The Twenty-Sixth Annual Scientific Meeting

For Health Sciences Research Trainees

Faculty of Health Sciences

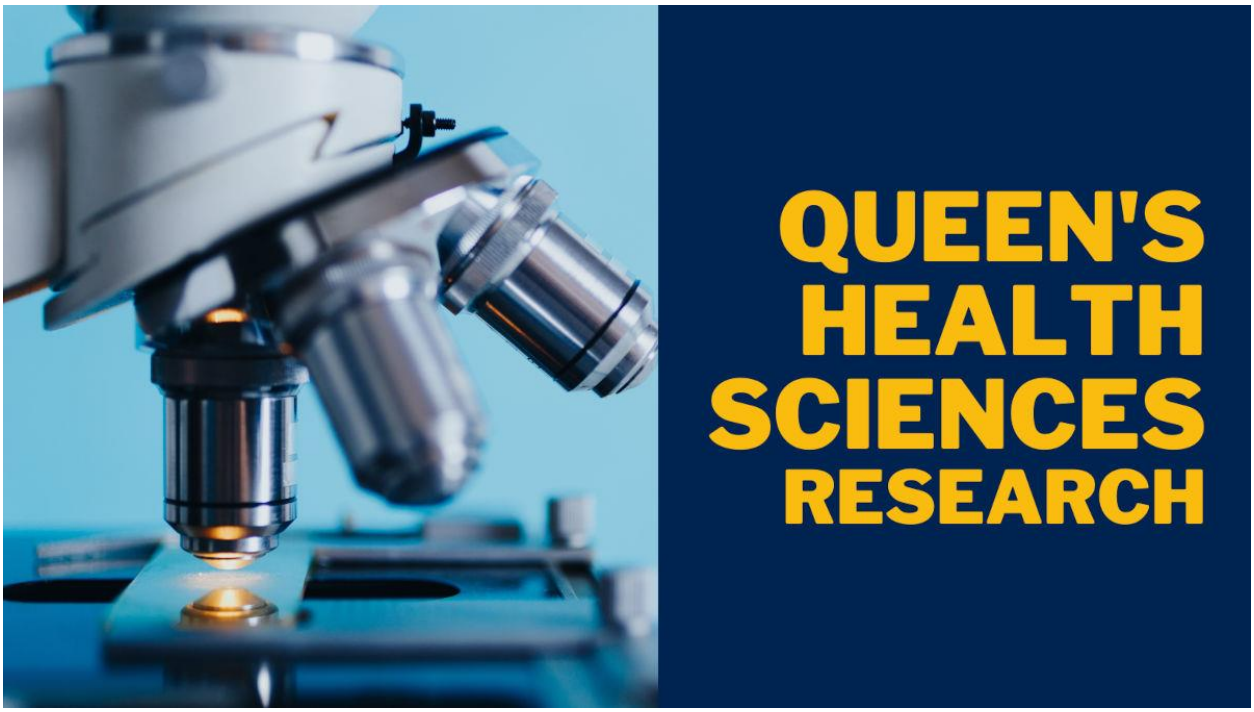
Queen's University

Queen's University
HEALTH SCIENCES

Wednesday, July 10th, 2024

Queen's Biosciences Complex

Room 1101



Thank You…….

We would like to extend a big thank you to everyone involved in the planning and adjudication of this event! Your time and efforts are truly appreciated.

Members of the Organizing Committee

Alan Lomax
Andrew Craig
David Natale
Eva Kauffman
Faith Brennan
Katrina Gee
Louise Winn
Madhuri Koti
Michael Adams
Nader Ghasemlou
Shetuan Zhang

Session Chairs

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Mark Ormiston

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Julien Miri
Kabeer Thaker
Kasthuri Ravishanker
Katie Zutautas
Kody Klupt
Laila Masalha
Mitchell Jeffs
Montserrat Mora Ochomogo
Nakeisha Lodge-Tulloch
Nasry Bouzeineddine
Safara Holder
Sakura Koner
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Victoria Velazquez Becerra
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Oral Presentation Adjudicators

Jean-Francois Pare
Tuany Eichwald

Flash Talk Adjudicators

Carolina Hernandorena
Ethan Thomas
Kabeer Thaker
Mia Wilkinson
Mikayla Erdelsky
Mitchell Jeffs

Acknowledgments

A special thank you to Mary White for her invaluable assistance in organizing this meeting.

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Wednesday, July 10th, 2024

Queen's Biosciences

Room 1101

8:00 – 8:30 am	Registration – Queen's Biosciences Atrium
	Poster set-up for morning participants – Biosciences Atrium
	Oral Presentations – Biosciences, Room 1101
8:30 – 8:40 am	**Welcome and Introduction** Dr. Chandra Tayade, Vice Dean, Basic and Public Health Sciences
	Introductory Remarks Dr. Jane Philpott, Dean, Queen's Health Sciences

Oral Presentations – Session 1: Biosciences, Room 1101

Chair: Mark Ormiston

8:40 – 8:52 am	Mitchell Jeffs - Development of a Luminescent Biosensor for the Clinical Detection of Carbapenemase-Producing Organisms
8:52 – 9:04 am	Declan Gainer - Stimulation of natural killer cell activity and metabolism by HCW9128 protects mice from hyperoxia-induced Bronchopulmonary Dysplasia
9:04 – 9:16 am	Natasha Iaboni - Using spatial metabolomics to distinguish in situ versus invasive ductal carcinoma
9:16 – 9:28 am	Jayne Dent - The chromosome-19 miRNA cluster (C19MC) promotes a dedifferentiated, metastatic phenotype in malignant melanoma
9:28 – 9:40 am	Angel Zhou - Real-world effectiveness of bevacizumab in metastatic cervical cancer patients in Ontario
9:40 – 9:52 am	Safara Holder - The Identification of Proteins in Proximity to Virion-Associated Herpes Simplex Virus Type-2 pUL21 Following Viral Entry

9:52 – 10:04 am	Dylan Weisman Zhao - Evaluating β-lactamase activity and antibiotic susceptibility in the changing urogenital environment – a step forward for urinary tract infections
10:04 – 10:30 am	Flash Talks (2.5-minute presentations) Presenters: Sumaiya Afrin, Monica Opoka, Emma LeBlanc, Elizabeth George, Danielle Sisnett, Rita Nakhle, Nakeisha Lodge-Tulloch
10:30 am – 12:00 pm	Coffee Break and Poster Presentations – Biosciences Atrium Virtual Poster Presentations – Biosciences Room, 1101
12:00 – 1:00 pm	Lunch – Biosciences Atrium Afternoon poster set up – Biosciences Atrium

Oral Presentations – Session 2: Biosciences, Room 1101

Chair: Alan Lomax

1:00 – 1:30 pm	Plenary speaker – Nader Ghasemlou - This explains a lot…my journey in science
1:30 – 1:42 pm	Katie Zutautas - Investigating the Relationship Between Group 2 Innate Lymphoid Cells and Multipotent Mesenchymal Stromal Cells in Endometriosis
1:42 – 1:54 pm	Helen Ngozichukwuka Obilor - Pregnant People's Cognitive and Emotional Responses to Climate Change and Relationship to Mental Well-being
1:54 – 2:06 pm	Sara Stickley - Network clusters of co-occurring gut microbes are associated with childhood atopy and asthma, environmental exposures, and host genomics in the CHILD Cohort Study
2:06 – 2:18 pm	Alexandra McDonald - Clonal Hematopoiesis and Alzheimer's Disease
2:18 – 2:30 pm	Abhishek Shastry - Multi-tissue metabolomics reveal mtDNA- and diet-specific metabolic profiles in a mouse model of diet-induced cardiometabolic disease
2:30 - 2:42 pm	Amanda Zacharias - Transcriptomics implicate neutrophil activity in the rhythmicity and chronicity of low back pain Background and Aims
2:42 – 3:15 pm	Flash Talks (2.5-minute presentations) Presenters: Andrew Garven, Anna Willmott, Julien Miri, Daniel Rivera, Elana Kertzman, Rohan Sampy, Abbey Politeski, Flourish Adebayo, Sofia Skebo
3:15 – 4:45 pm	Coffee Break & Poster Presentations – Biosciences Atrium
4:45 – 5:15 pm	Concluding Remarks and Awards
5:15 – 7:00 pm	Reception – Biosciences Atrium

Oral Presentations

Session 1

1. Mitchell Jeffs (Biochemistry and Cell Biology) Development of a Luminescent Biosensor for the Clinical Detection of Carbapenemase-Producing Organisms

   Mitchell Jeffs, Prameet M. Sheth, Christopher T. Lohans

   Carbapenemase-producing organisms (CPOs) pose an increasingly urgent global health threat due to their ability to inactivate carbapenems, a group of last resort antibiotics. Infections caused by these pathogens are associated with poor patient outcomes and high mortality rates; thus, early detection is vital to ensure optimal antimicrobial therapy and implementation of infection control practices. Current CPO testing strategies suffer from long turnaround times and low sensitivities for carbapenemases that exhibit weaker hydrolytic activity against carbapenems (e.g., OXA-type enzymes). In this study, we report the development of a luminescent biosensor assay for the rapid detection of CPOs. This biosensor can provide same-day positive test results within 2.5 hours inclusive of setup time and does not require the use of potentially costly colourimetric or fluorogenic substrates. Following initial optimization with laboratory strains of carbapenemase-producing E. coli, the assay was validated using a panel of clinical CPO isolates collected from patients at Kingston General and Sunnybrook hospitals. All CPOs tested positive with our assay, including eight isolates which produce the notoriously hard-to-detect OXA family of carbapenemases. Due to the rapid time-to-positivity, minimal setup requirements, and high sensitivity, this test could be suitable for use in clinical microbiology labs.

2. Declan Gainer (Translational Medicine) Stimulation of natural killer cell activity and metabolism by HCW9128 protects mice from hyperoxia-induced Bronchopulmonary Dysplasia.

   1Declan J. Gainer, 2Hing C. Wong, 2Niraj Shrestha, 1,3Mark L. Ormiston

   1. Department of Medicine, Queen's University, Kingston, ON, Canada
   2. HCW Biologics Inc., Miramar, Florida, United States
   3. Department of Biomedical and Molecular Sciences, Queen's University, Kingston, ON, Canada

   Background: Bronchopulmonary dysplasia (BPD) is a disease of impaired airway and pulmonary vascular development and a leading cause of mortality and morbidity in preterm infants. The accumulation of senescent cells and aberrant transforming growth factor-β (TGF-β) signaling have been identified as major contributors to preclinical models of BPD. We have shown that mice with TGF-β-insensitive natural killer (NK) cells (Tgfbr2NK-/-) exhibit a BPD-like phenotype, defined by impaired postnatal alveolarization and pulmonary vascular development. This finding suggests that the modulation of NK-mediated senolytic activity may be therapeutically relevant in BPD.

   Methods/Results: Single-cell RNA sequencing identified an excessive clearance of functionally critical

senescent endothelial cells in neonatal Tgfbr2NK-/- mice. As a model of BPD, wild-type neonates were exposed to 95% O2 from postnatal days 0-3, with or without NK cell stimulation with HCW9218, a Phase-I approved protein therapeutic, consisting of a bifunctional TGF-β antagonist fused to an IL-15/IL-15 receptor complex (10 mg/kg, S.Q.). HCW9218 treatment reduced senescent cell accumulation, protected against alveolar simplification and restored pulmonary vascular density in mice exposed to hyperoxia, relative to vehicle-treated controls.

Conclusions/Significance: The stimulation of NK cell senolytic activity is a viable therapeutic target in a preclinical model of BPD. Future work will evaluate the efficacy of HCW9218 in preventing long-term cardiorespiratory dysfunction in adult mice exposed to postnatal hyperoxia, with the ultimate goal of translating these findings to the clinic.

3. Natasha Iaboni (Cancer) Using spatial metabolomics to distinguish in situ versus invasive ductal carcinoma

Natasha Iabon1,2, Teaghan Kooster1, Martin Kaufmann3, Madeleine Carew1,2, Amoon Jamzad4, Abdulhameed Abdulhameed1, Rachel Rubino2, Kevin Ren1, John F. Rudan3, Parvin Mousavi4, Sonal Varma1, Christopher J.B. Nicol1,2.

Affiliations
1. Dept. of Pathology and Molecular Medicine, Queen's University, Kingston, ON
2. Cancer Biology and Genetics Division, Queen's University, Kingston, ON
3. Dept. of Surgery, Kingston Health Sciences Centre, Kingston, ON
4. School of Computing, Queen's University, Kingston, ON

Ductal carcinoma in situ (DCIS) is a breast cancer subtype contained within the ductal system, which may or may not progress to invasive ductal carcinoma (IDC). Women diagnosed with DCIS are often over-treated because of the prognostic challenges of knowing if DCIS will progress to IDC or not. To distinguish aggressive from non-aggressive DCIS tumours, we employ desorption electrospray ionization (DESI), a non-destructive mass spectrometry imaging technique that detects lipids using spectrums of mass to charge (m/z) ratios. We hypothesize DESI profiles of DCIS and IDC will reveal unique prognostic signatures. To test this, we performed a feasibility study using locally accrued human (DCIS/IDC, n=20) breast tumour samples. Sections of FFPE samples were analyzed by DESI using negative ionization, then H&E stained and annotated for tumour and non-tumour zones by pathologists. Multivariate analyses (m/z 50-1200) were performed on n≅200 randomized regions of interest per pathological zone. My data suggest a series of pro-tumourigenic, inflammatory ω-6 fatty acids are significantly upregulated in IDC, while anti-inflammatory prostaglandins, including 15d-PGJ2, are significantly upregulated in DCIS. As 15d-PGJ2 is an activator of PPARγ, a regulator of lipid metabolism and involved in breast tumour suppression, using immunohistochemistry, we observed a significant increase in PPARγ expression in DCIS samples compared to IDC. This suggests 15d-PGJ2 and PPARγ expression may help inform the aggressive potential of DCIS patients (Supported by Dean's Doctoral).

4. Jayne Dent (Cancer Biology and Genetics) The chromosome-19 miRNA cluster (C19MC) promotes a dedifferentiated, metastatic phenotype in malignant melanoma

Jayne Dent, Ainsley J. Cowan, Mike Vermeulen, Stephanie Young, Nicholas Fera, Kathleen Watt and Andrew Craig Department of Biomedical and Molecular Sciences, Queen's University; Cancer Biology and Genetics division, Queen's Cancer Research Institute

Cutaneous melanoma is an aggressive form of skin cancer that often progresses to metastatic and fatal disease. Despite recent advances in targeted therapies and immunotherapies, only a fraction of patients with metastases survive. This highlights the need for therapies that block metastasis. Recently, we profiled microRNA (miRNA) expression in the skin cutaneous melanoma (SKCM) cohort of the TCGA and discovered amplification of the Chromosome-19 miRNA cluster (C19MC) in ≈10% of SKCM tumors. C19MC is known to regulate placental trophoblast migration and differentiation and is considered an oncogenic driver in some embryonal brain cancers. The role of C19MC amplification in melanoma has not been reported and we hypothesized that C19MC activation drives a dedifferentiated, metastatic melanoma phenotype. We induced C19MC expression in A375 melanoma cells using a CRISPR/dCas9-based activation system and observed decreased cell growth, migration, and invasion in vitro. In a mouse model of human melanoma tumor xenografts, we observed decreased primary tumor growth with increased spontaneous liver metastases. C19MC expression also increased liver metastases in experimental in vivo metastasis assays. Profiling gene and protein expression revealed C19MC+ samples had increased pluripotency factors with decreased CDK2 expression, shedding light on the potential mechanisms underlying this phenotype. While future experiments are required to corroborate underlying mechanisms, the present study contributes new knowledge that will ultimately help to optimize treatments for this subset of melanoma patients.

Supporting Agencies: Cancer Research Society & Canadian Institutes of Health Research

5. Angel Zhou (Epidemiology) Real-world effectiveness of bevacizumab in metastatic cervical cancer patients in Ontario

Advanced cervical cancer is an aggressive disease with poor prognosis and stagnant treatment innovations. A potential shift occurred in 2016 when bevacizumab became publicly funded to treat this disease in Ontario driven by the promising results from the GOG-240 study. However, access and effectiveness of bevacizumab in everyday practice has not been explored since funding approval. Provincial administrative data was used to identify all cervical cancer patients who received frontline palliative treatment in Ontario between January 2006 to December 2022. The funding approval timing for bevacizumab of January 2016 served as the separation event between the two study cohorts "pre-bevacizumab" and "post-bevacizumab". Median overall survival (mOS) was demonstrated using Kaplan-Meier curves; overall survival (OS) of each era was compared with a multivariable Cox proportional-hazards (PH) model. Between January 2006 to December 2022, there were 208 adult women who met study inclusion. Bevacizumab uptake post funding approval was

44%; younger patients and patients with higher income were more likely to receive bevacizumab. Median OS was 7months and 11months in the pre-/post-bevacizumab cohort respectively (HR=0.56, 95%CI: 0.4, 0.8; p=0.0016). This study provides insight on the real-world effectiveness and potential uptake barriers for a costly cervical cancer therapy in Ontario. These findings can inform strategies to improve access to novel therapies for advanced cervical cancer patients, and guide practice changes for healthcare providers and funding parties.

6. Safara Holder (Microbes, Immunity and Inflammation) The Identification of Proteins in Proximity to Virion-Associated Herpes Simplex Virus Type-2 pUL21 Following Viral Entry

Safara M. Holder[1], Maike Bossert[1], and Bruce W. Banfield[1]
[1]Department of Biomedical and Molecular Sciences, Queen's University, Kingston, Ontario, Canada

Herpes simplex virus type 2 (HSV-2) virions contain a dsDNA genome encased within an icosahedral capsid, surrounded by a glycoprotein-studded lipid envelope. Situated between the nucleocapsid and viral envelope is a proteinaceous tegument layer which houses many proteins that function to enhance viral infection. One tegument component is pUL21, a multifunctional protein required for HSV-2 infection. During the late stages of infection, pUL21 regulates many crucial aspects of the viral replication cycle; however, its function immediately following viral entry remains elusive. To investigate these functions, a proximity-dependent biotin identification (BioID) approach was employed by constructing an HSV-2 strain encoding pUL21 fused to a non-specific biotin ligase, miniTurbo (pUL21-mT). Cells infected with this strain were treated with exogenous biotin following viral entry to induce the biotinylation of pUL21-mT proximal proteins and the identity of these proximal interactors was determined by LC-MS/MS. To ensure that these proteins were biotinylated by virion-associated pUL21-mT, de novo synthesis of pUL21-mT by the host cell was inhibited with cycloheximide, a protein synthesis inhibitor. Several cellular proteins such as innate immune system components, cell adhesion/junction, and nuclear membrane proteins were found to be in proximity to pUL21-mT following viral entry. This lead us to hypothesize that pUL21 interacts with these proteins to prime host cells for productive viral infection. Ongoing studies aim to evaluate the significance of these interactions during HSV-2 infection.

(Supported by CIHR grant 486466 and NSERC grant RGPIN-2018-04249)

7. Dylan Weisman Zhao (Bacteriology and Antibiotic Resistance) Evaluating β-lactamase activity and antibiotic susceptibility in the changing urogenital environment – a step forward for urinary tract infections

Dylan Zhao, Prameet Sheth, Christopher Lohans

The β-lactam class of antibiotics are cemented as one of the most effective antibacterial agents in modern medicine. Unfortunately, poor antibiotic stewardship has accelerated the emergence of β-lactam-resistant bacteria, which are responsible for more than 1 million deaths per year. Among high-risk pathogens, β-lactam-resistant Escherichia coli strains remain a significant burden to public

health, particularly in the realm of urinary tract infections (UTIs). A prominent resistance mechanism employed by E. coli involves the production of β-lactamase enzymes that hydrolytically degrade β-lactam antibiotics. In 2019, more than 40% of mortalities attributed to UTI treatment failure were caused by β-lactam resistance. However, our understanding of how the urogenital environment impacts β-lactam resistance in UTIs is incomplete. Thus, our work primarily seeks to understand how the properties of the urogenital environment impact E. coli physiology and β-lactamase-mediated resistance. Our preliminary findings suggest that culturing E. coli in artificial urine media has a dramatic impact on the activity of certain β-lactamases compared to standard lab media, influencing bacterial resistance against β-lactam antibiotics. Overall, more work is needed to fully unveil the complex interactions between the urogenital environment and β-lactam resistant E. coli, potentially offering avenues to exploit for the treatment of UTIs with β-lactams.

Session 2

Plenary Speaker – Nader Ghasemlou - This explains a lot…my journey in science

Dr. Nader Ghasemlou received the 2023-2024 Mihran and Mary Basmajian Award for Excellence in Health Research. As part of his presentation, he will outline his journey from graduate to post-graduate studies, and the past, present, and future research directions of his laboratory at Queen's University.

Nader is an Associate Professor at Queen's University and joined the Departments of Biomedical & Molecular Sciences and Anesthesiology in 2015. He completed his MSc at Queen's in Anatomy & Cell Biology, his PhD at McGill in Neuroscience, and was a Banting Postdoctoral Fellow at Harvard Medical School. He now leads the Pain Chronobiology & Neuroimmunology Lab and is co-chair of the Tissue Inflammation and Regeneration Research Excellence Cluster at Queen's. He also serves on the boards of various organizations including the International Association for the Study of Pain, Canadian Pain Society, Multiple Sclerosis Society of Canada, Chronic Pain Network, and Canada Brain Research Strategy. His research team uses a translational approach to study the intersection of neuroimmunology, pain physiology, and circadian biology. The lab is currently funded by grants from CIHR (Project Scheme and innovative Clinical Trial), NSERC, MS Canada, Craig Neilsen Foundation, and Brain Canada.

1. Katie Zutautas (Reproduction and Developmental Sciences) Investigating the Relationship Between Group 2 Innate Lymphoid Cells and Multipotent Mesenchymal Stromal Cells in Endometriosis

Katherine B Zutautas1 & Chandrakant Tayade1
1Department of Biomedical & Molecular Sciences, Queen's University, Kingston, Ontario

Background: Endometriosis (EM) is a chronic inflammatory disease defined by endometrial growth in extra-uterine locations. IL-33-driven group 2 innate lymphoid cells (ILC2) perpetuate cardinal EM

features like inflammation, fibrosis, and vascularization. One of the largest contributors of IL-33 are multipotent mesenchymal stromal cells (MSC), yet the interaction between MSCs, ILC2s, and IL-33 has yet to be investigated within EM pathophysiology. We look to identify and characterize these cell types within EM tissues to determine their role in disease.

