Nation were not included due to capacity from the Nation. Through member checking, informants felt this change in perspective on collaboration was useful however, were unclear how to apply it. In response, the researcher developed a series of exercises to support implementation.
Conclusion: By increasing awareness of shifting boundaries and boundary objects, healthcare professionals are better equipped to engage in collaboration. By better understanding how collaboration is co-produced this research could support primary care teams to deliver collaborative policy direction more efficiently.

RanBP2/Nup358 Enhances MicroRNA Activity by Smoylating Argonautes

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In the nucleus, messenger RNAs are synthesized and packaged into messenger ribonucleoprotein (mRNP) complexes, which undergo maturation events where the bound proteins are exchanged or post-translationally modified. Since all mRNPs need to cross the nuclear pore before translation, this provides a chance for nucleoporin proteins to modulate mRNP maturation and thus regulate gene expression. Ran-binding protein 2 (RanBP2), also known as Nucleoporin 358 (Nup358), is a SUMO (small ubiquitin-like modifiers) E3-ligase that resides at the cytoplasmic face of the nuclear pore. Mutations in RanBP2 are associated with a pediatric disease called acute necrotizing encephalopathy 1 (ANE1). Upon viral infection, such as influenza and SARS-CoV-2, ANE1 patients experience excessive cytokine production, causing neuropathology, coma, seizures and death. However, it is unclear how RanBP2 and its ANE1-associated mutations affect cytokine production. We showed that RanBP2 represses the translation of mRNAs that encode for ANE1-associated cytokines, such as interleukin6 (IL6). We found that Argonautes (AGOs), which are the core components of the RNA-induced silencing complex (RISC), are almost exclusively nuclear in transformed cancer cell lines as well as primary nasal epithelial cells. We showed that in the nucleus, AGOs are recruited to the IL6 mRNP through let-7 microRNA. Following nuclear export, RanBP2 sumoylates mRNP-bound AGO1, and this modification stabilizes the AGO-mRNA complex, enforcing silencing of the IL6 mRNA. When sumoylation is inhibited, AGO1 is ubiquitinated and RISC activity is impaired. These results present one of the few examples of how mRNPs undergo maturation at the nuclear pore, and how these maturation events affect the ultimate fate of the mRNAs in the cytoplasm. These findings also open the possibility that cells regulate ANE1-associated cytokines via the interplay between RanBP2, microRNA-mediated silencing pathways, and post-translational regulation of proteins.

Global Antimicrobial Resistance Regimes and Collective Action

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The ongoing COVID-19 pandemic has simultaneously proven that microbes care little for national borders and that the world's ability to cooperate around emerging health threats needs improvement. Fortunately, many political leaders now recognize the need to strengthen global health governance, and reforms in the wake of COVID-19 present an opportunity for bold action on other infectious disease threats such as antimicrobial resistance (AMR). AMR occurs when existing human pathogens develop resistance to our arsenal of antimicrobial medicines, effectively rendering essential medicines like antibiotics ineffective and turning curable infections into untreatable, deadly diseases. Resistant pathogens that arise from AMR also have the potential to spread into deadly pandemics, and the world is no more prepared to respond to these threats than it was for COVID-19. Much is at stake: current estimates suggest that annually, 5,400 people die from AMR in Canada and 1.27 million people die from AMR globally (4,5). If nothing is done, the global death toll is expected to climb to 10 million a year by 2050 and result in a total economic cost greater than the 2008 global recession. Urgent global collective action is needed to save these lives, prevent this potential global economic loss, and ensure that we do not further erode modern medicine's ability to treat infectious diseases. While there are national policies known to reduce antimicrobial use, no country can wholly mitigate the transnational threat of AMR on its own. Global cooperation is essential and well-designed global strategies and institutions can help countries collectively accomplish public health goals that they could not possibly accomplish alone. This social challenge means that we need to craft effective global governance mechanisms to mobilize collective action and support the coordinated implementation of national policies around the world. This research rigorously evaluates various political strategies that WHO, other United Nations agencies, national governments, NGOs, and individual leaders can use to improve the global response to AMR. In doing so, it directly informs WHO's current AMR efforts, the design of future evidence-informed global health strategies, and the science of global governance more broadly.