Methods: EM was induced in C57Bl6 mice and 3 intraperitoneal injections of IL-33 were administered every other day. Tissues were harvested (peritoneal fluid, uterus, adipose, lesion) and single cell suspensions enriched for ILC2s using magnetic separation. Cells were expanded in culture before flow cytometric characterization. To identify MSC expression, tissues were harvested from non-EM mice treated with PBS or IL-33 and characterized via flow cytometry.

Results: MSC-like cells were present in ILC2 cultures from the uterus and lesions of IL-33 treated mice. This presence was associated with reduced ILC2 expansion. Further, MSCs were more prevalent in adipose than uterine tissue and their proportions fluctuated with IL-33 treatment. These findings suggest a suppressive MSC/ILC2 crosstalk and highlight potential tissue specificity.

Conclusion: On-going experiments will investigate the presence of MSCs in EM lesions and identify the mechanism of MSC/ILC2 interaction to determine their roles in EM pathophysiology.

Work was supported with funding from Canadian Institutes of Health Research.

2. Helen Ngozichukwuka Obilor (Perinatal Mental Health) Pregnant People's Cognitive and Emotional Responses to Climate Change and Relationship to Mental Well-being

Helen Ngozichukwuka Obilor, RN, PhD; Gabriella Moraes Rae, BHSc; Amanda Ross-White, MLIS, AHIP; Shahirose Sadrudin Premji, RN, BSc, BScN, MScN, PhD, FAAN, FCAN.*
School of Nursing, Faculty of Health Sciences, Queen's University, Kingston, Ontario, Canada

Climate change impact is not limited to extreme weather events (EWEs). The gradual decline of the biosphere can affect individuals' mental health, with pregnant people being disproportionately affected worldwide. Our rapid review showed that there is limited knowledge on whether reactions to climate change, including cognitive appraisal (personal interpretation) and emotional responses, are maladaptive or protective. As such, we implemented a descriptive study (Canadian-based online survey) to determine the relationship between pregnant people's cognitive and emotional responses to climate change and their mental well-being. The review findings revealed that emotional and mental health issues among pregnant people were not assessed based on cognitive appraisal of climate change but rather on EWEs (e.g., floods, typhoons, and wildfires). EWEs resulted in emotional distress (e.g., anger, fear, and peritraumatic distress) and mental health conditions (e.g., depression, anxiety, and post-traumatic stress disorder). A preliminary analysis of 18 survey responses of participants aged 27 to 39 years who identified as women revealed solastalgia (i.e. distress experienced due to loss of solace and pain living in a changing environment) significantly correlated with cognitive appraisal of climate change ($r=0.54$; $p=0.02$), eco-anxiety ($r=0.59$; $p=0.01$),

and pregnancy-related anxiety (r=0.51; p=0.03). Pro-environmental action negatively correlated with pregnancy-related anxiety (r=-0.57; p=0.01) and solastalgia (r=-0.65; p=0.004). Evidence from our study could enhance healthcare providers' climate literacy to support pregnant people distraught over the climate crisis.

3. Sara Stickley (Experimental Medicine) Network clusters of co-occurring gut microbes are associated with childhood atopy and asthma, environmental exposures, and host genomics in the CHILD Cohort Study

Sara A. Stickley (PRESENTING AUTHOR), Zhi Y. Fang, Amirthagowri Ambalavanan, Yang Zhang, Charisse Petersen, Darlene Dai, Piushkumar J. Mandhane, Elinor Simons, Allan B. Becker, Jeffrey R. Brook, Theo J. Moraes, Malcolm R. Sears, Meghan B. Azad, Stuart E. Turvey, Padmaja Subbarao, Qingling Duan

Department: Biomedical And Molecular Sciences

Early-life taxa-specific gut microbial abundance shifts have been previously associated with development of childhood allergic diseases. These earlier studies, however, did not investigate connections among individual microbes and their combined effects on health and disease. In this investigation, we employ an unsupervised machine learning approach to determine network clusters of co-occurring microbes and examine their associations with childhood atopy and asthma prevalence. Moreover, we integrate host genomics and environmental exposures to explore their main and interaction effects on these microbial clusters. We leverage microbiota data from the CHILD Cohort Study generated from 16S rRNA sequencing of stool samples collected at two ages: 3 months (N=779) and 1 year (N=770), genomic profiles obtained from the Illumina HumanCoreExome Bead Chip, and environmental data (e.g. breastfeeding practices). We identified 10 microbial co-occurrence clusters at 3 months, such as one correlated with decreased sensitization to inhalant allergens (P=0.035). Genome-wide association analyses identified that a microbe within this cluster, Blautia obeum, was associated with variants on chromosome 2 (e.g. rs13031049, P=9.4E-11). Additionally, we observed 13 microbial clusters at 1 year, including one containing Streptococcus salivarius that is correlated with increased sensitization to food allergens (P=4.8E-3). This cluster was also associated with shorter breastfeeding duration (P=6.3E-3). Our findings suggest that early-life gut microbial communities are modulated by both environmental and genetic factors, which in turn may impact atopy and asthma susceptibility. Funding: CIHR

4. Alexandra McDonald (Pathology) Profiling CHIP to reveal genetic alterations associated with Alzheimer's Disease

Alexandra McDonald, Dr. Michael Rauh, Dr. Susan Crocker** *Key Words: Clonal Hematopoiesis, Alzheimer's Disease, neuroinflammation, somatic variants, germline variants*

Cross-disciplinary genomics research can expose pathogenic processes, so support early detection and treatment of progressive illnesses like Alzheimer's disease (AD). Curiously, immune system activation and successive recruitment of inflammatory cells is found to be both protective and harmful in AD. This may be linked to the point in which immune system activation occurs in relation

to an individual's broader health characteristics, necessitating further characterization of the changing microenvironment that accompanies disease progression. We use Clonal Hematopoiesis (CH), a condition involving pre-cancerous blood cells, to guide our exploration. AD and CH both involve inflammation and increased risk with age. CH is associated with a two-fold increase in cardiovascular risks and an inflammatory state linked to coronary artery disease, yet recent research suggests CH protects against AD. Building on these findings, our project aim is to expose future research and detection targets by investigating this surprising relationship. We have extracted Whole Exome Sequencing data from the UK biobank, a large-scale biomedical database, to investigate the relationship between CH and genetic alterations found to implicate AD pathology. We include genetic changes relevant to inflammation, focusing on microglia related genes and pathways. Integrating germline data helps build context for the role of acquired (somatic) mutations in blood cells - in AD pathology. This informs the development of a targeted panel to use with cell-free and genomic DNA in AD specific cohorts.

5. Abhishek Shastry (Cardiometabolic Disease) Multi-tissue metabolomics reveal mtDNA- and diet-specific metabolic profiles in a mouse model of diet-induced cardiometabolic disease

Abhishek Shastry1, Matthew Ryan Smith, MS, PhD2 Charles C. T. Hindmarch, MSc, PhD1,3,4, & Kimberly Dunham-Snary, MPS, PhD1,4

1Department of Medicine, Queen's University, Kingston, ON
2Department of Medicine, Emory University, Atlanta, GA
3Queen's CardioPulmonary Unit, Queen's University, Kingston, ON
4Department of Biomedical & Molecular Sciences, Queen's University, Kingston, ON

Background: Perturbations in mitochondrial metabolism have emerged as markers of cardiometabolic diseases (CMDs) and align closely with mitochondrial DNA (mtDNA) signature. Skeletal muscle (SM) dysfunction often precedes early cardiovascular dysfunction, but characterizing metabolite profiles while also isolating mtDNA-specific effects remains challenging in humans.

Methods: To isolate mtDNA effects, Mitochondrial-Nuclear eXchange (MNX) mice (harboring reciprocally exchanged nuclear (nDNA) and mitochondrial genomes) were used. Six-week-old male wild-type (WT) and MNX mice were placed on control (10% fat) or high fat diet (HFD, 45% fat) for six weeks. SM and plasma samples were collected and processed using dual column chromatography coupled with high-resolution mass spectrometry. Pairwise contrasts and supervised dimensionality-reduction analysis (PLS-DA) were applied to diet groups between nDNA-matched pairs.

Results: Numerous differentially abundant metabolites (p-adj < 0.1) differentiated the response of HFD-fed C57 WT and C57 MNX mice versus control, including glycerophospholipids, amino acids, and short-chain fatty acids. Prostaglandins, glycerophospholipids, amino acids, creatine, isocitric acid, and diacylglycerides differentiated C3H WT from C3H MNX mice under dietary stress. Pathway analysis revealed perturbations in lipid metabolism, phospholipid synthesis, and small-molecule

transport in C57nDNA mice and altered branched chain and non-branched chain amino acid metabolism in C3HnDNA mice.

Conclusions: Distinct nDNA-mtDNA combinations reveal metabolomic profiles that favour CMD resistance in mice harboring C3HmtDNA and CMD susceptibility in mice possessing C57mtDNA, which we propose directly contributes to differential susceptibility to CMD.

6. Amanda Zacarias (Experimental Medicine) Transcriptomics implicate neutrophil activity in the rhythmicity and chronicity of low back pain Background and Aims

   Chronic low back pain (CLBP) is highly prevalent (~8% globally) and is the leading cause of years lived with disability worldwide. Previous work shows that peripheral immune cells, such as neutrophils, monocytes, and T cells, have a role in the chronicity of pain. Indeed, neutrophils have been suggested to relieve pain by secreting opioid peptides at the site of inflammation and inhibiting T-cell responses. Neutrophils have also been shown to have circadian rhythms in their gene expression and trafficking in the naïve and injured states. Likewise, CLBP has been shown to vary in intensity throughout the day; however, the mechanisms underlying this variability remain unclear at the molecular and cellular levels. This study aims to investigate how CLBP pain rhythmicity affects immune cells and the transcriptomic changes underlying their responses.

   Methods: Pain rhythmicity phenotypes (constant-low, constant-high, rhythmic↑ and mixed) were characterized in 74 participants with CLBP. Peripheral blood samples (n=116) were collected at 8:00 and 20:00 for bulk RNA-sequencing. Reads were processed using FastQC/MultiQC, Hisat2, and StringTie. Normalization and outlier detection applied edgeR and arrayQualityMetrics. Transcripts with median absolute deviations ≥ the 70th quantile (n=101,0350) were kept for analysis. We identified differentially expressed transcripts (DETs) between the rhythmic↑ and other phenotypes using edgeR. Unsupervised network analysis identified transcript clusters associated with pain rhythmicity↑. Gprofiler2 ran pathway analysis on significant transcripts. Using the PainOMICs LBP cohort to replicate results, DESeq2 assessed whether a gene's trajectory between two visits differs between opioid users vs non-users. Enrichment analysis (fgsea) focused on Gene Ontology's biological processes (GO:BP) about cell activation.

   Results: We identified 40 to 170 significant DETs between the rhythmic↑ and other pain phenotypes. Pathway analyses determined significant enrichment of immune cell signaling and neutrophil degranulation pathways. Network analysis clustered transcripts into 82 and 77 clusters of co-expressed transcripts in the day and night networks, respectively. The rhythmic↑ phenotype was associated with 3 "day" clusters and 5 "night" clusters. Further, the neutrophil degranulation pathway was enriched in a night cluster negatively associated with rhythmicity↑ (Padj.<10e-57). Moreover, among all cell types of hematopoietic origin whose activation pathway were documented in GO:BP, only neutrophils showed significant activation. Finally, the neutrophil degranulation pathway's genes were over-expressed over time in opioid users versus non-users (enrichment score +0.31, P=2e-6).

Conclusions: Our results suggest that neutrophil activation may differentiate the rhythmic↑ pain phenotype, and potentially non-opioid users, from other pain phenotypes. Specifically, there is less neutrophil degranulation amongst patients with a rhythm of CLBP intensity and amongst those who do not consume opioids. These findings support previous evidence that neutrophils play a role in chronic pain and are under circadian control. However, the role of neutrophil activation in the rhythmicity of pain intensity has not previously been described. Hence, circadian rhythms of pain and neutrophil activation may guide novel interventions for individuals with chronic low back pain.
Funding: CIHR-SPOR Chronic Pain Network, CIHR

Poster Presentations

Morning Session

1. Tasha Jawa (Neuroscience/Critical Care) Neurological impairment in critically ill patients on dialysis: A feasibility study

   Jawa, NA; Silver, SA; Holden, RM; Scott, SH; Day, AG; Norman, PA; Kwan, BYM; Maslove, DM; Muscedere, J; Boyd, JG Department: Centre for Neuroscience Studies Introduction.*

   Acute kidney injury (AKI) resulting in kidney replacement therapy (KRT) is rising among critically ill adults. Long-term KRT and critical illness are independently linked to acute (i.e., delirium) and prolonged cognitive impairment/structural brain pathology. Poor regional cerebral oxygenation (rSO2) may contribute. This study sought to determine the feasibility of a longitudinal study of critically ill KRT patients, to identify barriers to enrolment/data collection and design mitigation strategies for a larger study. Methods. We enrolled adults ≥18 years with AKI within 12h of initiating continuous KRT (CKRT) or intermittent hemodialysis (iHD). rSO2 was monitored during the first 72h of CKRT, or throughout iHD. We measured acute neurological impairment by daily delirium screening, and long-term outcomes using the Kinarm robot, neurocognitive testing, and brain MRI. Feasibility metrics included monthly enrollment ≥1 participant, data capture ≥80%, and 3- and 12-month follow-up ≥70%. Results. Of 484 ICU patients, 26 met screening criteria. Two declined, and 13 met ≥1 exclusion criteria; 11 were enrolled. Eight died in ICU, one died within 2 months of discharge, and one declined follow-up. Data capture rates were high: rSO2/vitals (91.3%), delirium screening/demographics (100%). Longitudinal testing was completed in 50% (1/2) survivors. Conclusions. Challenges in reaching substitute decision makers within 12h of KRT initiation and high mortality and attrition hindered enrollment and follow-up, suggesting the need for deferred consent, increased awareness of participant eligibility, and improved follow-up strategies.

2. Tasha Jawa (Neuroscience/Critical Care) A pragmatic, mixed-methods, open-label randomized controlledtrial examining the effectiveness of a post-ICU follow-up clinic on improving clinical and qualitative outcomes among ICU survivors and their caregivers: Study protocol

   Jawa, NA; Boyd, JG.

Centre for Neuroscience Studies

Background/Rationale. 80% of ICU survivors experience post-intensive care syndrome (PICS), characterized by profound cognitive, physical, and psychiatric impairments. Caregivers similarly experience detrimental psychosocial effects. Despite this knowledge, follow-up care is limited. ICU follow-up clinics may mitigate these long-term impacts but lack evaluation.

Setting & participants. ICU survivors will be eligible if: age ³18y; life expectancy ³6m; and high risk for PICS defined as delirium >4d, mechanical ventilation >4d, tracheostomy, or lack of access to primary care. Caregivers of ICU survivors will also be eligible. Exclusion criteria: failure to consent, baseline communication difficulties, or inability to speak/read English.

Methods. This is a pragmatic, mixed-methods, open-label randomized (1:1) controlled trial to examine the effectiveness of an ICU follow-up clinic on improving clinical and qualitative outcomes among ICU survivors and caregivers. The intervention group will receive: 1) specialized follow-up care at 1- and 3-months following discharge, 2) information packages on expectations following discharge, and 3) diaries for the healthcare team, family, and patient to journal their experiences. The control group will receive generalized standard of care through their primary care provider. Focus groups will be used for qualitative assessment. Clinical assessments are described in *Table 1*.

Implications. ICU survivorship extends beyond surviving an ICU stay. This project will unravel the aspects of follow-up care needed to mitigate long-term impacts of PICS, improving outcomes for patients and caregivers.

Table 1: Clinical assessments

Outcome	Group	Measurement	Description
Neurocognitive function	ICU survivors	MoCA	Clinical screening tool for cognitive impairment. Assesses visuomotor, visuo-perceptual, task-switching ability, language, conceptual thinking, memory, and orientation.
Quality of life	ICU survivors Caregivers	SF-36	A 36-item self-reported short form health survey to evaluate quality of life.
Anxiety and depression	ICU survivors Caregivers	HADS	A 14-question instrument measuring anxiety and depression, with each question scored on a scale of 0-3 for a maximum of 21 points for each of anxiety and depression.

PTSD	ICU survivors Caregivers	PTSS-14	Screening questionnaire for post-traumatic stress disorder, validated in ICU patients.
Chronic pain	ICU survivors	CPGS	A 7-item valid and reliable tool for assessing pain intensity and pain-related disability in chronic pain conditions.
Chronic fatigue	ICU survivors	FSS	A 9-item scale evaluating severity of fatigue and its effect on a person's lifestyle and activities.
ADLs	ICU survivors	Katz Index of ADLs	A 6-item scale evaluating performance on ADLs.
IADLs	ICU survivors	Lawton IADL scale	Assessment of functional status through IADLs.
Adverse events	ICU survivors	# hospital and ICU readmissions # emergency department visits	Evaluation of adverse events including hospital and ICU readmissions and emergency department visits among ICU survivors.
Polypharmacy	ICU survivors	# concurrent medications	Evaluation of polypharmacy through the number of concurrent medications that a patient is prescribed.
Caregiver burden	Caregivers	CBS	22-item scale evaluating burden on caregivers of individuals with chronic illness across a variety of domains of functioning.
Sleep quality	Caregivers	PSQI	Self-reported scale assessing sleep quality over the previous one month.

3. Ethan Januszkiewicz (Alzheimer's Disease and Exercise) Tracking Skeletal Muscle Derived Fluorescent Extracellular Vesicles After Exercise in Myog-Cre/CD9-GFP Transgenic Mice

Ethan W. Januszkiewicz, Fernanda G.Q. Barros-Aragão, Natalia M. Lyra e Silva, Fernanda G. De Felice- Centre for Neuroscience Studies- Queen's University & CIHR

Exercise has been shown in various studies to improve the brain health as well as protect against many neurological conditions ranging from to depression to Alzheimer's disease. However, little is known about the mechanisms or pathways behind these positive effects. One proposed explanation stems from skeletal muscle derived extracellular vesicles (EVs) which contain messenger proteins from muscle called myokines and are increased in circulation during exercise. It is thought that these myokine filled EVs travel to organs through circulation and eventually make their way to the brain where they exhibit their neuroprotective effects. Therefore, the goal of this experiment is to quantify and provide evidence in mice models that skeletal muscle derived extracellular vesicles are transported to the brain during exercise. The mice models that are being used are called Myogenin-Cre/CD9-GFP transgenic mice and have muscle derived extracellular vesicles that express fluorescence when they are exposed to a chemical called tamoxifen. There are two main experimental groups those that exercise and those that are sedentary. The exercise protocol consisted of voluntary running on a wheel over the period of 8 weeks. Quantification of fluorescence in tissues was performed by isolating proteins, using specific antibodies, and performing western blots. If there's more fluorescence in the brain of the exercise group compared to the sedentary one, then this provides evidence that exercises increase muscle brain communication.

4. Sandy Luu (Psychiatry/Neuroscience) Developing a Digital Cognitive Behaviour Therapy Program for Perimenopausal Anxiety: A Feasibility and Acceptability Study

Luu S, 1 Yang M, 2 Omrani M, 2,3, Alavi N. 1,2, and Soares C. 2

1 Centre for Neuroscience Studies, Queen's University, Kingston, ON K7L 3N6, Canada

2 Department of Psychiatry, Queen's University, Kingston, ON, K7L 7X3 Canada

3 Online Psychotherapy Tool, Toronto, ON, Canada

During perimenopause, women may be especially vulnerable to developing anxiety. Current pharmacological and hormonal treatments for this population have shown mixed efficacy, stressing the importance of developing alternative treatment options. Cognitive Behavioural Therapy (CBT) is one of the leading anxiety disorder therapies and can be conveniently delivered fully online (e-CBT). This one-arm study seeks to examine the feasibility and usefulness of a 12-week e-CBT program for middle-aged women (n=25) in perimenopause diagnosed with Generalized Anxiety Disorder. As a secondary aim, we will examine program effectiveness by assessing anxiety symptom severity and other exploratory symptoms at baseline, at week 6, post-treatment, and the 3 and 6-month follow-up, using validated participant-reported mental and menopausal health questionnaires. As part of the study, participants will complete at-home e-CBT modules, receive feedback on e-CBT homework through a secure platform, and complete assessments, including a virtual post-study interview.

5. Edie Attwood (Psychiatry/Mental Health) The effect of mental health literacy interventions on university student health: A meta-analysis protocol and initial findings

Attwood E, Rivera D, Shania Sheth, Patten SB, and Duffy A.*
Carried out at: Department of Psychology, Queen's University; Centre for Neuroscience, Queen's University; School of Medicine, Queen's University; Department of Psychiatry, Queen's University; Faculty of Health

Sciences, Queen's University; Cumming School of Medicine, University of Calgary
Funding: Canadian Institute of Health Research, Rossy Family Foundation, Mach-Gaensslen Foundation

Background: Up to 60% of university students meet criteria for a mental health problem, challenging campus health services. Mental health literacy (MHL) is an important form of early intervention, yet whether MHL interventions improve health outcomes in university students remains inconclusive and is thus the focus of this systematic review and meta-analysis.

Methods: Searches of MEDLINE, EMBASE, PsycInfo, CENTRAL, CINAHL, AMED, Web of Science, and ERIC databases will be performed. Eligible studies include pre-post, cohort, or case-control studies, RCTs, and non-randomized studies of intervention. Study interventions must be offered via a university and have a priori mental health-related outcomes measured with validated instruments. Primary outcomes include changes in symptom scores for common mental health problems from baseline to post-intervention. Effects of interventions will be measured as the standardized mean differences (SMDs) for the changes in scores between control and intervention groups. Random-effects meta-analyses using the DerSimonian and Laird method will provide pooled SMDs.

Preliminary results: A PRISMA protocol was archived in PROSPERO. The search yielded the following results: MEDLINE (1494), EMBASE (3353), PsycInfo (1100), CENTRAL (596), CINAHL (891), AMED (18), Web of Science (1206), and ERIC (253). 52 articles are currently undergoing full text review.

Discussion: This study will be inform whether if and how MHL interventions affect student mental health outcomes and guide the refinement of such interventions to this end.