Exploring Policy Nursing in the Canadian Context: Barriers and Opportunities for Nursing Leadership in Policy and Governance

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Introduction: Global calls for nursing leadership in health and social policy are growing significantly, as the World Health Organization and other key international agencies acknowledge the contribution of nurses to global health and the necessity of including nurses in health care leadership as representatives of the largest health care workforce in the world. Indeed, the Government of Canada has recently launched recruitment for the first federal Chief Nursing Officer, signally national alignment with the global imperative to have nursing practice and knowledge at the policy table. However, there is a marked absence of literature, evidence, and practice supports to enable nurses actively engaged as leaders in policy environments.
Methods: In the context of the International Council of Nurses Global Nursing Leadership Institute (GNLI) programme, a national project was undertaken in Canada to examine the structural barriers, knowledge gaps, and opportunities for nurses practicing in policy roles. A series of fourteen one-on-one exploratory conversations were undertaken to explore the current state of policy nursing practice, national and provincial/territorial infrastructure for policy nursing, and opportunities for future growth of nursing leadership in this area. Analysis of data arising from these exploratory conversations was achieved using thematic analysis, with the Advocacy Coalition Framework as a grounding theoretical orientation.
Results: Seven key findings emerged from these exploratory conversations: the absence of policy knowledge, low levels of skill in policy work, the loss of consistent nurse representation in formalized policy roles in governments, little to no cross over of policy knowledge into nursing knowledge, the uniqueness of policy nursing as a nursing practice, the absence of interprovincial and interagency coordination, and available actions to grow the impact of nursing in health and social policy.
Conclusion: The novel use of policy science to ground an examination of nurses as policy actors provides unique insights into how nurses are currently engaging in nursing work at policy tables and the ways in which investments in policy knowledge, education for policy practices, and cascading structures within governments and nursing associations can enable nurses to answer the call for not only participation but true leadership in health and social policy.

Using Genomics to Guide the Development of Strategies to Predict and Prevent Cisplatin-Induced Ototoxicity

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Introduction: Cisplatin is a major chemotherapeutic agent and ototoxicity (hearing loss) is the most common adverse drug reaction associated with cisplatin treatment. Previous research has shown that 38-47% of the variability in this adverse drug reaction can be attributed to genetics and that susceptibility can be traced to multiple loci. Given the important role of genetics in cisplatin-induced ototoxicity (CIO), genomic data can be used to predict and prevent CIO.

Methods: Given that the genetic architecture of hearing loss and CIO overlap, we used large-scale hearing loss data from the UK Biobank to identify genetic factors contributing to diverse hearing traits. We used SBayesR to develop a polygenic score to predict hearing loss and S-MultiXcan to perform a transcriptome-wide association study to identify heritable gene expression profiles that were associated with hearing loss. We subsequently refined these results using publicly available data to increase their relevance to (i) hearing - based on murine inner ear single-cell RNA sequencing data and (ii) CIO - based on evidence of a drug-gene relationship, indicated by a correlation ($P<0.01$) between cisplatin cytotoxicity and gene expression. The relevance of the polygenic score to a pediatric CIO cohort, with various filters, was examined using ReACt. Additionally, CIO-associated gene expression profiles, with various filters, were compared to the gene expression profiles induced by various perturbagens using CLUE.

Results: We found that a hearing loss polygenic score was significantly associated with CIO ($P=7.6x10-8$). The significance of these results increased when only variants impacting genes that are expressed in the inner ear were included ($P=1.4x10-10$). Drug repurposing analyses revealed that the most promising otoprotectants (i.e., those showing the highest median connectivity scores) were identified for gene expression profiles that had undergone the most stringent filtering for CIO.

Conclusion: To the best of our knowledge, this is the first polygenic score developed to predict the risk of CIO. In addition, our novel filtering strategy showed an increase in confidence for drug repurposing results when applied to these data. Future clinical relevance includes the prediction of additional chemotherapy mediated adverse drug reactions using polygenic scores and investigating potential otoprotectants.

Characterizing How 1,4-Dihydropyridines Inhibit Transforming Growth Factor Beta Signalling

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Introduction: Transforming growth factor beta (TGFβ) is a multifunctional cytokine involved in various cellular processes, such as homeostasis, cell growth, and apoptosis. However, aberrant TGFβ signalling fuels epithelial-to-mesenchymal transition (EMT) and metastasis and is a distinctive trait of many epithelial-derived tumors, including lung carcinomas. Although a novel class of 1,4-dihydropyridines (DHPs) has been shown to specifically inhibit TGFβ receptor type II (TβRII) during cardiomyogenesis, the effect of DHPs on non-small cell lung tumorigenesis has not been investigated. This led us to our overall goal to characterize how DHPs affect TGFβ-related functional outcomes in non-small cell lung cancer (NSCLC) cells.

Methods: NSCLC cells were treated with DHPs with or without TGFβ1. Protein lysates were subjected to SDS-page and protein levels were measured by western blot. Western blotting for phospho-smad2/3 (P-smad2/3) assessed the state of TGFβ signalling, and N- and E-cadherin protein levels assessed EMT. Finally, TβRII protein levels were observed. Immunofluorescence microscopy was used to visualize trafficking of TßRII in a MvLu1 cell line containing an HAtagged TßRII. EMT was further analyzed by fluorescence microscopy where DHP-treated A549 cells were stained with phalloidin to visualize how TGFβ-induced stress fibers are affected. Finally, qPCR was utilized to determine if TβRII mRNA levels were affected by DHPs.

Results: We observed that DHPs reduced TGFβ-dependent signaling in NSCLC cells as well as TGFβ-dependent events such as E-cadherin to N-cadherin shift and decreased the formation of TGFβ-induced actin stress fibers. Interestingly, these outcomes correlated with a significant decrease in TβRII protein levels, although preliminary data suggests that TβRII endocytosis may not be affected by DHPs. qPCR results did not show a significant decrease in TβRII mRNA levels.

Conclusion: Our current work proposes that DHPs decrease total levels of TßRII and consequently P-smad2/3 signalling, but not by means of receptor endocytosis inhibition nor downregulation of TGFβ receptor mRNA. Further characterization of the mechanism that DHPs utilize to target TGFβ receptors will lay a foundation for the use of these small molecules as an effective anti-TGFβ lung carcinoma treatment strategy.

Sex Differences in the Acute Pharmacological and Subjective Effects of Smoked Cannabis Combined with Alcohol in Young Adults

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Introduction: The prevalence of co-use of alcohol and cannabis is increasing, particularly among young adults. Sex differences in the effects of alcohol alone and cannabis alone have been observed in animals and humans. However, sex differences in the acute pharmacological effects of cannabis combined with alcohol have not yet been studied. In young adults, aged 19-29 years, we aimed to examine sex differences following an intoxicating dose of alcohol (target 0.08% breath alcohol content) combined with a moderate dose of cannabis (12.5% Δ9-tetrahydrocannabinol; THC) using an ad libitum smoking procedure.

Methods: Using a within-subjects design, 28 regular cannabis users (16 males; 12 females) received in random order: 1) placebo alcohol and placebo cannabis, 2) active alcohol and placebo cannabis, 3) placebo alcohol and active cannabis, and 4) active alcohol and active cannabis. Blood samples for THC were collected and measures of vital signs, subjective drug effects, and cognition were collected.

Results: In the alcohol-cannabis combined condition, females smoked significantly less of the cannabis cigarette compared to males ($p<0.001$), although both sexes smoked similar amounts in the other conditions. There was minimal evidence that females and males differed in THC blood concentrations, vitals, subjective effects, or cognitive measures.

Conclusion: In the alcohol-cannabis combined condition, females experienced the same acute pharmacological and subjective effects of alcohol and cannabis as males, after smoking less cannabis, which has potential implications for informing education and policy. Further research is warranted on sex differences in cannabis pharmacology, as well as the combined effects of alcohol and cannabis.

Drug Resistance Prediction of *Mycobacterium Tuberculosis* Directly from Sputum

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Background: Tuberculosis is primarily a respiratory disease caused by the bacterium Mycobacterium tuberculosis (MTB). Global increases in drug resistant isolates highlight the need for rapid and accurate tests to detect and predict drug resistance of MTB as current culture-based identification methods are slow and labour-intensive. Extraction of DNA from sputum is a viable solution but challenging due to presence of other bacterial and host DNA. Rapid detection of MTB from patient sputum samples using whole genome sequencing could reduce turn-around-times and prevent costly, ineffective treatments.

Aim: To develop a method of mycobacterial DNA extraction directly from sputum for whole genome sequencing to detect and predict drug resistance. Validation will be done in 2 phases: (1) negative sputum spiked with *Mycobacterium bovis* BCG, (2) MTB positive sputum.