6. Jiale Xie (Critical Care Neurology, Neurovascular Physiology) NIRS-guided personalized blood pressure targets to combat ICU delirium and cognitive dysfunction

Jiale Xie (presenting), Jasmine M Khan, David M Maslove, John Muscedere, Stephanie Sibley, J Gordon Boyd
Department: Department of Critical Care, School of Medicine Supporting agency: Canadian Institutes of Health Research (CIHR) Project Grant

With the increasing success of Intensive Care Units (ICU) at saving lives, a new and growing patient population, ICU survivors, has emerged. Each year, ~230,000 Canadians receive ICU care, ~80% of them develop delirium, a condition that makes simple tasks, like recalling one's name and recognizing surroundings challenging. Delirium also triggers fear, anxiety, and paranoia and leads to detrimental long-term outcomes, such as cognitive dysfunction, which adversely affects the quality of life and employability of ICU survivors. While the causes of ICU delirium remain unclear, recent research hints to the role of inadequate blood flow to the brain. In some patients, their cerebral blood vessels lose their capacity to self-regulate, which is crucial for sustaining adequate oxygen and nutrient supply and cognitive function. Technological advances have enabled the use of near-infrared spectroscopy (NIRS), a portable, non-invasive method that monitors cerebral oxygenation. This technology facilitates the calculation of individualized blood pressure targets to optimize cerebral autoregulation. Our study evaluated the feasibility of calculating and implementing NIRS-guided

individualized blood pressure targets at the bedside of 12 ICU patients. Low recruitment rate, signal acquisition interruptions, and narrow target range are barriers in the evaluation and implementation of this precision medicine approach. Insights gained from this study are instrumental in the refinement and future evaluations of a personalized, neuromonitoring-guided intervention aimed at mitigating ICU delirium and its detrimental repercussions.

7. Nakeisha Lodge-Tulloch (Reproductive Immunology) Maternal innate immune reprogramming after complicated pregnancy

Nakeisha A. Lodge-Tulloch[1], Jean-François Paré[1], Camille Couture[2,3,4], Elsa Bernier[2,3,4], Tiziana Cotechini[1], Sylvie Girard[2,3], Charles H. Graham[1].
[1]Department of Biomedical and Molecular Sciences, Queen's University. [2]Department of Obstetrics and Gynecology; Department of Immunology, Mayo Clinic, Rochester, MN, USA. [3]Department of Microbiology, Infectiology and Immunology, Université de Montréal.

Introduction: Pre-eclampsia (PE) and fetal growth restriction (FGR) are often associated with maternal inflammation and an increased risk of cardiovascular and metabolic diseases. The mechanism responsible for this increased risk may involve reprogramming of innate immune cells through epigenetic histone modifications.

Study Method: Circulating monocytes from women with PE, FGR, or uncomplicated pregnancies (controls) were isolated before labor. Cytokine release from monocytes following lipopolysaccharide (LPS) exposure and the presence of lysine 4-trimethylated histone 3 (H3K4me3) within TNF promoter sequences were evaluated. Single-cell transcriptomic profiles of circulating monocytes from women with PE or uncomplicated pregnancies were assessed.

Results: Compared to controls, monocytes from women with PE or FGR exhibited increased IL-10 secretion and decreased IL-1β and GM-CSF secretion in response to LPS. While TNF secretion was not significantly different between cultures of control monocytes and those from complicated pregnancies with or without LPS exposure, monocytes from complicated pregnancies had significantly decreased levels of H3K4me3 associated with TNF promoter sequences. Cluster quantification and pathway analysis of differentially expressed genes revealed an increased proportion of anti-inflammatory myeloid cells and a lower proportion of inflammatory non-classical monocytes among the circulating monocyte population in women with PE.

Conclusions: Monocytes from women with PE and FGR exhibit an immune tolerance phenotype prior to labor. Further investigation is required to determine whether this tolerogenic phenotype persists after pregnancy and contributes to the increased risk of subsequent disease.

Funding Sources: CIHR, Mayo Clinic Foundation.

8. Mikayla Erdelsky (Cardiovascular Sciences/Experimental Medicine) Phosphodiesterase 4 activity uniquely regulates ciliary cAMP-dependent 3T3-L1 adipogenesis

Mikayla R Erdelsky, Sarah A Groves, Charmi Shah, Samantha B Delios, M Bibiana Umana, Donald H Maurice

Principle Investigator: Donald H Maurice

Recent evidence indicates that the presence of a primary cilium (PC), and of selective cAMP signaling within this smallest of organelles, promotes adipogenic differentiation of 3T3-L1 preadipocytes incubated in media supplemented with either a natural (docosahexaenoic acid, DHA), or a synthetic (TUG-891), free fatty acid receptor 4 (FFAR4) agonist. Indeed, in this earlier work, activation of ciliary FFAR4 in 3T3-L1 cells was correlated with selective increases in PC cAMP and adipogenesis in these cells. However, this study was silent on the role of local PC cAMP phosphodiesterases (PDEs)-mediated events in regulating these adipogenic responses and on the identity of cAMP PDEs that could regulate the "pool" of ciliary cAMP accessed by FFAR4 agonists. In this context, we have identified the PDEs expressed by 3T3-L1 preadipocytes and showed that of these, only PDE4 inhibition promotes FFAR4-mediated adipogenesis. We propose that this work will identify more selective therapeutic targets through which to control adipogenesis, and perhaps the differentiation of other stem cells in which ciliary cAMP is critical.

9. Isabella Martins (Biochemistry)

Supervisor: Dr. Zongchao Jia
Department of Biomedical and Molecular Sciences

Polyphosphate (polyP) is a ubiquitous polymer observed in all kingdoms of life. Accordingly, polyP has a diverse set of roles which range from phosphate storage to regulating gene expression and virulence signalling. Amongst these is the post-translational modification-like function of polyP, termed histidine polyphosphate modification (HPM), featured by strong but non-covalent interactions with histidine repeat proteins. Here, we show that polyP is also capable of modifying lysine residues, which we have since coined as lysine polyphosphate modification (KPM). Screening paired with an electrophoretic mobility shift assay, revealed several lysinerich hit proteins which include the human oncoprotein K-Ras4B, and the RNA helicase DDX55. Moreover, bacterial protein targets were identified which include the GTPase EngA and the putative ATP-dependent RNA helicase SrmB. Thus far, polyP has been shown as a critical regulator of virulence factors in *P. aeruginosa,* observed through wild-type (WT) and polyp knockout cell lines (D*ppk1*D*ppk2A*D*ppk2B*D*ppk2C* enzyme deletion, hereby referred to as DAll). For the first time, we demonstrate the role that KPM may play in regulating virulence factor expression through recombinant overexpression of the poly-K proteins EngA and SrmB in *P. aeruginosa*. Deletion of the lysine residues reduces virulence phenotypes like that of the polyp deficient strain. Given the abundance of lysine-rich proteins in mammalian and bacterial systems, analyzing polyP's interactome and its functional effect on proteins will allow for insights into downstream implications and possible therapeutic approaches.

10. Juliana Vicente (Candida albicans Als adhesins)

The human fungal pathogen Candida albicans forms stubborn biofilms using Als adhesin proteins that decorate their cell wall. Though some efforts have succeeded in modelling the structures of these adhesins, little is known about the mechanistic basis by which they form sticky protein aggregates. Recently, it was discovered that a unique, hydrophobic amino acid-rich segment of Als proteins, termed the amyloid-forming region (AFR), is responsible for driving formation of these aggregates and the subsequent assembly of C. albicans biofilms on host surfaces, such as skin, mucosa, and prosthetic devices. However, models predict that the AFR is concealed by the bordering N-terminal and T domains, and thus it remains unclear how the AFR is exposed and later associates with other AFRs to enable Als protein aggregation. We posit that partial unfolding of Als proteins may occur when their N-terminal domain adheres to a host surface and then is subjected to shear stress. Proving or disproving that this mechanism is what promotes AFR exposure and Als aggregation is a challenging problem. Our approach involves developing a surrogate cell system to host and test the adhesive activity of individual Als proteins that have been site-specifically altered to either restrict or facilitate exposure of their AFR under different cell adhesion conditions. These studies will help explain the mechanism of amyloid formation by Als adhesins and could reveal inhibition target sites for the prevention of C. albicans biofilm formation.

11. Conrad Pietrzak and Sarah Hopkins (Immunology) Deciphering the Influence of the Immune Environment on Trained Immunity Induction

Conrad Pietrzak1, Sarah Hopkins1, Gabriella Stefan1, Vidthiya Jeyanathan1, Colleen Tordoff1, Faith Brennan1, and Eva Kaufmann1

1Department of Biomedical and Molecular Sciences, Faculty of Health Sciences, Queen's University, Kingston, ON, Canada

Trained immunity enhances innate immune responses to secondary stimulation through epigenetic reprogramming. Trained immunity is initiated by vaccination-induced cytokine signaling to long-lived hematopoietic stem and progenitor cells (HSPC). HSPC are the progenitors of all short-lived, circulating innate immune cells. Targeting innate memory provides tremendous opportunities for future vaccine generation against infections and conditions in which classical, adaptive immunity failed to provide protection (e.g., Tuberculosis, sepsis). For clinical application, the robustness of a vaccination strategy in a heterogenous population is crucial. Diverse baseline immune statuses originate from genetics as well as persistent conditions, e.g., chronic infections and allergies. To date, the extent to which the host's baseline immune status directs the vaccination-induced cytokine signaling is unknown. To evaluate vaccine robustness across different immune environments, we test whether trained immunity-inducing vaccination is robust in mouse models with type 1 and type 2-skewed immune systems. Specifically, Proinflammatory-biased C57BL/6 mice and allergy-prone BALB/c mice are vaccinated intraperitoneally with β-glucan, a trained immunity inducer. Enhanced HSPC activation is observed in BALB/c mice, with elevated cytokine secretion from HSPC-derived macrophages in both strains upon secondary stimulation. Further research aims to explore strain-specific differences in vaccine response mechanisms and whether strain-specific targeting can

further enhance the immune responses. Ultimately, characterizing the impact of immune environments on trained immunity induction will strengthen clinical translation and enable host-directed adaptation of these next generation vaccines.

12. Doriana Taccardi (Microbes, Immunity and Inflammation)

    Chronic pain, which can fluctuate throughout the day, contributes to most years lived with disability worldwide. Whether pain rhythmicity affects biopsychosocial characteristics, medication use, and gene expression is unknown. Our prospective cohort study of 74 chronic low back pain (CLBP) participants including e-diary assessments tracking intra-day pain symptoms and biomarker analysis. Participants were divided into four groups of pain rhythmicity: constant-low, constant-high, rhythmic, and mixed. Blood was collected to determine circadian changes in immune cell populations and gene expression signatures. Analysis of an independent transcriptomics cohort of CLBP patients replicated and validated our findings. We recruited 74 adults with CLBP with 62 eligible for inclusion. A combination of statistical methods examining the average, standard deviation, and percent change in self-reported pain scores submitted over one week of sampling was used to group participants into four phenotypes. Four patient groups were identified based on mean pain score and variability: ~25% constant-low (3·0/10), ~23% constant-high (7·0/10), ~21% rhythmic increasing (↑) throughout the day (4·8/10), and ~31% unclear/mixed pattern of pain (5·1/10). The largest percentage of people using these medications were found in both the mixed and constant-high phenotypes compared to both constant-low and rhythmic↑; indeed, no participants in the rhythmic↑ phenotype were using opioids. Notably, this group still reported high pain intensity at night, comparable to the constant high group. The rhythmic↑ had no opioid users and also improved scores for pain catastrophizing, pain interference, depression, and sleep disturbance relative to all other groups. While no differences were found in circulating immune cell subtypes, RNA sequencing identified differentially expressed transcripts and gene pathways in the rhythmic↑ group relative to other pain phenotypes, with the neutrophil degranulation pathway notably enriched (p-valueadjusted=9·51e-57). Only the neutrophil activation pathway was enriched in differentially expressed genes (FDR<1%) in our replication cohort among all activation pathways for cells of hematopoietic origin. In summary, the experience of pain varies across different types of pain rhythmicity, which is correlated with pain intensity, gene expression, opioid use and other psychosocial variables on a molecular and biopsychosocial level. These findings suggest that circadian rhythms of pain and neutrophil degranulation may be key to distinguishing biopsychosocial profiles and opioid use in chronic pain. This study supported the idea that inter-individual variability, such as fluctuations in pain intensity across time and molecular changes associated with circadian rhythms, are important aspects to consider when treating pain. Often, when testing for the effectiveness of pain interventions, inter-individual differences are not considered. This can affect the applicability of these treatments in real life. Identifying profiles of rhythmicity in self-report pain rhythmicity and molecular profiles might help to create a more personalized intervention for people with CLBP, tailored to their pain profiles. Re-storing rhythmicity in people with arrhythmic profiles by using non-invasive treatment to synchronize circadian rhythms might present an innovative avenue to improve overall outcomes in

people suffering from chronic pain and other chronic conditions. (Supported by CIHR and CIHR-SPOR Chronic Pain Network)

13. Zhi Yi Fang (Bioinformatics and Genomics/Experimental Medicine) Networks of co-occurring human milk microbiota are associated with host genomics, childhood asthma and allergic sensitization

Zhi Yi Fang1, Sara A. Stickley1, Amirthagowri Ambalavanan1, Yang Zhang2, Amanda M. Zacharias1, Kelsey Fehr3,4,5, Shirin Moossavi3,4,5, Charisse Petersen6, Kozeta Miliku3,4,5,7, Piushkumar J. Mandhane8, Elinor Simons3,9, Theo J. Moraes10, Malcolm R. Sears11, Michael G. Surette12, Padmaja Subbarao10,13,14, Stuart E. Turvey6, Meghan B. Azad3,4,5, Qingling Duan1,2 1. Dept. of Biomedical and Molecular Sciences, Queen's University, Kingston, ON, Canada; 2. School of Computing, Queen's University, Kingston, ON, Canada; 3. Dept. of Pediatrics and Child Health, University of Manitoba, Winnipeg, MB, Canada; 4. Dept. of Food and Human Nutritional Sciences, University of Manitoba, Winnipeg, MB, Canada; 5. Manitoba Interdisciplinary Lactation Centre (MILC), Children's Hospital Research Institute of Manitoba, Winnipeg, MB, Canada; 6. Dept. of Pediatrics, British Columbia Children's Hospital, University of British Columbia, Vancouver, BC, Canada; 7. Dept. of Nutritional Sciences, University of Toronto, Toronto, ON, Canada; 8. Dept. of Pediatrics, University of Alberta, Edmonton, AB, Canada; 9. Section of Allergy and Immunology, University of Manitoba, Winnipeg, MB, Canada; 10. Dept. of Pediatrics, The Hospital for Sick Children, Toronto, ON, Canada; 11. Division of Respirology, Dept. of Medicine, McMaster University, Hamilton, Ontario, Canada; 12. Farncombe Family Digestive Health Research Institute, Dept. of Medicine, McMaster University, Hamilton, Canada; 13. Dept. of Medicine, McMaster University, Hamilton, ON, Canada; 14. Dalla Lana School of Public Health, University of Toronto, Toronto, ON, Canada;

The microbial community in human milk has been proposed to impact children's health such as atopy and asthma. Earlier studies have identified non-genetic determinants of the human milk microbiota (HMM). However, the influence of host genomics on HMM remains poorly understood. This is the first study to employ a network analysis approach to identify connections among co-occurring microbes in human milk and to determine their associations with maternal genomics and allergic outcomes in human milk-fed infants. HMM was assessed by 16S rRNA sequencing of 885 breastmilk samples in the CHILD Cohort Study. Genomic profiles of mothers and their children were obtained from the Illumina HumanCoreExome BeadChip. Using an unsupervised machine-learning method, we identified clusters of co-occurring microbes and determined their association with childhood atopy and asthma at age 5 years using a linear regression model. For example, we identified that a microbial cluster containing Pseudomonas-Stenotrophomonas in mother's milk is associated with their children's risk of asthma. In addition, increased alpha-diversity and the Veillonella-Prevotella containing cluster are associated with reduced risk of childhood atopy. Genome-wide association analyses of HMM revealed that genomic variants on chromosomes 11 (e.g., rs12275196, P=1.3×10-11) and 10 (e.g., rs11009644, P=1.6×10-8) are significantly associated with the microbial clusters associated with asthma and atopy, respectively. Thus, our findings suggest that maternal genomics influence the HMM, which may modulate risk of childhood allergic diseases. Supporting agency: Canadian Institutes of Health Research (MRT-168044, PJT-178390, FBD-181414).

14. Gabriella Stefan and Andisheh Liaghat (Microbes, Immunity and Inflammation) Investigating the Impact of Asthma on Hematopoietic Stem and Progenitor Cells

Gabriella Stefan1, Andisheh Liaghat1, Vidthiya Jeyanthan1, Conrad Pietrzak1, Makena Sceeles1, Lubnaa Hossenbaccus1, Mckenna Perlin1, Astha Patel1, Sarah Hopkins1, Sara Teimouri Nezhad1, and Eva Kaufmann1
Department of Biomedical and Molecular Sciences, Queen's University, Kingston, Ontario

Asthma is one of the most prevalent allergic diseases. Despite its devastating impacts on life quality and the medical system, to date, only symptomatic treatments are available. To develop novel therapeutic approaches, a better understanding of the pathomechanisms in asthma is urgently required. Asthma is characterized by abundant infiltration of immune cells into the lungs in response to allergen exposure, as well as airway smooth muscle hypertrophy. We and others have recently demonstrated that the progenitors of these innate immune cells, the hematopoietic stem and progenitor cells (HSPC) in the bone marrow (BM), can be epigenetically reprogrammed and long-term modify the host immune response in both beneficial and detrimental ways. We thus hypothesize that respiratory allergen exposure induces functional reprogramming in hematopoietic stem cells that leads to decreased host defense capacities in deriving innate immune cells. To investigate this hypothesis, C57BL/6J mice are sensitized with house dust mite extract and the lungs and BM are in-depth immunophenotyped by flow cytometry. We further analyze functional capacities of HSPC-deriving innate immune cells in response to both allergic and infectious stimulation. Interestingly, we find that asthma induction induces proliferation of HSPCs in the lung and decreased cytokine responses from bone marrow-derived macrophages to infectious stimulation. Together, these results suggest that immunophenotypes in asthma are driven by both quantitative and qualitative changes in BM and lung HSPCs.

15. James Rober (Histone Mutations) Uncovering biological effects of cancer-associated histone mutations

James Rober, Austin Macklem, Anna Panchenko, Daniel Espiritu, and Maria Aristizabal
Biomedical and Molecular Sciences

Histones play central roles in gene regulation, DNA packaging, and structural support to chromosomes. Mutations in histones that disrupt gene regulation can result in aberrant gene expression and potentially drive oncogenesis. Many histone mutations have been implicated in a variety of cancers such as brain tumours, chondroblastomas, and leukemia. Through screening of cancer genomic databases, hundreds of unique mutations have been identified in cancer patients. The best studied cancer-associated histone mutations have been shown to affect post translational modifications, nucleosome stability and gene expression. Using yeast as a model organism, I have screened nine cancer-associated mutations on histone H2B that have previously not been studied. These identified mutations are on residues not conserved between humans and yeast, therefore yeast strains humanized for the H2B protein were used. Four of these mutations displayed altered growth suggesting that they result in abnormal H2B activity. Most notably, the H2BC4-T122A mutation displayed a growth advantage compared to the H2BC4 mutants suggesting that it functions more similar to the H2B yeast histone. My work lays the foundation to expanding our understanding

of the role histone H2B mutations have in cancer biology by identifying several mutants that warrant further examination.

NSERC, NFRF, and the Cancer Research Society

16. Elizabeth George (Asthma) Polygenic risk scores of lung function interacts with early-life exposures to modulate the risk of Asthma in the CHILD Cohort Study

Elizabeth George1, Amirthagowri Ambalavanan1, Sara A. Stickley1, Zhi Y. Fang1, Zihang Lu12, Ruixue Dai8, Stuart E. Turvey3,4, Piushkumar J. Mandhane5, Elinor Simons6, Jeffrey R. Brook7, Theo J. Moraes8, Malcolm R. Sears9, Lars Bode10, Meghan B. Azad11, Padmaja Subbarao8, Qingling Duan1,2 Affiliations: 1Department of Biomedical and Molecular Sciences, Queen's University, Kingston, Ontario, Canada; 2School of Computing, Queen's University, Kingston, Ontario, Canada; 3Division of Allergy and Immunology, Department of Pediatrics, University of British Columbia, Vancouver, British Columbia, Canada; 4Department of Pediatrics, Child and Family Research Institute and British Columbia Children's Hospital, Vancouver, British Columbia, Canada; 5Department of Pediatrics, University of Alberta, Edmonton, Alberta, Canada; 6Section of Allergy and Immunology, Department of Pediatrics and Child Health, University of Manitoba, Winnipeg, Manitoba, Canada; 7Occupational & Environmental Health Division, Dalla Lana School of Public Health, University of Toronto, 8Department of Pediatrics, Hospital for Sick Children and University of Toronto, Toronto, Ontario, Canada; 9Department of Medicine, McMaster University, Hamilton, Ontario, Canada;10Dept. of Pediatrics and Larsson-Rosenquist Foundation Mother-Milk-Infant Center of Research Excellence (MOMI CORE), Human Milk Institute (HMI), University of California San Diego, La Jolla, CA, USA;11Manitoba Interdisciplinary Lactation Centre (MILC), Children's Hospital Research Institute of Manitoba, Winnipeg, Manitoba, Canada: 12Department of Public Health Sciences, Queen's University, Kingston, Ontario, Canada

RATIONALE: Asthma is the most prevalent chronic disease during childhood, affecting approximately 14% of children worldwide, and has been linked to reduced pulmonary function. Both genomics and environmental exposures such as diet and air quality have been reported to impact inter-individual variations in lung function1. In this study, we derived polygenic risk scores (PRSs) for lung function measures and determined their associations with lung function among preschool-aged children with and without asthma or recurrent wheeze. In addition, we explored how these PRSs interact with early-life exposures to influence lung function in childhood.

METHODS: We leveraged data from the Canadian CHILD Cohort Study. Lung function was assessed by spirometry as forced expiratory volume in 1 second (FEV1; N=1660), forced vital capacity (FVC; N=1309), and the ratio (FEV1/FVC; N=1278). Environmental exposures were documented through repeated parental questionnaires from prenatal to birth and until age 5 years. Genome-wide single nucleotide polymorphisms (SNPs) were genotyped using the Illumina Human-Core-Exome Bead-chip. PRSs were calculated for each child based on their genomic profiles and allelic weights from a published genome-wide association study (GWAS) of lung function2. Next, we applied generalized linear mixed models to determine the association of PRSs with lung function measures, comparing children with and without asthma (diagnosed by age 5 years) or recurrent wheeze (ages 2-5 years). We also investigated how potential gene-environment interactions (GxE) between these PRSs and exposures (e.g., maternal human milk components, pets) may influence children's lung function.