Methods: Sputum is decontaminated using a standard method to liquefy and remove contaminants. Samples are enzymatically-treated to degrade host DNA before bacterial genomic extraction. Prior to sequencing, extracts are subjected to two amplification methods: an in-house developed multiplex-PCR targeting known resistance genes and a random approach using GC-rich primers. Following sequencing on Illumina and/or Nanopore, sequences are subject to quality checks before being submitted to our bioinformatics pipelines: Kraken and Mykrobe Predictor which are used to asses sequence classification and resistance prediction, respectively.

Results: Phase 1 (n=72) tested 12 different conditions for sample decontamination and DNA extraction. We determined an optimal protocol that was shown to reduce host DNA by an average of 54% (0.3% to 95%). This protocol recovered the highest concentrations and increased mapped reads while decreasing non-covered bases. Random amplification increased DNA concentrations over 100-fold and the addition of GC-rich primers improved coverage while reducing non-covered bases, compared to standard conditions. When pooled with the multiplex-PCR reaction, all showed reduction in the relative abundance of host DNA and an increase in mycobacterial DNA. Simultaneously, this reduced errors in drug resistance predictions over unamplified DNA.

Conclusion: The optimized protocol was able to improve mycobacterial DNA quantity and quality while also improving drug resistance predictions using both targeted and random amplification. This could allow for accurate drug predictions without the use of culture.

Transcriptional Regulation of Insulin Action and Sensitivity via the GSK3β-FBXW7-ERRα Axis

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Insulin resistance (IR) is a forerunner of many metabolic diseases, where the body does not fully respond to the hormone insulin. Compelling evidence converges to highlight transcriptional dysregulation as a major contributor to insulin resistance. We recently demonstrated that global knockout of ERRα, an essential nuclear receptor manifested in mitochondrial functions, preserves insulin sensitivity during diet induced obesity (DIO). Still, how ERRα integrates insulin signaling with whole body metabolism remains unknown. Herein, we reveal that insulin-mediated gene expression greatly depends on the nuclear stabilization of ERRα by insulin through the GSK3β/FBXW7 axis. Liver-specific deletion of GSK3β or FBXW7, and mutating GSK3β/FBXW7 co-targeted ERRα phosphosites to alanines (3SA) result in accumulated ERRα proteins that no longer respond to insulin. ERRα 3SA mice display reprogrammed liver and muscle transcriptomes, resulting in insulin resistance and compromised metabolic homeostasis, which can be largely reversed by ERRα inhibitor C29. Our results hence support a pivotal role of ERRα in insulin action/resistance and suggest a putative way to ameliorate insulin resistance via intervening ERRα phosphorylation.

Cellular Dynamics of Immune Evasion during *Leishmania Major* Infection

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Introduction: There is a balance between the immune response and parasite escape mechanisms. Leishmaniasis is a neglected tropical disease with no cure or vaccination strategy. *Leishmania major* parasites elicit a strong T cell response, yet evade complete clearance and persistently infect a small pool of cells. This mechanism of immune evasion remains unclear.
Methods: A novel TCR transgenic mouse model, where T cells recognize the immunodominant Leishmania-glycosomal phosphoenolpyruvate carboxykinase (PEPCK) peptide on *L. major* parasites is combined with *in vitro* and *in vivo* 2-photon microscopy approaches to visualize the dynamics of anti-Leishmania CD4 T cell responses and to characterize mechanisms that restrain their effector functions.
Results: We show that monocyte-derived macrophage:T cell interaction dynamics were transient at steady-state, but prolonged upon antigen recognition. This activation leads to a production of high levels of IFNβ and can be significantly suppressed by PEPCK-specific Tregs *in vitro*, as compared to non-specific Treg controls. Co-culture of PEPCK-specific CD4+ T cells, *L. major*-infected monocyte-derived macrophages, and Tregs shows that antigen activation leads to a substantial increase in IL-10 levels, while decreasing IL-12, TNF, and IL-2 production in the culture. Intravital microscopy studies characterizing PEPCK-specific and control CD4+ T cell migration dynamics and tissue localization within skin lesions directly in live mice show a significant recruitment of adoptively transferred effector T cells to the lesion site *in vivo*, displaying cellular behaviors consistent with antigen recognition at early and late stages of infection, yet both cellular dynamics are augmented at the chronic stage, indicating a fundamentally altered environment. Upon secondary challenge with killed *L. major*, Tregs rapidly expand at the site of infection in healed mice. Currently we are evaluating how these Tregs are augmenting the cellular dynamics of Th1 cells *in vivo*.
Significance: The goal of this study is to identify barriers that need to be overcome in order to achieve complete parasite clearance in infected individuals. This work will describe the dynamic aspects of concomitant immunity generation and provide insights into the role of antigen-recognition in Treg suppression of immune responses.

A Clinically Translatable Immune-based Classification of HPV-associated Head and Neck Cancer with Implications for Biomarker-Driven Treatment Deintensification and Immunotherapy

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Purpose: Human papillomavirus-associated (HPV+) head and neck squamous cell carcinoma (HNSCC) is the fastest rising cancer in North America. There is significant interest in treatment de-escalation for these patients given the generally favourable prognosis. However, 15-30% of patients recur after primary treatment, reflecting a need for improved risk-stratification tools. We sought to develop a molecular test to predict the survival of patients with newly diagnosed HPV+ HNSCC.

Methods: We created a prognostic score (UWO3) that was successfully validated in six independent cohorts comprising 906 patients, including blinded retrospective and prospective external validations. Transcriptomic data from two aggressive radiation de-escalation cohorts were used to assess the ability of UWO3 to identify patients who recur. Multivariate Cox models were used to assess the associations between the UWO3 immune class and outcomes.

Results: A three-gene immune score classified patients into three immune classes (immune rich, mixed, or immune desert) and was strongly associated with disease-free survival in six datasets, including large retrospective and prospective datasets. Pooled analysis demonstrated that the immune rich group had superior disease-free survival at 5 years to the immune desert (HR= 9.0, 95% CI 3.2–25.5, P=3.6x10-5) and mixed (HR=6.4, 95%CI 2.2–18.7, P=0.006) groups after adjusting for age, sex, smoking status, and AJCC8 clinical stage. Finally, UWO3 was able to identify patients from two treatment de-escalation cohorts who remain disease-free after aggressive de-escalation to 30 Gy radiation.

Conclusions: The UWO3 immune score could enable biomarker-driven clinical decision-making for patients with HPV+ HNSCC based on robust outcome prediction across six independent cohorts. The superior survival of immune rich patients supports de-intensification strategies, while the inferior outcomes of the immune desert patients suggest the potential for intensification and/or immunotherapy. Prospective de-escalation and intensification clinical trials are currently being planned.

Neuregulin-1 Promotes Remyelination Through Regulation of Microglia Activity in Progressive Multiple Sclerosis