RESULTS: PRSs for FEV1, FVC and FEV1/FVC were derived from approximately 1.1M SNPs. We noted that lower PRSs were associated with decreased lung function (FEV1/FVC) as well as higher prevalence of childhood asthma (P=0.03) and recurrent wheeze (P=0.008). GxE analyses identified that these associations are modulated by exposures such as prenatal intake of iron (P=0.03) and dog ownership (P=0.008). Moreover, among children with high PRS, exposure to higher concentrations of the fucosylated human milk oligosacc.

17. Delaine Pereira (Developmental Toxicology) Exposure to a Teratogenic Dose of Valproic Acid Exhibited a Sex-Specific Increase in Placental Nutrient Transporters in CD-1 Mice

    Delaine Pereira (BScH Student, PRESENTING AUTHOR), Lauren Brown, Lihua Xue, and Louise Winn Department of Biomedical and Molecular Sciences, Queen's University

    Valproic acid (VPA), a medication for neurological disorders, is a potent teratogen causing multiple adverse pregnancy outcomes, including fetal growth restriction. FGR is often linked to placental insufficiency (insufficient transplacental nutrient transport to the fetus) which can be caused by decreased nutrient transporter expression. Specifically, decreased expression of nutrient transporters like glucose and folate has been implicated in placental insufficiency. Despite known teratogenic effects, the specific impact of VPA on placental nutrient transporters, glut-1 and folr1, remains poorly understood. This study investigated the effect of gestational VPA exposure on glut-1 and folr1 expression in CD-1 mice using RT-qPCR methods on placental tissues exposed to saline, 400 or 600 mg/kg VPA on gestational day 9. Additionally, this study explored sex-specific placental responses to VPA. Findings revealed a significant increase in glut-1 and folr1 mRNA levels in female placentas exposed to VPA, suggesting a sex-specific compensatory mechanism to maintain fetal nutrient supply. This adaptation was not observed in male placentas, demonstrating potential sex differences in placental responses to teratogenic exposure. These results contribute to mechanistic insights underlying VPA-induced FGR, emphasizing the importance of considering fetal sex in developmental research. Further investigation is needed on more glucose and folate transporters to develop strategies to mitigate the adverse outcomes of VPA use during pregnancy, leading to the creation of safer treatment options and enhanced maternal-fetal health outcomes.

18. Trina Dykstra-MacPherson (Biochemistry and Cell Biology) Limb girdle muscular dystrophy mutations and calpain-3 protease binding to titin

    Trina Dykstra-MacPherson, Mathias Bell, Peter L. Davies
    Department: Biomedical and Molecular Sciences
    Supporting Agency: CIHR

    Limb girdle muscular dystrophy recessive 1 (LGMDR1) leads to progressive weakness and atrophy within the hip and shoulder musculature with an incidence of 1 in 42,700 people worldwide. Its onset is caused by mutations to a muscle-specific, Ca2+-dependent, intracellular cysteine protease called calpain-3. There are over 500 loss-of-function mutations, found throughout the calpain-3 gene, which have been linked to LGMDR1. Calpain-3 is thought to play a role in clearing damaged muscle proteins after exercise. Previous work from our lab suggests that newly synthesized calpain-3

forms an inactive hexamer in the sarcomere until it binds titin whereupon it dissociates into functional dimers. This interaction is thought to occur between the unique insertion sequence 2 (IS2) of calpain-3 and the N2A region of titin. We hypothesize that LGMDR1 mutations in IS2 might cause dystrophy by spoiling binding to titin. Our approach is to recombinantly co-express calpain-3 with fragments of the titin N2A region in E. coli. If binding occurs a 320-kDa particle will appear on Blue Native PAGE. Mutations that block this interaction will produce the 500-kDa calpain-3 hexamer. This simple test may show which of the >500 mutations associated with LGMDR1 cause the dystrophy by blocking this protein-protein interaction.

Funded by CIHR

19. Logan Germain (Toxicology) An ethical in vitro alternative model to screen for alterations to the epigenome in embryonic cells following exposure to environmental chemicals

Authors: Logan Germain1, Lihua Xue1, Louise M. Winn1,2.
Department of Biomedical and Molecular Sciences1 and Department of Environmental Sciences2, Queen's University, Kingston, ON.

The ability for chemical exposures during fetal development to alter the epigenome are not considered in chemical safety regulatory decisions in Canada. There is a lack of easily reproducible, low-cost and ethical screening methods for alterations in the epigenome of embryonic cells following exposure to environmental chemicals. Certain fish models have been highlighted for their predictive potential due to the highly methylated nature of their DNA, responsiveness to their environment and highly conserved developmental pathways. This study established a method for assessing xenobiotic-induced changes to the epigenome and gene expression in embryonic cells using an aquatic in vitro model. An immortalized cell line derived from trout embryonic tissue was used to assess alterations to histone modifications and global DNA methylation following exposure to 0, 40 or 80 μM (24h) of the environmental pollutant triphenyl phosphate (TPhP). This model identified that histone H3 methylation, H3 acetylation and global DNA methylation were significantly reduced in the embryonic cells following TPhP exposure compared to control. Additionally, gene expression changes to estrogenic and metabolic pathways were identified via RT-qPCR, with the intention to assess gene promoter DNA methylation status in the near future. Statistical analysis included one-way ANOVAs and multiple comparisons tests. This model shows promising ability to be applied to other chemicals used in Canada as a screening tool for their ability to alter the epigenome of aquatic embryonic cells.

Funding sources include NSERC.

20. Eric B. P. Fernandes (Cardiovascular and Renal Diseases) Acute calciprotein particle responses to high dietary phosphate intake uniquely uncover marked loss of mineral buffering capacity in chronic kidney disease

Eric B. P. Fernandes BHScH,1 Trisha Singh,1 Rebecca Li BHSc,1 Nelson Chen,1 Heshanth Rasalingam,1 Tyler S. Rowsell MSc,1 Mandy E. Turner PhD,1,3 Rachel M. Holden MD,1,2 Michael A. Adams PhD1*

1 Department of Biomedical and Molecular Sciences, Queen's University, Kingston, ON, K7L 3V6, Canada

2 Department of Medicine, Queen's University, Kingston, ON, K7L 3V6, Canada

3 Division of Cardiovascular Medicine, Department of Medicine, Center for Interdisciplinary Cardiovascular Sciences, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, 02115, USA

**Presenting Author*

Calciprotein particles (CPPs) are bloodborne proteinaceous nanoparticles key to mineral homeostasis. While the saturation of these particles in chronic kidney disease (CKD) is believed to promote cardiovascular disease progression, how high phosphate intake may acutely perturb this system remains unclear. This study characterized acute CPP responses to oral phosphate challenges in both health and experimental CKD.

Male Sprague-Dawley rats were profiled before and after CKD induction (n=10, 0.25%-adenine). Fasted animals rapidly consumed a 1%-phosphate diet with serum collected at 0, 2, and 6hrs. Serum minerals and OsteoSense-CPPs were measured throughout, while functional mineral buffering was assessed at 0 and 6hrs via T50 transition-times and non-centrifugable [phosphate] following an ex vivo mineral challenge. All differences are $p<0.01$.

In healthy animals, despite no elevation in serum [phosphate], OsteoSense-CPPs were elevated (11.5%) at 2hrs but returned to baseline by 6hrs. Conversely, in CKD, both serum [phosphate] and OsteoSense-CPPs increased (12%) at 2hrs and by 30% and 16.6% at 6hrs. Further, functional mineral buffering was diminished in CKD as demonstrated by the 55.9% greater reduction in T50 time and 344.4% greater rise in pro-calcific non-centrifugable [phosphate].

In CKD, adaptive postprandial rises in CPPs were greater than in health, but unable to effectively compensate for the pro-calcific loss of functional mineral buffering. This supports the utility of profiling postprandial CPP responses for the nuanced assessment of mineral homeostasis in CKD.

21. Emma LeBlanc (Microbes, Immunology and Inflammation) Investigating the mechanisms of Toll-Like Receptor 4 activation in COVID-19 cytokine storm

Authors: Emmanuelle V. LeBlanc, Alice M. Ball, and Che C. Colpitts
Department of Biomedical and Molecular Sciences, Queen's University, Kingston, Canada

The COVID-19 pandemic highlights the threat posed by respiratory viruses to human health and the healthcare system. Severe cases of COVID-19 are characterized by a disproportionate release of proinflammatory cytokines, known as cytokine storm, which damages organs and can lead to death. The immune protein Toll-like receptor 4 (TLR4) drives bacterial sepsis as well as virus-associated cytokine storm. The mechanisms by which viral glycoproteins, such as SARS-CoV-2 spike, induce TLR4 signaling remain unclear. We hypothesize that the under-processed glycosylation pattern of spike is recognized as a non-self moiety and leads to the activation of pro-inflammatory TLR4 signaling. We

have shown that exposure of THP1-derived macrophages to lentiviral particles pseudotyped with SARS-CoV-2 spike induces the expression of pro-inflammatory cytokines in a TLR4-dependent manner. Next, we produced spike pseudoparticles in cells deficient in MGAT1, an enzyme required for the elaboration of complex N-glycans. We observed an increased expression of pro-inflammatory cytokines when THP1 cells were exposed to SARS-CoV-2 pseudoparticles produced in MGAT1-/- cells, which are expected to have more high-mannose (under-processed) glycans compared to wild-type pseudoparticles. Additionally, we have generated a panel of spike glycosylation site mutants which have revealed roles of specific glycosylation sites for viral entry and TLR4 activation. Evaluating the viral factors that induce cytokine storm will impact our understanding and therapeutic approach to severe COVID-19 and other viral infections driven by hyperinflammatory responses.

22. Trisha Singh (Chronic Kidney Disease and Vascular Calcification) Fetuin-A ELISA Detection is Altered in the Presence of Calciprotein Particles and by Protein Acidification Status

Trisha Singh,1 Eric B P Fernandes BHScH,1 Heshanth Rasalingam,1 Nelson Chen,1* Emilie Ward,1 Laura van Staalduinen PhD,1 Rachel M Holden MD,1,2 Michael A Adams PhD1*
**contributed equally as co-third author*

1. Department of Biomedical and Molecular Sciences, Queen's University, Kingston, ON, K7L 3V6, Canada
2. Department of Medicine, Queen's University, Kingston, ON, K7L 3V6, Canada

In chronic kidney disease (CKD), impaired renal excretion causes fetuin-A to bind excess minerals, forming calciprotein particles (CPPs). These CPPs inhibit vascular calcification (VC), suggesting fetuin-A as a CKD/VC biomarker. However, conflicting literature on fetuin-A levels in CKD may stem from inaccurate ELISA detection in CPP presence. This study investigates CPP impacts on fetuin-A detection and methods to mitigate CPP-interference.

To minimize ELISA interference, in vitro CPPs underwent dialysis or EDTA-treatment. Acidified-CPPs (pH 4.5) were dialyzed before neutralization. Alternatively, CPPs were dissolved using 20mM EDTA. An ELISA compared untreated, acidified-dialyzed, and EDTA-treated CPP impacts on fetuin-A detection. The efficacy of purification methods was evaluated by comparing acidified and non-acidified fetuin-A using mineral-sequestration assays, T50 assays, and circular dichroism (CD). o-Cresolphthalein calcium and Dc protein assays confirmed mineral removal and sample retention following dialysis.

Previously acidified fetuin-A exhibited increased mineral sequestration in CPP-Is and larger CPP-II formation. Acidified protein CD profiling revealed a structural shift, restored after neutralization. Dialysis effectively removed minerals without sample loss; however, a 35% reduction in ELISA detection suggests altered antibody affinity following acidification. Both EDTA-treated and untreated CPPs artificially increased fetuin-A detection by 61% possibly due to enhanced epitope access or CPP presence during antibody synthesis.

Varying physiological conditions artificially alter fetuin-A detection, reducing its biomarker potential. Consequently, standardized sample preparation is crucial for determining fetuin-A's association with CKD/VC clinical outcomes.

23. Danielle Shibi Rosen (Immunology) Exploring the function and origin of kidney capsule macrophages

Danielle Shibi Rosen1, Seily Shrestha1, Sarah A. Dick1
1 Department of Biomedical and Molecular Sciences, Queen's University, Kingston, CA

Macrophages are a type of innate immune cell that have roles in pathogen detection, homeostasis, tissue development, and inflammation. In general, macrophages can be tissue resident and self-renewing, or recruited from the bone marrow via a monocyte intermediate. Previously, it was proven that are three main subsets of tissue resident macrophages, with conserved ontogeny and self-renewal capacity across tissues. These include TLF+ (expressing TIMD4, LYVE1, and FOLR2), MHCIIhi, and CCR2+. The kidney parenchyma is notably lacking the TLF+ macrophages that are present in all other tissues and constitute a yolk-sac derived subset, capable of self-renewal and contributes to wound repair. The kidney capsule is a fibrous tissue that encapsules the kidney parenchyma, that remains vastly understudied. It was recently shown that the kidney capsule contains TLF+ macrophages, however the function and origin of this population within the capsule is not known. Here we demonstrate a specific role for fibroblast-derived macrophage colony stimulating factor (mCSF) in the maintenance of this population, which is dispensable for macrophage maintenance in the parenchyma. We show that, unlike most TRMs, the renal capsule TLF+ macrophages do not appear to self-renew, and are replaced by infiltrating monocytes. This supports their dependence on fibroblast-mCSF, which we have shown is required for monocyte differentiation in the setting of injury. Future work will explore the functional role of these cells in injury.

Funding: NSERC

24. Sophia Stegeman (Inflammation, Immunity and Microbes) IL-27 influences the localization of endosomal TLR7/8 following IAV infection

Sophia K. Stegeman, Che Colpitts, Katrina Gee
Department of Biomedical and Molecular Sciences, Faculty of Queen's University, Kingston, Ontario, Canada

Innate immune responses are the first line of defence to prevent viral invasion or replication, and the main cells involved in this response are macrophages. Macrophages recognize pathogens via pattern recognition receptors (PRRs), leading to the production of cytokines and chemokines which regulate the immune response to infection. These PRRs recognize pathogen associated molecular patterns, such as nucleic acids from viruses. The main PRRs which recognize influenza are Toll-like receptor (TLR) 3, 7 and 8, where TLR7 and 8 recognize ssRNA and TLR3 recognizes dsRNA. These TLRs will signal and lead to cytokine expression, including the cytokine IL-27. However, it is not known if IL-27 and TLRs interact with one another. Therefore, we sought to identify the interaction between TLR7/8 and IL-27 during influenza infection. To accomplish this, we first treated A549s, which are a lung cell

line, with IL-27 (150ng/mL) for 24h and then infected with a pandemic influenza virus. We then imaged the cells to look for the localization of TLR7 and TLR8 post infection. We noticed that in the groups pre-treated with IL-27 there were modifications within the localization and expression levels of TLR7 and TLR8 over hours post infection when compared to those not treated with IL-27. Suggesting, that IL-27 may influence the function of TLR7/8 through modification of the cellular location.

Funding: KG is funded by an NSERC Grant, and SKS is funded by OGS

25. Rita Nakhle (Anatomical Sciences) Direct activation of dorsal root ganglia neurons by a bacterium associated with inflammatory bowel disease

Rita Nakhle, Hannah M. Wood, Corey C. Baker, David E. Reed, Alan E. Lomax
1. Gastrointestinal diseases research unit, Queen's University, Kingston, ON

Objective: Inflammatory bowel disease (IBD) is characterized by chronic relapsing inflammation of the gastrointestinal tract and abdominal pain. Recently, proteolytic activity from the gut bacterium Bacteroides vulgatus has been correlated with IBD disease severity. We hypothesize that B. vulgatus will directly activate visceral afferent neurons involved in nociceptive signalling. Methods: The effects of culture supernatant from B. vulgatus on cytosolic [Ca2+] signaling in dissociated mouse dorsal root ganglia (DRG) neurons was measured using fura-2 ratiometric Ca2+ imaging. An increase in [Ca2+] serves as an indicator of neuronal excitation. Results: Superfusion of B. vulgatus supernatant (dilution here) increased intracellular calcium by 26% ($p < 0.0001$, $n = 74$). Further, the amplitude of Ca2+ influx in response to the TRPV1 agonist capsaicin (x nM) was significantly elevated following exposure of neurons to B. vulgatus supernatant ($p = 0.0332$, $n = 60$). Conclusion: These data suggest that B. vulgatus can directly modulate Ca2+ handling in DRG neurons and sensitize these neurons to the pronociceptive mediator capsaicin. Future experiments will interrogate the role of proteases and their receptors in this effect. Supported by CIHR.

26. Julia Vassalakis (Cancer) SMARCA4 loss in endometrial cancer cells drives progression by enabling a state of sustained lineage plasticity

Mackenzie Coatham[1], Julia Vassalakis[2], Hannah Plummer[2], Hamid Ghaedi[3], David Knapp[4], Tyler Cooper[2,5], Jiahui Liu[1], Guihua Zhang[1], David Berman[3], Cheng-Han Lee[6], Lynne-Marie Postovit[2]

[1] Department of Oncology, University of Alberta, AB, CA
[2] Department of Biomedical and Molecular Sciences, Queen's University, Kingston, ON, CA
[3] Department of Pathology and Molecular Medicine, Queen's University, ON, CA
[4] Department of Pathology and Cell Biology, Université de Montréal, QC, CA
[5] Department of Biochemistry, The University of Western Ontario, ON, CA
[6] Department of Laboratory Medicine and Pathology, University of Alberta, AB, CA

Dedifferentiation in endometrial carcinoma denotes an aggressive phenotype, posing challenges in diagnosis and treatment. This phenotype features undifferentiated and well-differentiated regions

within the same tumor. Undifferentiated parts of Dedifferentiated Endometrial Carcinoma show mutations in chromatin remodelers, loss of epithelial differentiation markers, and acquisition of stem cells characteristics. Knockouts cell lines were generated for the catalytic subunit of the chromatin remodeling SWI/SNF complex SMARCA4. Transcriptome analysis showed downregulation of adherent junctions of epithelial cells and upregulation of stemness factors. Loss of SMARCA4 initially induced a senescent-like phenotype, characterized by the presence of increased protein expression of senescent-associated secretory phenotype. When serially transferred into in vivo models, SMARCA4 loss recapitulated clinical DDEC features observed in patients. Dedifferentiation was shown histologically, with the loss of differentiation and epithelial markers. When treated with chemotherapy such as carboplatin, tumors with SMARCA4 loss behaved as treatment-resistant undifferentiated regions of clinical DDEC. Singel-cell sequencing showed that gene expression alterations occur overtime in SMARCA4 knockout tumors and that these are associated with changes in chromatin occupancy. This progression was not hierarchical, and clones were not selected via mutations. Trajectory analysis comparing initially generated SMARCA4 knockout cells and more terminally dedifferentiated tumors suggest that high tumor heterogeneity observed overtime occurs stochastically and is multidirectional. Overall, this model suggests that SMARCA4 loss enables progression by permitting epigenomic chaos, resulting in the acquisition of undifferentiated phenotypes.

27. Monica Opoka (Biochemistry and Cell Biology) Mitochondrial Dynamics Regulate Activation of the Integrated Stress Response

Monica Opoka, Kaylee Punter, Sheela Abraham, and Edmond Chan.
Department of Biomedical and Molecular Sciences, Queen's University, Kingston, ON, Canada.
Cancer Research Society Canada.

Cancer cells that survive cytotoxic therapies, termed persister cells, can lead to cancer recurrence. New studies suggest that the aggressive phenotype of persister cells is driven in part by incomplete apoptotic commitment. Specifically, sublethal cytochrome c release from mitochondria activates the integrated stress response (ISR) and downstream adaptive transcriptional changes. Accordingly, the mitochondrial ISR is a promising target to potentially reduce persister cell formation. However, the ISR has dual roles in promoting both cell survival and apoptosis, supporting the need for more detailed understanding to effectively target this pathway. Related to this, during apoptosis initiation, remodelling of the mitochondrial membrane is required for cytochrome c release. The dynamic remodelling of mitochondria involves fission and fusion events, each controlled by GTPase proteins. Thus, we hypothesized that mitochondrial dynamics can regulate the activation of the ISR via modulation of intrinsic apoptosis. We utilized a class of chemotherapeutics, BH3 mimetics, to activate the mitochondrial intrinsic apoptotic pathway in human triple-negative breast cancer cells. We observed a notable increase in apoptosis and ISR activation by the BH3 mimetics upon silencing the mitochondrial fission protein, Drp1, but not the mitochondrial fusion protein, Opa1. These findings suggest that Drp1 may function to suppress ISR activation, while Opa1 promotes the ISR.

Thus, targeting mitochondrial dynamics could represent a promising strategy to increase the efficacy of chemotherapy and reduce persister cell development.

28. Tristin Wilson (Osteology) A novel bone health marker: increased calcium excretion in females with osteoporosis following an acute dietary phosphate challenge

    Authors: Tristin Wilson, Mandy Turner, Bikram Sidhu, Michael Adams, Rachel Holden

    Background: Osteoporosis is a prevalent and destructive skeletal disorder among postmenopausal females. Bone mineral density (BMD) scans diagnose osteoporosis but have significant limitations. The oral phosphate tolerance test (OPTT) reveals dysregulation in calcium and phosphate homeostasis.

    Objectives: (1) Utilize the OPTT to identify females with altered bone remodeling. (2) Develop an early response marker which overcomes the limitations of BMDs, reflects the dynamic state of the skeleton, and enhances clinical decision making.

    Methods: In an ongoing longitudinal cohort study, 59 females aged 65 and older underwent baseline BMDs and OPTTs. BMD was determined using DXA for the lumber spine and femoral neck of the hip. OPTTs measured urinary calcium and phosphate excretion in response to an oral phosphate drink.

    Results: Baseline urinary phosphate to creatinine and calcium to creatinine ratios did not differ between females with varying bone health status. Females with osteoporosis excreted ~2.7x more calcium than healthy females (0.51±0.7 vs 1.4±0.6, $p<0.05$) and ~1.9x more calcium than females with osteopenia (0.73±0.7 vs 1.4±0.6, $p<0.05$) over the 4 hours following the oral phosphate challenge.

    Conclusion: The OPTT reveals dysregulated mineral homeostasis in females with osteoporosis. Findings support the continuation of this trial to further develop the OPTT as a dynamic marker of bone health.