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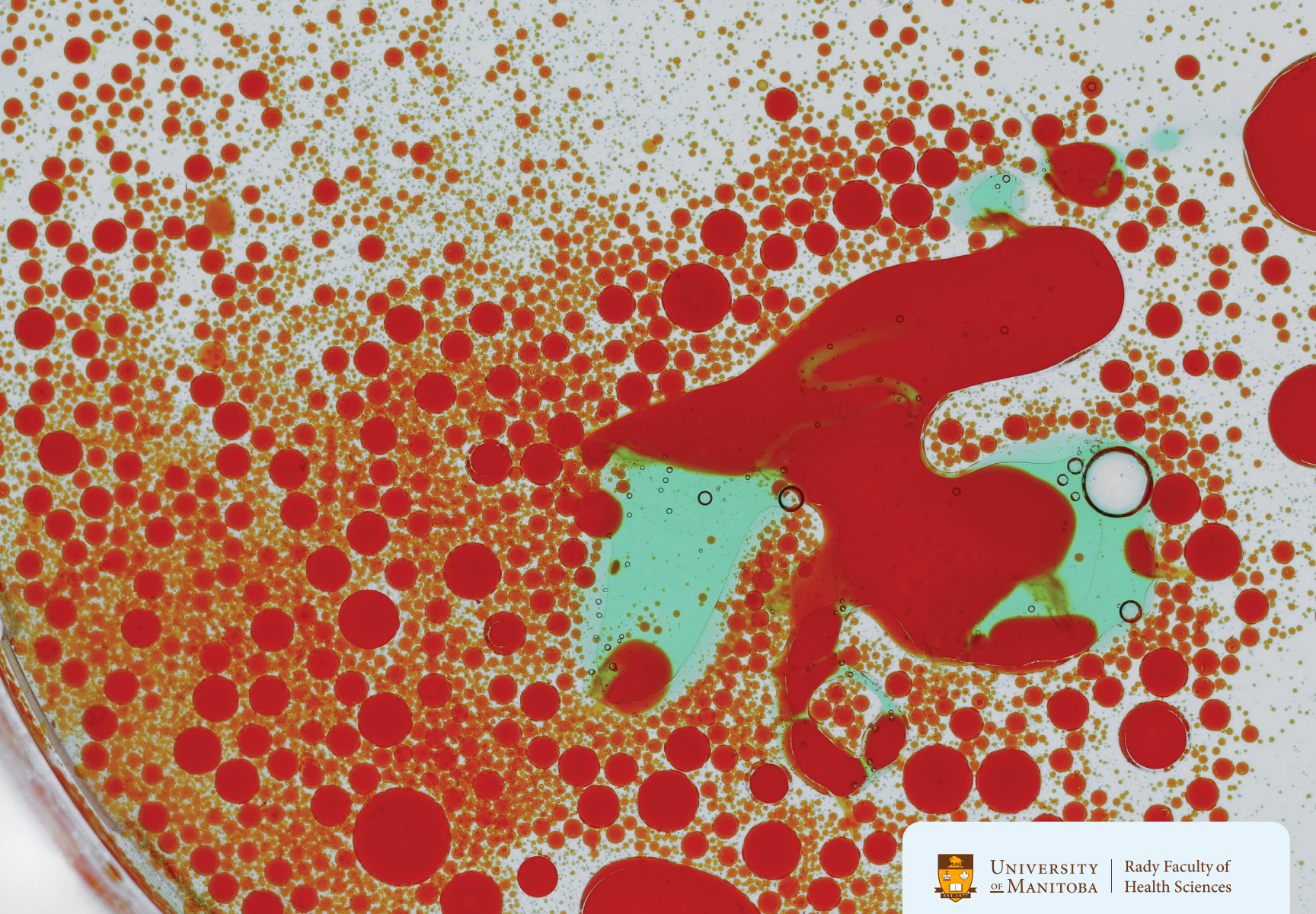
Multiple sclerosis (MS) is a chronic inflammatory disease of the central nervous system (CNS), characterized by multifocal demyelinating lesions, subsequently leading to progressive neurodegeneration. Remyelination by generation of new oligodendrocytes is a critical repair process in MS. Although spontaneous remyelination occurs completely or partially during the remission period at the early stages, it declines with disease progression. There is currently a critical need to uncover the underlying mechanisms of remyelination to allow development of effective reparative treatments for progressive MS. The pro-inflammatory CNS resident microglia and infiltrating macrophages are involved in the initial destruction of myelin sheath in MS lesions. These cells can also acquire anti-inflammatory, disease-resolving phenotypes required for remyelination. Anti-inflammatory microglia and macrophages have higher capacity for phagocytosis of cholesterol rich myelin debris in MS lesions; and promoting the reparative potential of these cells has paramount importance for remyelination. We previously reported that Neuregulin-1 (Nrg-1), a key signaling protein for CNS myelination, is significantly reduced in MS lesions. Restoring the Nrg-1 levels in the MS mouse model, experimental autoimmune encephalomyelitis (EAE), promoted pro-regenerative phenotypes in microglia and macrophages. Thus, we hypothesized that Nrg-1 treatment promotes oligodendrocyte progenitor cells (OPCs) maturation into myelinating oligodendrocytes in chronic demyelinating lesions by enhancing myelin phagocytosis and cholesterol biosynthesis of microglia and macrophages. Using *in vitro* model systems of primary cultures of microglia and bone marrow derived macrophages (BMDMs), we found that Nrg-1 promotes phagocytosis of myelin debris in both microglia and BMDM populations in a time-dependent manner. Moreover, Nrg-1 elevates the cellular levels of esterified and free cholesterol in microglia following myelin phagocytosis resulting in an increase in efflux of cholesterol from these cells through upregulation of cholesterol efflux transporter ABCA1. Exposure of OPCs to conditioned media from activated microglia treated with Nrg-1 promoted maturation of OPCs into myelinating oligodendrocytes. These *in vitro* finding were corroborated in our studies in chronic Cuprizone mouse model of demyelination where Nrg-1 treatment promoted remyelination in a microglia-dependent manner. Our findings unravel new regulatory mechanisms of remyelination in MS, and introduce Nrg-1 as a potential therapeutic strategy for enhancing myelin repair in progressive MS.



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