    Supporting Agency: SEAMO

29. Kasthuri Ravishanker (Reproduction and Developmental Sciences) Determining the Role of Cannabinoids in Modulating Neuroinflammatory Pathways in Endometriosis

    Kasthuri Ravishanker, Alison McCallion, Harshavardhan Lingegowda, Chandrakant Tayade
    DBMS

    Objective: Characterize immune cell composition of dorsal root ganglia (DRGs) in endometriosis (EM) and assess cannabinoid treatment effects on the DRG immune microenvironment.

Study Methods: Lumbar DRGs from C57BL/6 mice were enzymatically digested and cultured to determine cytokine profiles. EM mice (n=5) were treated with CBD (15 mg/kg) or vehicle. Cytokine analysis was conducted on plasma. Flow cytometry was performed on peritoneal fluid (PF) and spleen cells. Lesions underwent immunohistochemistry (IHC) to identify endothelial structures and proliferative cells. In an EM vs. sham-operated mouse study (n=1), total RNA was extracted from thoracic and lumbar DRGs to identify inflammation-related genes.

Results: Protocols facilitated DRG isolation and culture. Flow cytometry of PF and spleen cells in CBD and vehicle-treated mice revealed differences in CD3+ T cells and CD11b+ myeloid cells. Cytokine analysis indicated significant differences in IL-9 and IL-13 concentrations between post-treatment plasma samples in CBD and vehicle groups. Differential gene expression was observed between thoracic and lumbar DRGs.

Conclusion: Preliminary findings suggest CBD-induced changes in immune cell populations and modulation of the inflammatory response in EM-induced mice. Analyzing gene expression in DRGs from different treatment states may uncover inflammation-related gene expression differences, enhancing understanding of cannabinoid effects on EM-associated pain pathways.

Funding Source: CIHR

30. Eileen O'Brien (Microbes, Immunity and Inflammation) Cytokine multi-omics and immunohistochemistry identifies ccl3 as a potential mediator of inflammatory bowel disease severity

Eileen O'Brien, BSc1,2, Chinmay Potdar, BSc1,2, Daniel J. Mulder, MD, PhD1,2,3
1. Department of Pediatrics, Gastrointestinal Diseases Research Unit, Queen's University, Kingston, Ontario
2. Department of Biomedical and Molecular Sciences, Queen's University, Kingston, Ontario
3. Department of Medicine and the Translational Institute of Medicine, Queen's University, Kingston, Ontario

Background: Chemokine Ligand 3 (CCL3) is a proinflammatory chemokine previously shown to be increased in murine models of colitis but has not been established to be related to inflammation in humans. We aimed to characterize the changes in CCL3 expression in relation to a range of inflammatory bowel disease (IBD) severity.

Hypothesis: CCL3 production is increased in IBD patient gut mucosal biopsies and localization correlates with inflammation severity.

Approach: In this study (HSREB 6033229), 17-plex multi-fluorescent bead-based immunoassay (Abcam, Cambridge, UK) was used to investigate cytokine profiles of a subset of IBD and control patients. Gastrointestinal mucosal biopsies were collected from IMAGINE study IBD participants (N=21, n=58). Immunohistochemistry was performed with a CCL3 antibody (ab32609). CCL3 expression was quantified using QuPath image analysis software (v.0.4.4). Inflammation severity of

each biopsy is graded using the Naini & Cortina Score (Naini & Cortina, 2012). Using R script, mucosal CCL3 expression is compared to inflammation severity.

Results: An extreme gradient boost ML model was found to have optimal sensitivity and specificity for predicting disease activity based on serum cytokine levels. Within this model lower levels of serum CCL3 were associated with higher severity of IBD activity. Among ulcerative colitis (UC) biopsies, CCL3 expression increases with the severity of gut inflammation. CCL3 was found to be expressed on macrophages, eosinophils, and neutrophils. Expression is rarely on epithelial cells.

Conclusions: With the data collected, we identify CCL3 as a possible biomarker and therapeutic target for severe IBD.

31. Danielle Sisnett (Reproduction and Developmental Sciences) The IL-23/Th17/IL-17 axis and its role in endometriosis pathophysiology

Authors: Danielle J. Sisnett, Katherine B. Zutautas, Jessica E. Miller, Harshavardhan Lingegowda, Soo Hyun Ahn, Alison McCallion, Olga Bougie, Bruce A. Lessey, and Chandrakant Tayade
Department: Biomedical and Molecular Sciences, Queen's University, Kingston, Ontario, Canada
Program: Reproduction and Developmental sciences
Funding/Supporting Agency: Work is supported by funding from CIHR and NSERC

Introduction: Endometriosis is a chronic inflammatory disease perpetuated by immune dysfunction. Indeed, the IL-23/T-helper(Th)17/IL-17 axis has been increasingly implicated in endometriosis pathophysiology and associated infertility. IL-23 is a key mediator in development/differentiation of Th17 cells, driving pathogenic Th17 cells producing pro-inflammatory IL-17, known to contribute to endometriosis pathophysiology. IL-23, IL-17, and Th17 cells are elevated in endometriosis and associated with disease severity. Thus, it is postulated that IL-23 promotes pathogenic dysregulation of Th17 cells in endometriosis to exacerbate disease.

Aims: Investigate endometriotic lesions as source of IL-23/Th17/IL-17 axis mediators and examine IL-23 function in pathogenic dysregulation of Th17 cells. Further, define unique functional profile of pathogenic/non-pathogenic Th17 cells in endometriosis.

Methods: Quantified IL-23/Th17 axis mediators in patient tissues and plasma via qPCR/ELISA. Representative human cell lines (endometriotic epithelial, endometrial epithelial, endothelial, stromal, primary Th cells) stimulated with rhIL-23 underwent cytokine/chemokine analysis. Further, immune dysfunction was investigated using a syngeneic endometriosis mouse model.

Results: Key genes in IL-23/Th17 axis were dysregulated in endometriosis ectopic/eutopic tissues. In-vitro rhIL-23 stimulation of aforementioned cell lines influenced secretion of cytokines known to promote lesions. In-vivo rmIL-23 treatment captured immune dysfunction at lesion-level. Th17 cells

were isolated from endometriosis/control blood via FACS. Continued analysis underway.

Impact: Results suggest dysregulation of the IL-23/Th17 axis and support that IL-23 potentiates endometriosis-associated inflammation. This may reveal potential diagnostic/therapeutic targeting of IL-23/Th17 axis in endometriosis

32. Sumaiya Afrin (Biomedical Informatics) Genetic haplotypes associated with human milk oligosaccharides also impact maternal health

Sumaiya Afrin (presenter), Qingling Duan
Biomedical and Molecular Sciences

It is well established that breast milk and its many components, including human milk oligosaccharides (HMOs), benefit the health of milk-fed infants. Earlier studies showed that HMOs contribute to immune system development and the infant gut microbiota. However, the genetic determinants that influence HMO composition and secretor status—an individual's ability to secrete blood group antigens into bodily fluids—remain poorly understood, along with their implications for maternal health. While previous research has largely focused on individual genetic variants, we hypothesize that a combination of alleles (e.g., haplotypes) may influence the secretion of HMOs in human milk, secretor status, and maternal health.

Our analysis was conducted using the haplo.stats package in R, utilizing SNPs previously associated with HMOs and secretor status by our team. We then associated the haplotypes with maternal traits by computing the regression of a trait on haplotypes. Our analysis revealed that haplotypes on chromosomes 19p and 19q are significantly associated with maternal HMOs, secretor status, and maternal health outcomes such as anemia, diabetes etc.

Our study provides novel insight into the genetic basis of interindividual variations in HMOs and secretor status, as well as their potential contribution to maternal and infant health. These nuanced studies of the mother-milk-infant triad inform the benefits of breastfeeding and breast milk, guiding strategies for protecting the health of infants and mothers.

33. Kartik Sachdeva (Cancer Research) Unraveling the association between circulating t follicular helper cells and atypical b cells in non-muscle invasive bladder cancer progression

Kartik Sachdeva1, Priyanka Yolmo1, Sadaf Rahimi1, Gwenaelle Conseil1, Nick Vanin2, D. Robert Siemens1,2 and Madhuri Koti1
1Queen's Cancer Research Institute, 2Department of Urology, Queen's University, Kingston, ON, Canada

Introduction: Recurrence and progression following treatment remain a major challenge in the management of non-muscle invasive bladder cancer (NMIBC). Our previous findings revealed a significant correlation between an elevated B cell densities within tumors and unfavorable clinical outcomes in individuals diagnosed with NMIBC. Specifically, we have identified a potential role of

subset of B cells called 'atypical B cells (ABCs)', in disease progression and treatment outcomes. Given the role of TFH cells in the regulation of B cell responses, hypothesize that investigating the role of TFH cells could offer insights into mechanisms underlying B cell exhaustion, which are intricately linked to tumor progression and treatment response.

Methods: We investigated the correlation between TFH-associated transcript abundance and clinical outcomes in tumors from patients with NMIBC. Two publicly available tumor whole transcriptome profiles from patients with NMIBC were analyzed. Multi-parametric flow cytometry-based profiling of circulating TFH cells (cTFH) and atypical ABCs was performed using peripheral blood collected from patients undergoing transurethral bladder tumor resection (TURBT) at the Kingston Health Sciences Center. Profiles of TFH cells were measured at multiple time points during disease progression in a carcinogen induced murine model of bladder cancer.

Results: Bulk-RNA sequencing-based tumor whole transcriptome analysis revealed patient sex, tumor grade, and stage-associated differences in TFH profiles possibly linked to B and T cell interaction. Preliminary results showed an inverse correlation between cTFH and circulating ABCs in patients (n=35), mirrored in carcinogen-exposed mice.

Conclusion: Findings from this study indicate a potential role of TFH in mediating B cell exhaustion during the progression of NMIBC. These findings hold the promise of uncovering immune monitoring biomarkers and potential therapeutic targets within the pathways involving cTFH cell mediated mucosal immune exhaustion in bladder cancer.

Funding: Research funded by Bladder Cancer Canada and the Cancer Research Society.

34. Michelle Kuriakose (Experimental Medicine) The influence of mitochondrial DNA (mtDNA) signature on the transcriptome of the hypothalamic arcuate nucleus (ARC) of the brain in a model of high-fat diet induced obesity

Michelle Kuriakose1, Prusha Balaratnam1, Ethan Morad1, Aleksa Groen in ‘t Woud1, Alex Choe1, Kate Zucconi2, Janet Menard2, Kimberly J Dunham-Snary3,4*, Charles Hindmarch3,4*

1 The Discovery Program, Department of Biomedical and Molecular Sciences, Queen’s University, Kingston, Ontario, Canada
2 Department of Psychology, Queen’s University, Kingston, Ontario, Canada
3 Department of Biomedical and Molecular Sciences, Queen’s University, Kingston, Ontario, Canada
4 Translational Institute of Medicine, Department of Medicine, Queen’s University, Kingston, Ontario, Canada
**These authors contributed equally to this work*

Background: Cardiometabolic diseases (CMD) are risk factors for cardiovascular disease and Type 2 diabetes, the leading causes of global mortality. There remains an urgent and unmet need to investigate the molecular mechanisms underpinning CMD. Mitochondrial genetic variation has been identified as an area of interest for studying CMD and obesity, specifically to explain ancestral variation in disease susceptibility, severity, and prognosis.

Mouse Model: Here, we use the Mitochondrial-Nuclear eXchange (MNX) mouse, which was designed to investigate mitochondrial-nuclear genome interactions. We have previously demonstrated transcriptional differences in adipose tissue depots between MNX and wild-type mice. While several tissues have been explored in this model, the brain has not yet been studied despite its role in feeding dynamics and energy homeostasis. A key hypothalamic structure underpinning food intake and metabolism is the arcuate nucleus (ARC).

Hypothesis: Mitochondrial DNA signature will influence expression of genes associated with energy homeostasis and feeding regulation in the ARC, and differences will be during high fat diet exposure.

Methods: ARC was isolated from brain slices obtained from wild-type and MNX mice fed chow or highfat diet for six weeks. 3'Next-Generation RNA Sequencing was used to profile the ARC transcriptome. Gene expression patterns that are specific to mtDNA background and high fat diet were presented and placed into biological context using functional analysis.

35. Farzaneh Afzali (Experimental Medicine)

36. Priyanka Yolmo (Cancer Research) Investigating bacillus Calmette-Guérin induced B cell responses in patients with non-muscle invasive bladder cancer

Priyanka Yolmo[1,2], Kartik Sachdeva[1,2], Sindhuja Pattabhi[3], Alanna Brewer[4], Abdulhameed Abdulhamed[5], Ashley Griffin[6], Gwenaëlle Conseil[1,2], Haocheng Yu[7], David M. Berman[5], Amir Horowitz[7], Morgan Roberts[3], Peter Black[3], D. Robert Siemens[2,4,6] and Madhuri Koti[1,2,6,8]

[1]Queen's Cancer Research Institute, Kingston, ON, Canada; [2]Department of Biomedical and Molecular Sciences, Queen's University, Kingston, ON, Canada; [3]Vancouver Prostate Centre, Department of Urologic Sciences, University of British Columbia, Vancouver, BC, Canda; [4]Department of Urology, Kingston Health Sciences Center, Kingston, ON, Canada; [5]Pathology and Molecular Medicine, Queen's University, Kingston, ON, Canada; [6]Department of Urology, Queen's University, Kingston, ON, Canada; [7]The Marc and Jennifer Lipschultz Precision Immunology Institute, Icahn School of Medicine at Mount Sinai, New York, NY, USA; [8]Department of Obstetrics and Gynecology, Queen's University, Kingston, ON, Canada

Background: Despite the proven efficacy of bacillus Calmette-Guérin (BCG) immunotherapy treatment for non-muscle invasive bladder cancer (NMIBC), over 50% of patients experience early disease recurrence or progression. An improved understanding of BCG induced anti-tumor mucosal immune responses is needed to identify biomarkers of response and alternative therapies.

Rationale: Our previous research revealed high intra-tumoral B cell density correlates with shorter recurrence- and progression-free survival in BCG-treated patients. Using a carcinogen-induced murine model, we reported that repeated BCG administration expanded an exhausted B cell subset, atypical B cells in the bladder microenvironment. Depletion of exhausted B cells during BCG treatment in BBN-exposed mice promoted urothelial recovery and reduced PD-L1 immune checkpoint expression. Thus, we hypothesize that expansion of ABCs during repeated BCG instillation dampens the anti-tumor immune responses, leading to early recurrence or progression to muscle invasive disease.

Methods: B cells from peripheral blood of patients undergoing BCG treatment were characterized using multispectral flow cytometry. Matched plasma samples were analyzed using the Olink Target-96 Immuno-Oncology panel. Corresponding index TURBT specimens underwent histopathological evaluation.

Results: Flow cytometry-based profiling of circulating B cells revealed a variable trend in ABC profiles post-BCG treatment, with a significant expansion in majority of the recurrent cases. Olink platform-based analysis of plasma revealed differential protein expression post-BCG group compared to the pre-BCG. Patients exhibiting early recurrence had significantly increased levels of secreted PD-L1 and IL-6, whereas those who did not recur had elevated CD40, CD5, and IL-12, after four intravesical BCG instillations.

Conclusion: Novel findings from this study indicate a potential influence of BCG-induced innate and adaptive functions of B cells in response. Targeting B cell exhaustion could be a novel therapeutic approach for patients at risk of early recurrence.

37. Nick Denniston (Virology) The Role of HSV pUL16 in Altering Host Mitochondrial Physiology

Authors: N.G. Denniston, A. Lubinsky, S.M. Holder, M. Wilkinson, M. Bossert, K. Dunham-Snary, B.W. Banfield

The conserved, Herpes simplex virus (HSV) tegument protein, pUL16, is implicated in several viral processes, including virion morphogenesis and cell-to-cell spread of infection. Recent work has shown that HSV-2 pUL16 localizes to distinct regions of the host cell mitochondria; however, the implications of this interaction remain unclear. Our proximity-dependent Bio-ID experiments have identified both mitochondrial-associated membrane and matrix components in proximity to pUL16 throughout infection. Electron microscopy experiments have also shown dramatic alterations in mitochondrial ultrastructure between HSV-2 186 wild-type (WT), HSV-2 virus deleted for pUL16 (Δ16), and mock-infected HaCaT cells. We hypothesize that the interaction between pUL16 and mitochondrial components regulates mitochondrial physiology to promote viral replication. Our preliminary analysis of mitochondrial oxygen consumption and extracellular acidification rates in HSV-2 186 WT, HSV-2 Δ16 and mock-infected HaCaT cells suggest key differences in mitochondrial respiration. At 6-9hpi, the levels of cellular basal respiration, spare respiratory capacity, and maximal respiration were decreased in Δ16 infected cells, while these parameters were preserved in the WT infected cells relative to mock infection. These findings suggest that HSV-2 pUL16 may function to preserve mitochondrial respiration throughout infection. Future experiments will characterize the differences in mitochondrial respiration, glycolytic rate, and ATP production in multiple strains of HSV-1 and -2, to better understand how HSV infection impacts cellular metabolism.

38. Ethan Thomas (Biomedical and Molecular Sciences) The Multifunctional Viral Proteins pUL21 and pUL16 Facilitate Redundant Roles in Herpes Simplex Virus Type-1 (HSV-1) Nuclear Egress

Ethan C.M. Thomas[1], Renée L. Finnen[1], and Bruce W. Banfield[1]

[1]Department of Biomedical and Molecular Sciences, Queen's University, Kingston, Ontario, Canada

Inside the nucleus of HSV infected cells, transcription of viral genes, viral genome synthesis, capsid assembly, and genome packaging into nascent capsids takes place. Nucleocapsids then transit from the nucleoplasm to the cytoplasm for the final stages of virion maturation, in a process called nuclear egress. Two multifunctional viral proteins and binding partners, pUL21 and pUL16, have been implicated in the nuclear egress of HSV-2 nucleocapsids, however, this is not the case for HSV-1. HSV-2 mutants deleted for pUL21 (Δ21) or pUL16 (Δ16) alone display severe nuclear egress defects, while single deletion mutants in HSV-1 do not. Interestingly, we found that simultaneous deletion of both pUL21 and pUL16 (Δ21/Δ16) impairs HSV-1 nuclear egress. This defect resulted in downstream effects including impaired cell-to-cell spread and viral replication of the Δ21/Δ16 mutant in comparison to the single Δ21 and Δ16 mutants. These data suggest that pUL21 and pUL16 facilitate redundant roles in HSV-1 nuclear egress. Since HSV-1 nuclear egress functions in the sole absence of pUL21 or pUL16, we hypothesize that pUL21 and pUL16 complex formation is not necessary to facilitate this process. To test this, we are constructing an HSV-1 mutant that contains a point mutation that impairs the interaction between pUL21 and pUL16 and expect that this mutant will not have a nuclear egress defect. Understanding the roles pUL21 and pUL16 play individually, and in complex, will enhance our understanding of HSV assembly and the differences between HSV-1 and HSV-2 morphogenesis.

39. Hannah Plummer (Cancer) SMARCA4 loss as a driver of cellular plasticity and dedifferentiation in endometrial cancer

Department: Biomedical and Molecular Sciences – Experimental Medicine

Dedifferentiated endometrial carcinoma (DDEC) arises in the endometrial lining of the uterus and is characterized by the mixed presence of dedifferentiated and differentiated regions. In two-thirds of cases, the dedifferentiated regions are associated with the loss of a component of the SWItch/Sucrose-non fermentable (SWI/SNF) complex, often SMARCA4, the catalytic domain. The SWI/SNF complex is a chromatin remodelling complex responsible for altering chromatin accessibility by sliding and evicting nucleosomes. Our lab has previously shown loss of SMARCA4 drives the dedifferentiation observed in DDEC. Microenvironment stresses encountered by the tumour such as hypoxia, induce an adaptive response including epigenetic modifications, transcriptional changes, and translational reprogramming. Data from our lab has shown that when SMARCA4 is knocked out (KO) of endometrial cancer cells the DDEC disease phenotype is recapitulated, including reduction in DNA methylation, and loss of epithelial markers, in addition to the induction of a senescent-like phenotype (increased secretion of the senescent associated secretory phenotype, etc…) that the cells overcome with serial *in vivo* passaging. An increase in mTOR signalling has been linked to an increase in SASP secretion. Therefore, we hypothesize that SMARCA4 loss leads to reductions in histone methylation which enable dedifferentiation, in addition to alterations in mTOR signaling enabling senescence escape. Using western blot analysis and functional assays we have shown that SMARCA4 KO cells have an increase in mTOR activation. In addition to a global reduction in DNA methylation, histone methylation is significantly reduced both under hypoxia and in the presence of demethylase inhibitors in the KO cells.

40. Linnea Soon (Translational Medicine) DAYS: A study to assess the safety and efficiency of a simplified diagnostic approach for deep vein thrombosis

Authors: Soon L, Clayton N, James P, Good D, Tarulli E, Parpia S, de Wit K This study is conducted in the Department of Emergency Medicine

Current evidence-based diagnostic algorithms for deep vein thrombosis (DVT) were developed in the 1990s and designed for the clinic setting. Due to the complexity of evidence-based DVT testing algorithms, they are seldom used by physicians in the emergency department (ED) and may entail multiple visits for patients. To reduce emergency physician cognitive load and improve patient experience, we propose the DAYS algorithm. The DAYS algorithm consists of one clinical item answered by the physician and the blood D-dimer result. The clinical item is the physician's estimate of whether DVT is the patient's most likely diagnosis. If DVT is the most likely diagnosis, the standard D-dimer limit excludes DVT. If DVT is not the most likely diagnosis, the patient's age-adjusted D-dimer limit excludes DVT. If DVT cannot be excluded based on the D-dimer, the patient undergoes ultrasonography. Patients tested for a lower limb DVT at the Kingston Health Sciences Centre ED or urgent care centre are eligible. Our aim is to determine the safety and efficiency of the DAYS algorithm for DVT testing by conducting a retrospective medical record review. The primary outcome is the 90-day false negative rate of the DAYS algorithm. If the DAYS algorithm is safe and efficient, it can change emergency physician practice and improve patient care in the ED.

41. Dalia Miller (Cardiometabolic Disease) Mitochondrial DNA signature modulates the progression of cardiometabolic disease in mice

Dalia Miller, Mia Wilkinson, Kimberly Dunham-Snary
Department: Department of Medicine & Department of Biomedical and Molecular Sciences
Supporting Agencies: Canadian Institutes for Health Research, Canada Research Chairs, Canada Foundation for Innovation, Banting Research Foundation

Background: Cardiometabolic disease (CMD) affects 20% of Canadians, and is a cluster of inter-related disorders including non-alcoholic steatohepatitis (NASH), abdominal obesity, and insulin resistance. Current interventions do not adequately address underlying genetic and biomolecular factors impacting disease etiology.
Objective: Our objective is to investigate the significance of mtDNA signature on the progression of CMD.

Methods: Mitochondrial Nuclear eXchange (MNX) mice harboring reciprocally exchanged nuclear and mitochondrial genomes, were generated using CMD-prone C57BL6/J and CMD-resistant C3H/HeN mice. Comparing phenotypes of MNX mice to their nDNA-matched control allows for isolation of mtDNA-specific effects on CMD phenotype. 6-week-old wild-type and MNX mice were fed either control (13% fat, 12% sucrose by weight), or a 'Western-style diet' (WSD; 42% fat, 34% sucrose by weight) for 3, 6, 12, and 18 weeks. Liver histology was performed using hematoxylin and eosin and the NASH Clinical Research Network (CRN) scoring system was used to assess disease progression.

Results: 3 weeks of WSD was sufficient to noticeably increase lipid accumulation in male mice, compared to age- and sex-matched controls. Induction of pre-clinical hepatosteatosis was confirmed in WSD-fed male mice at the 6-week timepoint versus sex-matched mice only exposed to WSD for 3 weeks.

Conclusions: Preliminary results suggest that the early stages of NASH are emerging in as little as 3 weeks of WSD, and ongoing studies will determine if mice with differing mtDNA signature exhibit diverging disease progression.

42. Cassidy Laub (Cancer Health Services Research) A scoping review of the quality of research quantifying the association between the cancer diagnosis to treatment interval and overall survival

Authors: Laub C (1,2), Jalink M (1,3), Hanna TP (1,2,3,4)

Author Affiliations: (1) Division of Cancer Care and Epidemiology, Cancer Research Institute at Queen's University, Kingston ON, Canada, (2) Department of Medicine, Queen's University, Kingston ON, Canada, (3) Department of Public Health Sciences, Queen's University, Kingston ON, Canada, (4) Department of Oncology, Queen's University, Kingston ON, Canada

Background: Studies investigating the association between the cancer diagnosis to treatment interval (DTI) and overall survival (OS) have been plagued by poor validity and inconsistent methods, affecting practice and policy relevance. We evaluated the quality of methodologies to explore whether renewed interest in this topic during the COVID-19 pandemic was associated with improved study quality.

Methods: We performed a scoping review to identify studies evaluating the association between cancer DTI and OS, published between April 11, 2020, and October 15, 2023, on curative treatment for breast, lung, colon, rectum, cervix, bladder, and head and neck cancers. This period follows the publication of an influential systematic review identifying limitations in the quality of historic studies on the topic.

Results: We identified 51 studies and the methodologies of a random sample of 30 studies were evaluated. All were retrospective cohort studies. There were inconsistencies in the index date for measuring OS with 33% using date of diagnosis, 40% using date of treatment initiation, and 27% not stating the index date. None of the studies that stated the index date managed bias appropriately (i.e., immortal time bias, lead-time bias). All studies used multivariable regression adjustment, but 47% stated missing variables as a limitation.

Conclusions: This study highlights poor validity and inconsistency of methods amongst recent cancer DTI and OS studies. Guidelines are needed to improve the quality of this literature to inform health policy.

43. Gabriella Torretto (Cancer Biology and Genetics) Assessing the Clinical Relevance of BRCA1 BRCT Domain Variants of Uncertain Significance

Departments: 1. Department of Pathology and Molecular Medicine, Queen's University 2. Cancer Research Institute, Division of Cancer Biology and Genetics, Queen's University

Authors: Gabriella Torretto, Matthew Martin, Nicole Archer, Kaamraan Islam, Harriet Feilotter PhD and Scott Davey MBA, PhD Presenting Author: Gabriella Torretto

Up to 10% of breast and 25% of ovarian cancers are caused by inherited genetic mutations, most commonly in the BRCA1 and 2 genes. BRCA1 is tumour suppressor gene involved in various mechanisms that promote genomic stability, such as DNA repair. Therefore, mutations that disrupt its normal functioning significantly increase the risk of developing cancer. Genetic testing is used to identify carriers of pathogenic BRCA1 mutations, who would then be eligible for enhanced surveillance, risk-reducing strategies, targeted treatments as well as reflex family testing. Contrarily, those with benign mutations can avoid any unnecessary interventions. The widespread implementation of genetic testing however has resulted in the discovery of thousands of variants of uncertain significance (VUS) - DNA mutations with unknown effects on gene function and disease risk. VUSs pose a serious clinical challenge as clinicians are unable to effectively recommend appropriate treatment and management steps to patients with VUSs. Approximately half of all documented BRCA1 variants have some level of uncertain significance. BRCA1's BRCT domain plays essential roles in mediating DNA repair. Thus, assessing VUSs in this domain is of high interest. Here, we developed a BRCA1 BRCT domain-specific machine learning classifier trained to predict VUS pathogenicity with enhanced accuracy and stratify variants to conduct functional analyses on. Pull-down assays were then used to assess the impact of select VUSs on BRCT functionality. Along with providing strong evidence for the classification of select BRCA1 VUSs, this study reveals the utility of domain-specific approaches for VUS interpretation.

Supporting Agencies: Canadian Cancer Society, Canadian Breast Cancer Foundation

44. Matt Martin (Breast Cancer)

Matthew Martin1,2, Gabriella Torretto1,2, Kaamraan Islam2, Nicole Archer2, Harriet Feilotter, PhD1,2 & Scott Davey, MBA, PhD1,2

Departments:
1Department of Pathology and Molecular Medicine, Queen's University, Kingston, Ontario
2Cancer Research Institute, Division of Cancer Biology and Genetics, Queen's University, Kingston, Ontario

The BRCA1 protein serves an essential function in maintaining genomic integrity, guarding against the development of cancer. Consequently, inheriting a copy of the BRCA1 gene with a mutation that impairs this function can increase a woman's risk of breast cancer sixfold. These genetic variants are deemed pathogenic, with carriers benefiting from risk-reducing interventions like surgery. Alternatively, benign variants preserve BRCA1 function and do not impact cancer risk or require intervention. Accordingly, the ability to discern between these two variant types is critical to

appropriately prescribe care. Unfortunately, around 40% of all BRCA1 variants have uncertain significance (VUS) with unknown impacts on protein function and overall cancer risk, providing no information to inform patient care. To address this problem, our lab obtains evidence to resolves the true nature of BRCA1 VUS as pathogenic or benign. This project focused on VUS within the N-terminal RING domain of BRCA1, and took had two evidence approaches. First, an artificial intelligence algorithm was trained to predict the cancer risk of VUS based on patterns that distinguish currently validated pathogenic and benign variants. Then, to confirm algorithm predictions on a molecular basis, we designed a test to measure the functional level of VUS; considering the protein's essential role in cancer prevention, observing reduced activity served as evidence that the variant increases cancer risk and is pathogenic. Future work will strengthen evidence through further functional testing and pedigree analysis.

Supporting Agencies: Canadian Cancer Society, Canadian Breast Cancer Foundation

45. Juliana Shizas (Cancer Research) Investigating the role of the RET receptor in pancreatic ductal adenocarcinoma cell invasion

Julie C Shizas, Timothy J Walker, Brandy D Hyndman, Lois M Mulligan.

Pancreatic ductal adenocarcinoma (PDAC) constitutes 90% of pancreatic neoplasms and is the third leading cause of cancer-related death in Canada. This therapeutically challenging disease is characterized by minimal early-onset symptoms, late detection, and rapid progression. REaranged during Transfection is a proto-oncogene and receptor tyrosine kinase present in approximately 50-65% of PDAC cases, where elevated expression is correlated with poorer patient prognoses. Previously, our lab established that activation of RET induces directional cell motility and increases the invasive potential of PDAC cells. Despite these preliminary findings, the mechanisms and signaling events underlying RET-mediated PDAC cell invasion remain unclear. Another potential oncoprotein in PDAC specimens is TKS5 (tyrosine kinase substrate with 5 SH3 domains), an SRC-kinase substrate required to form actin-rich, proteolytic cell protrusions termed invadopodia. Preliminary data suggests that RET activation promotes phosphorylation of SRC kinase and other downstream mediators involved in cell migration invasion, including FAK and AKT. We also found that sustained RET activation enhances RET-TKS5 colocalization in PDAC cells. Future studies will investigate whether colocalization occurs at proteolytically active cell extensions and explore whether genetically and chemically inhibiting RET, SRC, or TKS5 abolishes this process. Together, we hope to elucidate the molecular mechanisms underlying RET-mediated motility and invasion in PDAC cells.

46. Montdher Hussain (Cancer Research) A role for the cell adhesion molecule NCAM1 in mediating activity of the receptor tyrosine kinase RET in cancer

Montdher H. Hussain,1 Brandy D. Hyndman,1 Frederick S. Vizeacoumar,2 Mathieu J. Crupi,3 Franco J. Vizeacoumar,2 and Lois. M. Mulligan.1
1. Division of Cancer Biology and Genetics, Cancer Research Institute, and Department of Pathology and Molecular Medicine, Queen's University, Kingston, ON.

2. Cancer Research, Saskatchewan Cancer Agency and Division of Oncology, University of Saskatchewan, Saskatoon, SK.
3. Cancer Therapeutics Program, Ottawa Hospital Research Institute, Ottawa, Ontario, Canada

RET (REarranged during Transfection) is a receptor tyrosine kinase with essential roles in early development; however, genetic alterations lead to aberrant RET activity that contributes to oncogenic processes in several cancers. While some of the mechanisms by which RET mediates these processes are known, the network of proteins facilitating its activity remains largely undiscovered. To address this, we performed a genome-wide synthetic dosage lethal screen which identified NCAM1 (Neural Cell Adhesion Molecule 1) as one potential candidate facilitating RET function. Using an NCAM1 knockdown (KD) in SH-SY5Y neuroblastoma cells to assess the relationship between RET and NCAM1, we show that loss of NCAM1 reduces total mRNA transcript and protein levels of RET. Furthermore, we observe reduced cell-surface expression of RET in NCAM1 KD cells and a subsequent reduction in GDNF-dependent activation of RET. We also show reduced GDNF-dependent activation of proliferative signaling pathways downstream of RET. Functionally, SH-SY5Y cells exhibit significantly reduced cell viability when RET is activated in the absence of NCAM1. Together, our data suggest a role for NCAM1 in affecting total RET protein available for activation by GDNF, thereby reducing RET-mediated viability and proliferation of cells. Our work has uncovered a role for NCAM1 in mediating RET activity in cancer and validates the use of our synthetic dosage lethal approach in uncovering proteins facilitating oncogenic RET activity.

47. Timothy Walker (Cancer Research) Investigating the Role of MEN2-RET Receptor Localization in Tumorigenesis

Timothy J. Walker (presenting author), Eduardo Reyes-Alvarez, Costin N. Antonescu, and Lois M. Mulligan

The RET proto-oncogene encodes a single-pass receptor tyrosine kinase with important roles in development. Aberrant changes in RET expression or activity are associated with several cancers, including multiple endocrine neoplasia type 2 (MEN2) tumour syndromes characterized by the development of aggressive medullary thyroid carcinoma (MTC). The MEN2A and MEN2B subtypes are driven by distinct constitutively activating RET mutations, with MEN2B exhibiting the more aggressive phenotype. Previous studies of 2A- and 2B-RET have not identified definitive mechanistic differences to explain the distinctions in their respective disease pathologies. Here we use an MTC cell-model to begin characterizing the trafficking and localization of MEN2-RET. Our preliminary work demonstrated significantly increased internalization and early endosome colocalization of both MEN2-RET receptors, as well as increased recycling. Using immunofluorescence in the plane of the cell-membrane, we showed increased constitutive colocalization of MEN2-RET with clathrin coated pits that facilitate receptor internalization. Additionally, these receptors demonstrated increased half lives, and increased localization on the cell membrane in lipid rafts, consistent with increased recycling. Interestingly, we found differences between the MEN2A- and MEN2B-RET mutants with 2A showing increased recycling and localization on the cell surface, while 2B showed more localization within early endosomes. Our preliminary data show that MEN2-RET mutants exhibit constitutive and unique intracellular trafficking, which may be altering their signalling activity and eventual

transforming ability beyond their constitutive phosphorylation alone.

Department of Pathology and Molecular Medicine

48. Deirdre Finnigan (Hematology and Oncology) Investigating the Relationship Between Red Blood Cell Biomechanical Properties and Anemia in Cancer Patients Undergoing Chemotherapy

Deirdre Finnigan1 (presenting author), Regan Bucciol1, Nick Cruickshanks2, Anita Agrawal3, Mihaela Mates4, Susan Evans5, Maha Othman1,6,7

1 Department of Biomedical and Molecular Sciences, School of Medicine, Queen's University, Kingston, Ontario, Canada
2 Department of Physiology and Pharmacology, Schulich School of Medicine and Dentistry, Western University, London, Ontario, Canada
3 KHSC and Department of Obstetrics and Gynecology, Queen's University, Kingston, Ontario, Canada
4 KHSC and Department of Oncology, Queen's University, Kingston, Ontario, Canada
5 ALCOR® SCIENTIFIC
6 School of Baccalaureate Nursing, St. Lawrence College, Kingston, Ontario, Canada
7 Faculty of Medicine, Mansoura University, Mansoura, Egypt

Human red blood cells (RBCs) possess unique characteristics to effectively deliver oxygen throughout the body: aggregability, deformability, and elasticity. Anemia is a huge burden in cancer patients and impacts treatment protocol. This study aims to assess the relationship between anemia, conventional blood parameters, and the biomechanical properties of RBCs in cancer and following chemotherapy. Blood from controls and patients with female cancers was tested pre- and post-chemotherapy; these data were compared. Biomechanical RBC properties and conventional blood parameters (hemoglobin (Hb), RBC count, hematocrit (Hct), red cell distribution width (RDW), RBCs indices; mean corpuscular volume, Hb and concentration respectively (MCV, MCH, MCHC) were analyzed. Pre-chemotherapy data showed a significant increase in RBC aggregability and a significant reduction in MCHC compared to controls. Following chemotherapy, there was a significant reduction in RBC deformability, RBC count, Hb, and Hct, and a significantly higher RDW. Only 3% of patients had anemia pre-chemotherapy, versus 8% post-chemotherapy. There was a positive correlation in the post-chemotherapy group between the time for RBCs to regain their original shape and both MCV and MCH. We report that chemotherapy impairs RBCs' biomechanical properties which can hinder oxygen delivery; these changes correlate with conventional blood parameters and anemia. This is novel and has implications in patient care. In future, we hope to further elucidate the relationship between these changes and their relation to thrombotic events.

Funding: SLC IGNITE Fund, Alcor Scientific support grant

49. Elana Fridman and Emily Dephoure (Neonatal and Parental Health) Parental Administered Sensorimotor Intervention: Exploring its Impact on Parental Stress, Self-Efficacy and Satisfaction in the NICU Environment

Fridman E(a), Dephoure E(a), Smith L MSc(a), Snider L PhD(b), Fucile S PhD(a)
(a)School of Rehabilitaion Therapy and Department of Pediatrics, Queen's University
(b) School of Physical and Occupational Therapy, McGill University

The inherently stressful NICU environment may disturb parent-infant relationships due to the physical separation and reduced involvement in infant care, affecting parental stress and self-efficacy.1-3 Parents of hospitalized infants can experience psychological distress, guilt, and loss of parental role.4,5 Early parental engagement programs, such as parent administered sensorimotor interventions, aim to optimize preterm infant and parental outcomes.6 A significant gap in the literature regarding the impact of parental administered sensorimotor programs on parental stress, self-efficacy, and satisfaction remains. The objectives of this study are to evaluate the effect of a Parent Administered Sensorimotor Intervention (PASI) program on parental stress, self-efficacy, and satisfaction. We intend to conduct a secondary analysis of a previous randomized controlled trial. Ninety-four preterm infants (< 34 weeks gestation) were recruited and randomized into the experimental (PASI group) or control group (standard care) using a block size of four per arm. The PASI intervention consisted of tactile (whole body) and oral input, facilitated by the parent(s), administered once a day for 10 days, within a 14-day period. Upon culmination of the PASI program, the Parental Stress Scale (PSS), Parental Sense of Competence Scale (PSOC), and Parental Satisfaction Survey (Psat) were completed by parents, investigating the impact of PASI on parental stress, self-efficacy, and satisfaction. This study may inform future NICU practices involving parental-facilitated care.

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50. Mia Wilkinson (Mitochondrial Biology/ Preclinical Health Research) Platelet bioenergetics correlate with skeletal muscle metabolism in C57BL/6J mice.

    Skeletal muscle insulin resistance is a key step in progression of cardiometabolic disease, and impaired mitochondrial bioenergetics has been implicated. However, mitochondrial bioenergetic research in skeletal muscle is limited by the need for muscle biopsies. We sought to identify a liquid biopsy for early cardiometabolic disease by assessing if platelet bioenergetics are a minimally invasive surrogate for muscle bioenergetics. Multiple parameters of mitochondrial respiration, measured by high resolution respirometry, correlated between platelets and gastrocnemius muscle in healthy male C57Bl/6J mice (n=30). Both skeletal muscle ATP-linked respiration and respiratory control ratio (RCR) correlated negatively with platelet basal respiration and proton leak (all $p < 0.05$). Platelet reserve capacity positively correlated with skeletal muscle ATP-linked respiration and RCR (all $p < 0.05$). These relationships glean insight into the coupling state, or efficiency of the mitochondria at producing ATP per oxygen consumed, of the skeletal muscle. We propose the coupling state of platelet mitochondria reflects that of skeletal muscle in mice, providing a foundation for future research on using platelets as a liquid biopsy for muscle mitochondrial health in cardiometabolic disease and offering early insights into muscle metabolism to enhance clinical biomarker implementation.

Virtual Posters

1. Laura A. Killam (Nursing) Learner Experiences of Co-Creating Assessments with Educators During a Course: A Phenomenographic Study

    Authors: Laura A. Killam, Mercedes Lock, and Marian Luctkar-Flude

    Incorporating learners in simulation design processes enhances the potential for effective simulated learning experiences. In this process, called learner-educator co-creation (LECC), decision-making power is shared with students, with the aim of creating a more equitable, meaningful and authentic learning experience. Limited research on LECC, particularly involving assessment, makes it challenging for educators to assess benefits and challenges of incorporating simulation co-creation into a course. This study helps to fill this gap through a qualitative investigation of student experiences of co-creation of interprofessional virtual simulation games as a course assignment. It is guided by the question: 'What are different ways in which students can experience LECC of a virtual simulation assessment?' Exploring differences in student experiences of co-creation is essential for informing decisions about engaging in simulation co-creation as a tool to maximize learning. Note: This poster was previously presented at a simulation conference.

2. Hanna Kerr (Nursing)

   Background: Canada has a nursing workforce crisis, recently reaching an all-time high 32,400 Registered Nurse job vacancies. Specifically, newly graduated nurses (NGNs) have the highest rates of attrition in the profession. Structural empowerment (SE), that is, how workplaces influence work effectiveness, and psychological empowerment (PE), reflecting a worker's orientation towards their role, may be useful in mitigating NGN attrition. However, minimal synthesis has left it unclear how SE and PE have been examined with NGNs. A literature review is warranted to inform future research.

   Methods: A systematic literature review was conducted to identify primary research studies that explored NGNs' SE and/or PE.

   Findings: Despite similar objectives, definitions of NGNs varied, ranging from nurses with less than six months to three years of experience. 23 of the 24 studies utilized quantitative methodologies to measure NGNs' perceived SE and/or PE; frequently reported to be moderate-to-high. Gaps include minimal qualitative or longitudinal research, few studies on psychological empowerment, little program evaluation, outdated workforce data, and minimal diversity in samples and healthcare settings.

   Implications: Improved consistency in how NGNs are defined and diversified methodological approaches would facilitate a richer understanding of NGNs' empowerment. This would enable policymakers to create initiatives that reflect NGNs' empowerment needs. Nursing practice would benefit from improved work conditions. Lastly, findings could be integrated into nursing curricula to better prepare nursing students for the workforce.

3. Laura A. Killam (Nursing) Principles for equity-centered learner-educator co-creation: A reflection

   Authors: Laura A. Killam, Mercedes Lock, and Marian Luctkar-Flude

   Learner-educator co-creation means actively involving students in decision-making about the design, implementation, or evaluation of course components. Embedding co-creation into has numerous potential benefits that may include improved equity, inclusion, learning, and relationships in a course. However, achieving these benefits is contingent on how co-creation is enacted. What is missing in existing literature is clear guidance on how to navigate the complex process of learner-educator co-creation.

   In this poster we share recently published principles for equity-centered learner-educator co-creation to promote dialogue about ways in which educators may share power with students during courses. Principles and strategies to guide co-creation efforts were developed based on our experiences in a nursing context, practices, and existing interdisciplinary literature.

   The eight principles guiding equity-centered co-creation include: prioritizing equity, ongoing reflection on values, negotiation of power sharing, active and honest dialogue, integration of choice and flexibility, respectful trusting-caring relationships, and promoting a psychologically safer environment.

When enacted through these principles we believe that learner-educator co-creation may be used as a tool to help students from diverse backgrounds achieve their learning goals. Research exploring educator and student views of co-creation during a course is needed to evaluate this potential.

Note: This poster was submitted for consideration for presentation at the CASN conference.

Reference

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Afternoon Session

1. Kim Huynh (Mild Traumatic Brain Injury) Imaging-Based Analysis in Military Snipers Experiencing Repetitive Subconcussive Impacts

K.M. Huynh, C.C. Hambly, G. Ramirez-Garcia, N.S. Coverdale, D.J. Cook
Centre for Neuroscience Studies, Queen's University

Subconcussive impacts are head impacts without symptoms, but accumulating these may lead to cognitive and neurological symptoms. Military snipers are continually exposed to subconcussive impacts as the stock of their weapon impacts the shoulder and transfers recoil forces to the head. Anecdotally, some snipers report either constant neurological symptoms after an accumulation of high caliber rifle recoil forces throughout their careers or a decreased threshold for symptoms as their careers progress. Previous work done in our lab with magnetic resonance imaging (MRI) revealed elevated cerebrovascular reactivity (CVR) in athletes with a history of sports-related concussion. CVR reflects the ability of the cerebral blood vessels to dilate in response to an increased demand for blood. This study will determine the effects of repeated subconcussive impacts on brain physiology in elite military snipers.

In the current study, 15 military snipers (37±6 years) and 10 healthy controls (36±7 years) are recruited. Snipers are scanned using multi-sequence MRI before and after a sniper training course involving repetitive subconcussive impacts. The control group will undergo the same scanning protocol. The MRI protocol includes a Blood Oxygenation Level Dependent sequence with a hypercapnic challenge. The data is processed using FSL and the seeVR toolbox.

2. Kristen Kyone (Health Policy, Population Health, and Epidemiology) The associations between screen time, mental health, and academic performance among first-year undergraduate students: a key target for health intervention

Kyone K, Dephoure E, King N, Pickett W, Pankow K, Brar S, & Duffy A

First-year undergraduate students confront increased responsibilities and unique stressors during their transition to university. With screen time becoming embedded in daily life, concerns have arisen regarding its impact on mental health. This study explored the associations between screen time, mental health and academic performance among first-year undergraduates. Data was collected from the Queen's U-Flourish Well-Being Survey in September 2021, using validated measures of screen time, anxiety (GAD-7) and depressive symptoms (PHQ-9). GPA data was obtained from the university database. Multivariable log-binomial and linear regressions were employed. Nearly half (49%) and one-third (31%) of students (n=1,415) reported spending ≥4 hours/day engaged in leisure and social screen time, respectively. Leisure screen time (e.g., TV, gaming) was more common in males (58% vs 46%, p<.001), while social screen time was more common in females (32% vs 26%, p=.04). Students engaging in ≥4 hours of leisure screen time were significantly more likely to screen positive for anxiety (RR: 1.12; 95% CI: 1.01-1.25) and depression (RR: 1.32; 95% CI: 1.17-1.49) than those engaging in ≤3 hours. Similar, albeit smaller effects, were observed for social screen time. Both leisure (β= -0.24; 95% CI: -0.33,-0.15) and social (β= -0.14; 95% CI: -0.24,-0.04) screen time were negatively associated with GPA. Excessive screen time poses significant risk for worse mental health and lower academic performance, emphasizing the need for targeted interventions upon university entry.

3. Ahmad Chahin (Cancer Biology) Ezh2-Mediated Cell Cycle Regulation of Granule Neuron Precursors and Medulloblastoma Cells.

   Ahmad Chahin1, James Purzner1,2, Teresa Purzner1,2, 1Centre for Neuroscience Studies, Queen's *University, Kingston, ON, Canada, 2Department of Surgery, Queen's University, Kingston, ON, Canada*

   During normal cerebellar development, granule neuron precursors (GNPs) undergo a period of Hedgehog (Hh)-driven transit amplification, followed by a rapid switch from proliferation to differentiation. If Hh signaling is aberrantly retained due to mutations in the Hh signaling pathway, GNPs will fail to exit the proliferative stage, leading to the formation of medulloblastoma (MB), the most common pediatric brain tumor. In both GNPs and MB cells, the H3K27me3 modification plays a central role in delaying the activation of genes required for differentiation. Paradoxically, during periods of peak GNP proliferation and in Sonic Hedgehog (SHH) MBs with unrestrained proliferation, we observe dense H3K27me3 marks on the pro-proliferative genes Cyclin D1 and Cyclin D2. The H3K27me3 modification is generated by a component of the PRC2 complex, Ezh2. We demonstrate that conditional knockout (cKO) of Ezh2 significantly increases Cyclin D1 expression, and Ezh2 overexpression induces G0 cell cycle arrest. Additionally, Ezh2 expression depends on the abundance of the S-phase gene expression complex pRb/E2f1, as shown by E2f1 overexpression. A consequence of this model is that inhibition of Ezh2 will abrogate the effects of drugs that impact cell cycle by reducing Cyclin D1 transcript levels. As anticipated, we show that Ezh2 inhibitors can rescue cells from Vismodegib, a clinically approved SHH inhibitor currently used as standard of care in children diagnosed with SHH MB.

4. Daniel Rivera (Psychiatry) Post-secondary students and campus mental health care: Does initial care received reflect health need at presentation?

Rivera D, Patten SB, Keown-Stoneman C, King N, and Duffy A.
Carried out at: Centre for Neuroscience, Queen's University; School of Medicine, Queen's University, Department of Psychiatry, Queen's University, Cumming School of Medicine, University of Calgary; Applied Health Research Centre, Unity Health Toronto
Funding: Canadian Institute of Health Research, Mach-Gaensslen Foundation

Objectives: Students' need for university mental health support continues to grow, outpacing institutional resources. Timely access to a level of care appropriate for their health needs is important for efficient and rational care provision. This study will investigate the association between student symptom levels for common mental health problems and types of care providers accessed by students.

Methods: The U-Flourish survey is an ongoing cohort study of university students. Survey data was linked to administrative care data for students accessing university mental health services between 2018-2023. U-Flourish data included validated screening measures of anxiety (GAD-7), depression (PHQ-9) and well-being (WEMWBS). ANOVA and logistic regressions examined associations between symptom levels and the care provider type at their first appointment.

Results: The study included 1752 students. Anxiety and depression scores were significantly higher in students first seeing psychiatry or a MD/NP compared to counselling and lower for counselling compared to nursing ($p<0.05$). Screen-positive rates followed a similar trend, with students seeing psychiatrists having the highest and counselling having the lowest rates. Symptom scores and screen positive rates were comparable between nursing and MD/NPs. Functional impairment related to anxiety and depression screens was comparable across providers.

Conclusions: Overall, there was some evidence of rationalized care based on symptom levels but not on functional impairment. Future studies will investigate whether students' flow through care follows the principles of stepped-care.

5. Megan Cull (Therapeutics, Drug Development, and Human Toxicology) Accounting for intra-litter variability is necessary to identify xenobiotic-induced developmental toxicities in a mouse model using benzene as a toxicant

Authors: Megan E. Cull1, Lauren T. L. Brown1, Lihua Xue1, Perri M. Grant1, Louise M. Winn1,2 Author Department Affiliations: 1. Department of Biomedical and Molecular Sciences, Queen's University, Kingston, Ontario. 2. School of Environmental Sciences, Queen's University, Kingston, Ontario.

Background: The objective was to establish a data normalization approach to account for intra-litter variability in xenobiotic-induced developmental toxicity studies. Mouse models are commonly used for these studies; however, mouse pregnancies are multiparous and have intra-litter variability dependent upon litter size, maternal weight, fetal sex, and intrauterine position (IUP). This study used benzene, an environmental pollutant and carcinogen, to develop this approach. Methods:

Pregnant CD-1 mice were exposed to vehicle control or 200 mg/kg benzene between gestational days (GD) 8-14, then sacrificed on GD19. Fetoplacental units were stratified into groups based on exposure, sex and IUP. Raw fetus and placenta weights were recorded and normalized to maternal weight and the number of live fetuses. Results: Normalizing fetus and placenta weights elucidated intra-litter variability and revealed benzene-induced developmental toxicities that were not observed following raw data analysis. Analysis of the normalized compared to the raw fetal and placenta weight data revealed more significant interactions between IUPs, sexes and exposure groups. For example, normalized weight data identified that benzene (but not control) exposure caused significantly increased fetus and placenta weights in the left compared to the right IUPs. This result was not observed using the raw data. Conclusion: This highlights the importance of accounting for intra-litter variables when using mouse models for xenobiotic-induced developmental toxicity studies and argues the necessity of using this approach in the future. Supporting agency: CIHR

6. Montserrat Mora-Ochomogo (Microbiology)

   Increased antibiotic degradation by β-lactamase-producing bacteria in the presence of D-amino acids β-lactams are the most used class of antibiotics worldwide. However, their clinical utility is severely threatened by the spread of resistant bacteria that produce β-lactamases, enzymes that inactivate β-lactams. This β-lactamase production can also protect other bacteria that would otherwise be susceptible to β-lactams, in what has been described as a sheltering effect. Bacteria produce D-amino acids for many cellular processes, including peptidoglycan synthesis. D-amino acids can be incorporated into the peptidoglycan layer, and may impact the functions of proteins located in the outer membrane and periplasm. We hypothesize that the incorporation of D-amino acids into peptidoglycan impacts cell wall stability, and may lead to a greater level of antibiotic sheltering. The hydrolytic activity of supernatants of β-lactamase-producing Escherichia coli cultures supplemented with different D and L amino acids was measured with nitrocefin. We observed increased β-lactamase activity when bacterial cells were grown with certain D-amino acids. Western blotting was used to measure the amount of β-lactamase in the culture supernatant, and preliminary results reveal greater amounts of enzyme in the supernatants cultured with D-Trp than with L-Trp and the no amino acid control. Results suggest that D-amino acid production by bacteria can impact the cell wall stability of other bacteria in the same environment. This can lead to increased release of their periplasmic contents into the extracellular space, benefitting the bacteria that produced the D-amino acids. These microbe-microbe interactions may increase antibiotic sheltering, potentially complicating bacterial infection treatment.

7. Nicholas Ricci (Cardiopulmonary Physiology)

   The influence of acute sleep deprivation (ASD) on cardiopulmonary modulation during exercise is poorly understood. ASD may or may not alter minute ventilation (Ve) and reduce maximal oxygen consumption (VO2max), but ASD consistently reduces time to exhaustion and increases perception of exertion during exercise. This study will investigate the impact of ASD on perceptual (i.e., dyspnea, leg discomfort) and physiologic responses to incremental exercise in forty healthy non-athletes after 30-33 hours of ASD via ventilatory drive (measured by crural diaphragmatic electromyography, EMGdi; a robust correlate of dyspnea in health and disease) and muscle oxygenation measured by

functional near-infrared spectroscopy (fNIRS). Participants (20M, 20F; 18–30 years old) will perform two randomized incremental cycling cardiopulmonary exercise tests to exhaustion after: (i) one night of normal sleep, and (ii) 30-33 consecutive hours of wakefulness (ASD). Continuous measurement of EMGdi and fNIRS (quadriceps and cerebral oxygenation) will be made alongside standard cardiopulmonary and metabolic measures. These physiologic measures will be compared to perceptual responses obtained via Borg CR10 (to quantify dyspnea and leg discomfort during exercise) and Rating of Fatigue (ROF) scale after exercise termination. It's hypothesized that ASD will significantly increase EMGdi and reduce muscle oxygenation, correlating with increased Borg dyspnea, leg discomfort, and ROF scores, respectively. Sex-stratified analysis will be performed. This study will fill gaps in how ASD influences exercise performance and tolerance in non-athletes.

8. Ciara O'Connor (Neuroimmunology) Microglial activation is regulated by circadian rhythms in neuropathic pain

Ciara D. O'Connor, Olivia M.A. Smith, Nader Ghasemlou

Background: Microglia have been shown to be drivers of pain hypersensitivity in the spared nerve injury (SNI) model of neuropathic pain in mice. Following SNI, microglia in the dorsal horn of the spinal cord proliferate and transition from a homeostatic phenotype to a pro-inflammatory phenotype. These microglia have been shown to retain their morphology and pro-inflammatory phenotype from acute to chronic timepoints (>3 months). These cellular changes are accompanied by behavioural changes as mice develop mechanical and thermal (cold) allodynia, which they retain into the chronic phase. Clinical studies have shown that pain can be rhythmic in various chronic pain states. Recent work has now shown that microglial gene expression patterns, activation states, and physiological function are governed by circadian rhythms, with diverse regional and context dependent heterogeneity. However, it remains unknown whether microglia exhibit circadian rhythmicity in chronic pain states.

Methods: To investigate this gap in knowledge, male and female C57BL/6 mice received a spared nerve injury (SNI), with tissues collected at 3, 7, 10, 14, 28, and 84 days following injury. Animals were sacrificed at ZT2 and ZT14, corresponding to 2 hours after start of the light- and dark-phases. The spinal cord was immunostained for markers of homeostatic and pro-inflammatory microglia, as well as transcriptional regulators known to be under circadian control, and confocal z-stacks were taken to create 3D surface renderings of cells. 3D surface renderings were analyzed to characterize microglial morphology and activation state.

Results: We found changes in both microglial morphology and activation state (using key cell markers) in both the naïve and injured state. In the naïve state, microglia exhibited more ramified, extended processes indicative of a homeostatic surveillance phenotype during the dark-phase, and in the light phase, microglia had shorter less ramified processes and a more amoeboid morphology indicative of a pro-inflammatory phenotype. During peak periods of microglial activation following SNI, which occur between 7 and 14 days following injury, microglia in the dorsal horn took on an extremely pro-inflammatory phenotype at during the light-phase, but more retained a more (relative to the light-phase in injured animals) homeostatic phenotype during the dark phase. Further

understanding of microglial activation states across male and female mice, and during the circadian cycle, may provide new insight into mechanisms regulating their activity and function in the pathophysiology of chronic neuropathic pain.

9. Heidi Scott (Microbes, Immunity, and Inflammation) Investigating Genetic and Viral Factors Influencing Filovirus Activation of Toll-like Receptor

4 Heidi M. Scott1, Rory P. Mulloy2, Floriana Maswa Pondi2, Marceline Côté2, Katrina Gee1 and Che C. Colpitts1

1Department of Biomedical and Molecular Sciences, Queen's University, Kingston, Ontario, Canada.
2Department of Biochemistry, Microbiology and Immunology, University of Ottawa, Ottawa, Ontario, Canada.

Filoviruses, such as Ebola virus (EBOV), cause outbreaks with high mortality rates. EBOV glycoprotein (GP) activates Toll-like receptor 4 (TLR4), a pattern recognition receptor that classically recognizes bacterial lipopolysaccharide (LPS). This interaction contributes to EBOV pathogenesis by promoting a hyperinflammatory response and is associated with severe infection. Thus, the severity of filovirus disease may be influenced by modulation of TLR4 signaling pathways and TLR4 genetic variants present within the population may affect this. We hypothesize that common TLR4 polymorphisms (e.g., D299G, T399I) affect its activation by filovirus GPs. However, how filovirus GPs activate TLR4 and how TLR4 polymorphisms may influence EBOV disease is still unclear. In a TLR4/NF-B reporter assay in human epithelial 293T cells, transfection of EBOV GP robustly activates TLR4 compared to transfection of non-pathogenic Reston virus GP. The GPs of other filoviruses differentially activate TLR4. Furthermore, TLR4 D299G exhibits enhanced responsiveness to EBOV GP in this assay. We have generated THP1 monocytic cells that lack or stably re-express TLR4 (WT or D299G) and tested LPS responsiveness. Ongoing work aims to study TLR4 polymorphisms in THP1 cells, a more physiologically relevant model of inflammatory responses, and to identify structural discrepancies in filovirus GPs to explain differential TLR4 activation. This research will improve our understanding of why some individuals develop severe filovirus disease, and could inform development of novel treatment modalities.

10. Julien Miri (Immunology/Virology)

Effects of IL-27 and Cyclosporine A on Macrophage Polarization in Dengue Infection Dengue virus (DENV) is a pervasive positive-stranded RNA virus that threatens close to half the global population. Infection can lead to severe symptoms such as Dengue hemorrhagic fever causing systemic inflammation and internal bleeding resulting in fatal outcomes. Central to the host response are macrophages, pivotal players in the innate immune system, which exhibit distinct polarization states: M1, implicated in antiviral responses, and M2, involved in immune modulation and inflammation resolution. Modulating polarization holds promise in altering immune responses by altering macrophage populations; IL-27, a heterodimeric cytokine, has shown potential to skew macrophages towards an M1 phenotype. Using THP-1 as the model, PMA is used to differentiate the monocytes into macrophages. These macrophages are either left untreated as M0 or polarized with IFN-y and LPS or IL-4 and IL-13 to achieve M1 and M2 macrophages respectively. They are then infected at an MOI of 0.5, which is followed by treatment of IL-27. qPCR is used to observe mRNA levels of

polarization markers as well as DENV RNA. Our findings revealed that DENV infection is significantly reduced in M1 macrophages, while being increased in M2 cells. Furthermore, DENV has been shown to markedly reduce M1 markers and increase M2 polarization. This effect is observed at an MOI of 0.5 suggesting that uninfected cells are being affected as well. Future aims of this project look to investigate characteristics of M1 cells that prevent viral replication, mechanisms of DENV in downregulating M1 and upregulating M2 polarization and identifying the polarization pathway(s) of IL-27 and how DENV suppresses it. By uncovering these mechanisms, our research offers the potential for novel therapeutic targets in combating Dengue virus-associated complications, potentially informing strategies for immunomodulation and antiviral interventions.

Authors: Julien Miri, Madison Roth, Che Colpitts, Katrina Gee

11. Andrew Garven (Cell Biology)

The Prognostic and Molecular Impact of Transposable Element Expression in Bladder Cancer
Transposable elements (TE) are mobile genetic sequences derived from endogenous retroviruses constituting approximately 45% of the human genome. In bladder cancers (BC), the prominence of epigenetic instability leads to an inordinate incidence of TE transcripts. Previous studies suggest that TE expression improves anti-tumour immunity. Since immunotherapy is a vital therapeutic modality for the treatment of BC, we leveraged transcriptional profiling and immunohistochemistry to investigate the relationship between TE expression and immunologic response. Transcriptional expression of both gene and TE sequences from early-(non-muscle invasive, n=535) and later-stage (muscle invasive, n=412) cancers were quantified and subjected to unsupervised clustering. TE clusters were correlated with molecular subtypes and clinically relevant endpoints (e.g., time to recurrence). To identify cellular pathways enriched in response to TE expression, pathway enrichment analysis was performed on genes correlated with TE transcript expression. These findings were validated using immunohistochemical staining for the LINE-1 TE protein, ORF-1 in an independent BC cohort (n=371). Heightened TE expression correlated with the activation of an integrated stress response, including the suppression of genes involved in RNA processing, antigen presentation and chemokine production. Contrary to previous work, these findings indicate that TE expression correlates with immune evasion/exhaustion and inferior treatment outcomes. This work provides a novel classification of the TE transcriptional landscape in BC. heightened TE expression was associated with both a reduction in chemokine production and inferior treatment outcomes in BC patients. These findings highlight the significance of TE expression as a prospective biomarker and therapeutic target for BC management.

13. Rohan Sampy (Bladder Cancer) BCG treatment outcome in patients with non-muscle invasive bladder cancer (NMIBC) is associated with distinct transcriptomic profiles in peripheral blood mononuclear cells (PBMCs)

Rohan Sampy (1), Jean-François Paré (1), Andrew Garven (2,3,4), D. Robert Siemens (5), Charles H. Graham (1) 1 Department of Biomedical and Molecular Sciences, Queen's University, Kingston, ON, Canada 2 Department

of Pathology and Molecular Medicine, Queen's University, Kingston, ON, Canada 3 Division of Cancer Biology and Genetics, Queen's University, Kingston, ON, Canada 4 Cancer Research Institute, Queen's University, Kingston, ON, Canada 5 Department of Urology, Queen's University, Kingston, ON, Canada

Bacillus Calmette-Guérin (BCG) is a mainstay immunotherapy in the treatment of patients with intermediate to high-risk non-muscle invasive bladder cancer (NMIBC). Unfortunately, 40%-70% of patients do not respond adequately to BCG therapy, and tumors recur, with some patients progressing to life-threatening muscle-invasive disease. Previous studies revealed an association between the acquisition of innate immune reprogramming in monocytes and recurrence-free survival. To obtain a more comprehensive view of BCG-associated immune reprogramming in the context of NMIBC treatment, we performed combined transcriptomic and epigenomic profiling at the single-cell level (scRNA-seq + scATAC-seq) on circulating peripheral blood mononuclear cells collected from patients with NMIBC just before the sixth BCG intravesical instillation of induction treatment. Our initial analysis at the transcriptomic level indicates that lack of early disease recurrence is associated with an increased proportion of anti-tumorigenic monocyte subpopulations, whereas early recurrence is associated with an increased proportion of pro-tumorigenic monocytes. We have also identified distinct profiles in the lymphoid compartment associated with treatment outcomes. Further analysis will help elucidate mechanisms involved in the reprogramming of circulating immune cells associated with recurrence-free survival of patients with NMIBC. Identification of such mechanisms may lead to the development of new therapeutic approaches and the identification of biomarkers to predict outcomes in patients with high-risk NMIBC.

Funding: Canadian Institutes for Health Research, Bladder Cancer Canada, Canadian Urological Association

14. Vidthiya Jeyanathan (Immunology) Mold exposure-induced epigenetic reprogramming in hematopoietic stem cells and innate immune cells

Vidthiya Jeyanathan, Gabriella Stefan, Conrad Pietrzak, Sara Teimouri Nezhad, Makena Sceeles, Andisheh Liaghat, Astha Patel, Sarah Hopkins, and Eva Kaufmann

Mold exposure occurs in approximately 40% of houses with suspected implications for immune functions even in immunocompetent individuals. These implications can reach from mucosal irritation, increased infection susceptibility, to neurological symptoms. In contrast, the fungal cell wall component and vaccine adjuvant β-glucan has been described to initiate host protection against a range of infections through reprogramming of hematopoietic stem cells in the bone marrow, known as innate immune training. We suspect based on similar studies, that related pathogens can exert opposing effects in immune reprogramming based on their pathogenicity. Thus, the overall goal of this study is to investigate in preclinical disease models the impact of mold exposure on reprogramming in hematopoietic stem cells (HSC) and its role in host immunity to viral infection. To this end, we establish an immunocompetent C57BL/6 mouse model of intranasal A.fumigatus allergen exposure to delineate subclinical and clinical implications. We determine the fungal exposure dose that causes no clinical disease, and examine its effects on HSCs and immune cells. We further investigate the functional implications of mold allergen exposure upon secondary in vitro and

in vivo re-exposure to infections and environmental allergens. Understanding the mechanisms of mold-induced alterations in immune functions will ultimately allow us to therapeutically target and alleviate long-term health implications in mold-exposed individuals.

15. Suruthy Sivanathan (Experimental Medicine/Bioinformatics)

    Earlier studies of infant gut microbiota reported associations with childhood asthma and atopy. These studies, however, investigated microbial abundances at a single time point, which do not consider if and how microbial dysbiosis over time may impact risk of atopic diseases. Our study aims to determine the main effects of longitudinal microbial changes during the first year of life as well as interaction effects with host genomics on risk of childhood asthma and atopy. First, we calculated the change in the abundances of 29 common microbes at two time points (e.g., slope), which were derived from 16S rRNA sequencing of stool samples collected from infants in the CHILD study at 3-months and 1-year of age (N=584) . Next, regression analyses identified microbial changes associated with health outcomes in pre-school aged children (e.g., recurrent wheeze at 2-5 years, asthma at 5 years, atopic dermatitis at 1-5 years, and food/inhalant sensitization evaluated by skin prick tests). We determined that changes in Flavonifractor sp. (PBH=0.015, Beta=0.13), Anaerostipes sp.8 (PBH=0.011, Beta=0.14) and Clostridium innocuum sp. (PBH=0.011, Beta=0.15) abundances are associated with food sensitization at 3 years . Moreover, we performed gene-environment interaction (GxE) analyses using polygenic risk scores (PRS) of the infants previously associated with lung function by our team (G) and the changes in microbial abundances as environment exposures (E). This analysis identified additional changes in gut microbes such as Flavonifractor sp. that was associated with asthma diagnosed by age 5 years (PBH=0.028, Beta=0.12), only when simultaneously considering the infants' individual polygenic risk for asthma. Finally, single nucleotide polymorphisms (SNPs) genotyped from the Illumina HumanCoreExome Bead Chip were used in genome-wide association studies (GWAS) to determine genetic factors contributing to changes in gut microbial composition in the first year of life. Interestingly, GWAS detected SNPs such as rs1487669249 in the KCNN2 gene, encoding a calcium-activated potassium channel, which were associated with the slope of Bacteroides sp. 6 (P=1.4e-08). In conclusion, our study reports that changes in the gut microbiota during the first year of life may contribute to asthma and atopic disease susceptibility. These may represent potential microbial biomarkers for disease-related dysbiosis which can be further investigated for therapeutic and preventive applications.

16. Laila Masalha (Biochemistry) Use of a Whole-Cell Biosensor for Identifying β-Lactamase Inhibitor Proteins with Broader Inhibitory Activities

    Authors: Laila Masalha (First), Mitchell Jeffs, Dr. Christopher Lohans
    Department: Biomedical and Molecular Sciences
    Supporting agencies: Queen's University, New Frontiers in Research Fund

    The clinical use of β-lactam antibiotics is threatened by the spread of antimicrobial resistance (AMR). One of the most important bacterial resistance mechanisms involves the production of β-lactam-

hydrolyzing enzymes, β-lactamases. As resistance to clinical β-lactamase inhibitors (BLIs) continues to emerge, the development of new inhibitors is needed. β-lactamase-inhibitor proteins (BLIPs) potently inhibit certain β-lactamases; however, their use is hindered by inconsistent inhibitory efficacy and challenges in accessing their targets.

This project aims to address these hurdles by preparing and testing BLIP variants using a luminescent biosensor developed for screening BLIs. β-lactam antibiotics activate the AmpR/AmpC system of the biosensor, resulting in a luminescent signal. When β-lactamase-producing cells are also present, the β-lactams are degraded, reducing luminescence; however, in the presence of an effective BLI the β-lactam is rescued, and luminescence persists.

Genes encoding BLIPs were cloned into pNIC28-Bsa4 through ligation-independent cloning, and these proteins were expressed in Escherichia coli BL21(DE3). Preliminary results show inhibition of the β-lactamase KPC-2 by the recombinant BLIPs. Further experiments are underway, using the luminescent biosensor to screen for BLIP variants which target a broader range of β-lactamases.

As AMR continues to emerge, novel countermeasures become crucial. Our work explores the potential of expanding the inhibitory activity of BLIPs against β-lactamase enzymes. These BLIP variants could inform efforts for developing new BLIs, or may serve as diagnostic tools for the detection of β-lactamase-producing bacteria.

17. Heshanth Rasalingam (Renal and Cardiovascular Research) Characterizing the Influence of Fetuin-A Sialylation on Calciprotein Particle Formation

Authors: Heshanth Rasalingam1, Eric B P Fernandes BHScH1, Nelson Chen1, Trisha Singh1*, Laura van Staalduinen PhD1, Rachel M Holden MD1, 2, Michael A Adams PhD1*

1Department of Biomedical and Molecular Sciences, Queen's University, Kingston, ON K7L 3V6, Canada
2Department of Medicine, Queen's University, Kingston, ON K7L 3N6, Canada
**NC and TS contributed equally to this work as co-authors*

Presenting Author: Heshanth Rasalingam, Queen's University, hr40@queensu.ca

Calciprotein particles (CPPs) are colloidal, fetuin-A complexes responsible for chaperoning circulating phosphate and calcium (P&C). An increased amount and size of CPPs are linked to kidney and cardiovascular diseases; however, mechanistic understandings for this phenomenon are unclear. Simultaneously, while fetuin-A desialylation has been demonstrated in disease state populations, its impact on CPP formation is unknown. Therefore, this study investigates the impact of fetuin-A sialylation on CPP formation.

Mature CPPs were synthesized with two protein preparations: (1) 0.5g/L native (NF) and desialylated fetuin-A (AF) with 40g/L albumin and (2) 1g/L NF and DF. Solutions were incubated, for 2 and 12

hours respectively, with P&C until particle maturation. Nanoparticle tracking analysis was conducted to obtain particle size and charge. Functional changes in CPP maturation were assessed using the T50 assay. Finally, a differential centrifugation protocol was used to obtain total P&C mineral sequestration between CPP preparations.
AF CPPs were 9% larger ($p<0.0001$) and more positively charged ($p<0.0004$) than NF CPPs. T50 peak optical density (OD), compared to time, was more indicative of fetuin-A sialylation; AF CPP peak OD was higher than NF CPPs ($p<0.0001$). Finally, mineral assays demonstrated CPP-mediated P&C sequestration is dependent on fetuin-A, albumin, and novelly, sialylation.
Fetuin-A sialylation does impact CPP formation and provides potential mechanistic insight into disease-based changes in CPPs. Future studies should elucidate fetuin-A differences across populations to gain further insight into mineral homeostasis dysregulation mechanisms.

19. Hailey Gowdy (Neuroimmunology) A cross-sectional study of the circadian control of biopsychosocial outcomes in chronic pain

Hailey G.M. Gowdy1, Doriana Taccardi1, Amanda M. Zacharias1, Élisabeth Lamoureux2, Lesley Norris Singer3, Jennifer Daly-Cyr3, Etienne J. Bisson4, Qingling Duan1, Manon Choinière2, Zihang Lu5, M. Gabrielle Pagé2, and Nader Ghasemlou1,4,6.

1Department of Biomedical and Molecular Science, Queen's University, Canada. 2Department of Anesthesiology and Pain Medicine, Université de Montréal, Canada. 3Chronic Pain Network, McMaster University, Canada. 4Department of Anesthesiology and Perioperative Medicine; 5Department of Public Health Sciences; 6Centre for Neuroscience Studies, Queen's University, Canada.

Introduction: 20% of the Canadian population lives with chronic pain, for which current treatments are insufficient. In the pursuit of individualised management strategies, it is crucial to know why and when someone has pain. Time-dependent fluctuations in acute thermal nociception have been found to be regulated by majority endogenous circadian rhythms; we hypothesize that circadian rhythms also play a role in mechanisms of chronic pain. Our study explored how pain rhythmicity may be associated with well-being.

Methods: Following an initial questionnaire, recruited participants (n=907) completed electronic symptom-tracking diaries, in which they rated their pain intensity, affect, and fatigue on a 0-10 scale at 3 timepoints (08:00, 14:00, 20:00) daily for 1 week. Pain ratings were used to identify phenotypic patterns of pain rhythmicity.

Results: Five key pain rhythmicity phenotypes were identified: constant low (23.0% of total), constant high (27.0%), rhythmic↑ (16.0%), rhythmic↓ (4.1%), and mixed (arrhythmic; 29.9%). Despite rhythmic↑ (n=102) participants reporting similar pain intensities in the evening to constant high (n=172) participants, they reported significantly less pain interference in daily activities ($p=0.0066$), fatigue ($p=0.0225$), and depressive symptoms ($p<0.0001$).

Discussion: The associations between pain phenotypes and well-being measures observed in our sample present a tool to characterise and potentially help manage chronic pain. This work will be expanded with the collection of blood from participants at multiple times of day to explore potential biomarkers of pain rhythmicity.

This work is supported by CIHR and the CIHR-SPOR Chronic Pain Network.

20. Nelson Chen (CKD, CVD, Nanoparticles) Fetuin-A and albumin play synergic roles in calciprotein particle-based chaperoning of calcium and phosphate

Nelson Chen,1 Eric B P Fernandes BHScH,1 Heshanth Rasalingam,1 Trisha Singh,1* Laura van Staalduinen PhD,1 Rachel M Holden MD,1,2 Michael A Adams PhD1*
**contributed equally as co-third author*

1Department of Biomedical and Molecular Sciences, Queen's University, Kingston, ON, K7L 3V6, Canada
2Department of Medicine, Queen's University, Kingston, ON, K7L 3V6, Canada

Fetuin-A and albumin (Fet-A/Alb) play integral roles in calciprotein particle-based (CPP) chaperoning of calcium and phosphate (CaP), protecting against mineral accrual in the vasculature. While the deficiency of both proteins in chronic kidney disease (CKD) contributes to cardiovascular risk, the impact on CPP function is unclear. This study characterized the kinetics and functional capacity of CPPs formed under varying concentrations of Fet-A/Alb.

Mature CPPs were synthesized in-vitro with bovine serum albumin at 0, 20, 40, or 60g/L in combination with 0.25, 0.5, or 1.0g/L fetuin-A (physiological concentration: 0.5g/L fetuin-A, 40g/L albumin). The capacity of CPPs to inhibit CaP precipitation was assessed via differential centrifugation to preferentially isolate free mineral, protein-bound mineral, and CaP precipitate. CPP maturation was assessed by T50 transition times following an in-vitro mineral challenge.

Without albumin, physiological [fetuin-A] is unable to prevent CaP precipitation in solution. CPP maturation is also abolished but is retained at supraphysiological [fetuin-A]. In CPPs synthesized with Fet-A/Alb, T50 time is significantly decreased in reduced [fetuin-A; 0.25g/L] (1.3x-decreased, $p<0.0001$) compared to physiological [fetuin-A; 0.5g/L]. Further, reduced [fetuin-A; 0.25g/L] decreases protein-bound phosphate compared to [fetuin-A; 0.5g/L] (1.5x-decreased, $p<0.0001$), and is dramatically decreased with [fetuin-A, 0.25g/L; albumin, 20g/L] (3.1x-decreased, $p<0.0001$).

The findings demonstrate the dose-dependent and synergistic behaviour of Fet-A/Alb in CPP-based chaperoning of CaP, indicating a need to assess fetuin-A levels alongside albumin to evaluate cardiovascular risk in CKD.

21. Eduard Popescu and Daniel Wang (Cardiovascular and Renal Diseases) Investigating the Effects of Bisphosphonates on Calciprotein Particle Maturation and Buffering Capacity

A. Eduard Popescu1, Daniel Wang1*, Eric B. P. Fernandes1, Rachel M. Holden1,2, Michael A. Adams1*
1 Department of Biomedical and Molecular Sciences, Queen's University, Kingston, ON, K7L 3V6, Canada
2 Department of Medicine, Queen's University, Kingston, ON, K7L 3V6, Canada
**Co-First Authors, Presenting Authors*

Fetuin-A based calciprotein particles (CPPs) are responsible for buffering excess circulatory calcium and phosphate. Characterizing primary CPP-Is is challenging due to lability and rapid maturation to CPP-IIs. This study explores utilizing bisphosphonates (alendronate and OsteoSense) binding to crystalline CaPi to slow CPP maturation and enable CPP-I characterization.

Alendronate infusions were conducted 15 and 30 minutes following in vitro CPP synthesis initiation. Mineral sequestration and particle maturation were assessed via differential centrifugation (1K RPM and 20K xG). Calcium and phosphate sequestration was quantified using o-Cresolphthalein and malachite green assays, respectively. Functional particle transition was assessed via alendronate addition 15 minutes into a T50 assay. In separate alendronate-infused samples, trace OsteoSense fluorescently tracked and quantified the distribution of bisphosphonates in post-centrifugation supernatants and pellets.

Increased mineral precipitation was observed in alendronate-infused samples following 1K RPM centrifugation, and decreased precipitation following 20K xG centrifugation. T50 alendronate infusion resulted in a 26% decreased end optical density and 12% increased T50 time ($p<0.0001$). Fluorescent detection suggests OsteoSense incorporation into CPP-Is prior to CPP-II maturation. Fluorescence was detected within 1K RPM and 20K xG reconstituted pellets, with minimal detection in 20K xG supernatant.

The effects of alendronate on mineral sequestration and T50 profile indicate alterations in CPP maturation. OsteoSense detection suggests bisphosphonate incorporation into CPP-Is and calcium-phosphate precipitate. Findings support continued exploration of bisphosphonates in arresting immature CPPs for future investigations.

22. Claire Zanin (Study in Anatomical Sciences, Research in Biomechanical Engineering) Shape Variability in the Human Knee and Shoulder: Implications of Locomotor Constraints on Joint Morphology

Claire Zanin, Erin Lee, Nathan Young, Sharon Swartz, Michael Rainbow
Department of Mechanical and Materials Engineering, Queen's University, Kingston, ON, Canada

In able-bodied people, the knee is required for locomotion (walking and running), whereas the shoulder is free from locomotor constraints, and accommodates complex non-locomotor actions. As a result of these differences, it has been suggested that the knee has less variation in shape compared to the shoulder; however, this theory has not been rigorously tested. We aim to test the idea that the knee is more constrained and less variable than the shoulder in humans. We have

created statistical shape models of a large collection of scapulae (n=125) and distal femora (n=105). As a first step, we computed the variance, V, in the scapulae. With a V of 0.0082, humans show the highest scapula variance among primates, who all use their upper limbs for locomotion. This supports the idea that freedom from locomotor constraints increases variation. One challenge with this approach for comparing knees to shoulders is that the functional significance of shape variation is difficult to compare across bones belonging to different joints. To address this, we aim to use joint disease and injury to calibrate comparisons in the knee and shoulder by analyzing the proximity of injured shapes to the asymptomatic mean shape. This will enable us to determine whether the knee or shoulder is more/less tolerant to variation, with the hypothesis that joints under tighter constraints will tolerate less variation in shape.

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23. Elana Kertzman (Microbiology – Virology) The host protein cyclophilin A promotes evasion of interferon signaling by hepatitis C virus non-structural protein 5A

Elana R. Kertzman, Carla E. Gallardo Flores, Dr. Che C. Colpitts
Department of Biomedical and Molecular Sciences, Queen's University, Kingston, Canada

Hepatitis C virus (HCV) is a bloodborne, single-stranded RNA virus which causes over 58 million infections globally. Most people develop chronic HCV infection, putting them at risk for liver disease and hepatocellular carcinoma, resulting in approximately 290, 000 deaths annually. HCV establishes chronic infection and complications by evading immune responses and replicating undetected. Cytoplasmic immune sensors such as RIG-I are crucial for detecting RNA and viral infection, thus activating the interferon (IFN) response which establishes an antiviral state within cells. We deduced that HCV non-structural 5A (NS5A) protein antagonizes the RIG-I and IFN pathways, indicated by decreased IFN-β and interferon-stimulated response element (ISRE) reporter activity, respectively, in the presence of NS5A. Another focus of this research is on cyclophilin A (CypA), a cellular protein that positively regulates these pathways by enhancing RIG-I signalling and STAT1 phosphorylation, a key event in the IFN response. However, CypA also promotes HCV infection through its interaction with the NS5A protein. Interestingly, we found that CypA is required for the NS5A-mediated evasion of the IFN pathway, but not the RIG-I pathway. As such, the exact mechanisms behind the CypA-NS5A evasion of the IFN response requires further evaluation, with a focus on the STAT1 aspect of this pathway. This research aims to further understand how the interaction between HCV NS5A and CypA facilitates viral replication and aids in immunomodulatory strategies.

24. Ruilin Zhao (Neuroimmunology)

Galectin-9 regulation of neuro-immune responses in inflammatory pain Ruilin Zhao1, Laurel Ballantyne1, Pascale Patenaude1, Madeline Robinson1 and Nader Ghasemlou1,2,3 1Department of Biomedical & Molecular Sciences; 2Department of Anesthesiology & Perioperative Medicine; 3Centre for Neuroscience Studies, Queen's University, Kingston, ON K7L 3N6, Canada Inflammatory

pain is driven by inflammatory mediators secreted by immune cells and neuropeptides derived from sensory neurons. Interactions between these two systems are important in development and maintenance of pain. Our lab identified galectin-9 (gal9) as a potential nociceptor-specific neuropeptide; previous research suggests that gal9 may regulate peripheral inflammation. We therefore sought to investigate the mechanisms regulating gal9 secretion after skin injury and determine whether gal9 can alter neuroimmune interactions and the pain response. We performed incisional wounds in 6-12 week old male and female C57BL/6J mice and collected the dorsal root ganglia (DRGs), spinal cord, and footpad at 3h, 6h, 24h, 72h, and 144h post-injury. DRG and spinal cords will be sectioned and immunofluorescence used to determine co-expression of gal9 with peptidergic/non-peptidergic neurons. We also assessed the activity of gal9 on dendritic cells in vitro by activating DC2.4 cells with LPS and IL-1β. Cells (for mRNA) and supernatant (for protein) were collected at 1h, 3h, 6h, and 24h to assess expression of key pain mediators CCL22 and CCL17. This work lays the foundation for better understanding the contribution of gal9 to pain neuroimmunity.
Funding source: CIHR

25. Jaskaran Singh Hora (Biochemistry and Cell Biology) Understanding the Role of cGAS-STING in Modulating Chemoresistance to Mitochondrial-Targeting anti-cancer therapies

    Cancer cells often find routes to evade traditional chemotherapeutic treatments through development of drug-resistant states via adaptive signalling mechanisms. In this regard, the cyclic GMP-AMP synthase (cGAS)-STING innate immunity pathway that detects cytoplasmic DNA has been suggested to be critical for cancer persistor cell formation. This study aims to explore the potential role of cGAS-STING in the modulation of chemoresistance following treatment with mitochondria-targeting anti-cancer drugs that might trigger unwanted release of mitochondrial DNA. Towards this, we have treated MDA-MB-231 triple negative breast cancer cells to range of compounds including the electron transport chain inhibitor IACS-10759 and the BH3 mimetics ABT-199 and ABT-737 that target the mitochondrial outer membrane.

    Analyses of the resulting drug resistant cells indicated that each chemotherapeutic triggered a distinct profile of changes including clear sets of innate immunity genes along with activation of the integrated stress response. These data suggest that cGAS-STING pathways might be engaged following mitochondrial damage in breast cancer cells. To further explore this mechanism, we are currently testing if loss of mitochondrial maintenance genes similarly cause release of mitochondrial DNA and cGAS-STING activation.

    Lastly, we are directly testing if inactivation of cGAS-STING signalling can improve chemo-sensitivity to mitochondrial inhibitors in cancer cells. This investigation provides critical insights into the interplay between mitochondrial dysfunction and immune signalling pathways that drive cell survival, offering potential avenues for the development of combinational therapeutic strategies to more effectively tackle tumor cell persistence.

    Authors: Jaskaran Singh Hora, Monica Opoka, Kaylee Punter, Sheela Abraham, Edmond Chan

Department of Biomedical and Molecular Sciences (DBMS)
Supported by: Cancer Research Society Canada

26. Stefania Coroneos (Women's Health Research) Advancing knowledge of thrombosis risk in hormonal contraception users: a thromboelastography study and educational outreach (study proposal)

Stefania Coroneos 1 (BHSc 4th year), Maha Othman (MD PhD) 1,2
1. Department of Biomedical and Molecular Sciences, Queen's University, Kingston, Ontario, Canada
2. School of Baccalaureate Nursing, St Lawrence College, Kingston, Ontario, Canada

The use of hormonal contraception (HC) is increasing globally. Previous research investigated misconceptions and identified knowledge gaps among post-secondary female students particularly regarding thrombotic risk. Unlike conventional haemostasis tests, thromboelastography (TEG®) provides comprehensive information about coagulation and potentially predicts this risk. A prior TEG study found trends towards hypercoagulability and fibrinolysis among HC users, but the small sample size limited generalizable conclusions. This study aims to: 1) assess the coagulation profile of a larger sample of HC users compared to non-users. 2) evaluate the effectiveness of an existing educational infographic about HC risks. Females aged 19-29 will be recruited. Data on age, HC type, duration of use, and smoking status, will be collected. Coagulation will be tested by TEG® 5000 analyzer, evaluating time to clot formation, rate of clot formation, speed of clot propagation, strength of clot, clot lysis after 30 minutes, and thrombin generation. The differences between HC users and non-users, stratified by smoking status, will be assessed using ANOVA and Chi-Square tests. For the educational initiative, a QR code will be incorporated into the infographic that links to an online survey. This will be disseminated on campus and via social media. Tracking scans and subsequent interactions will gauge the infographic's outreach, assessing its effectiveness in educating the target audience. This study shall provide a more comprehensive understanding of the coagulation profiles of HC users and non-users. The infographic's initiative shall highlight the needs for further education among post-secondary females.

Funding: IGNITE Funds, St Lawrence College

27. Temeara Barrett (Reproductive and Developmental Science) Fetal, maternal anemia in placental transcriptomic analysis of FGR Δ9-THC-exposed rat pregnancies.

Temeara Barrett1, Kendrick Lee2, Daniel B. Hardy (PhD)2,3, David Natale (PhD)1,4

1Dept. of Biomedical and Molecular Sciences, Queen's University, Kingston, Ontario, Canada.
2Dept. of Physiology and Pharmacology, Schulich School of Medicine and Dentistry, Western University, London, Ontario, Canada.
3Dept. of Obstetrics and Gynaecology, Schulich School of Medicine and Dentistry, Western University, London, Ontario, Canada.

Background: Maternal Cannabis use has increased in Canada following its 2018 recreational

legalization. Cannabis use in pregnancy is associated with maternal anemia at time of admission. In-utero Cannabis exposure is associated with adverse fetal outcomes: impaired long-term cardiovascular, metabolic, and neurodevelopmental functioning. In-utero Δ9-THC exposure causes fetal growth restriction with associated labyrinth-zone placental deficits in rats.

Aim: This project aims to characterize the placental transcriptome in Δ9-THC-exposed rat pregnancies.

Methods: Daily IP Δ9-THC or vehicle injections were administered to pregnant Wistar rat dams from GD6.5-19.5. Fetal weights and placentae were collected at GD19.5 and placentae stored in RNALater (ThermoFisher) before bulk RNA sequencing. Raw paired-end sequencing reads were aligned with indexed reference genome (mRatBN7.2) and annotated with same genome. Gene counts were generated using featureCounts (Rsubread) and pre-processed (filterByExpr, normLibSizes (edgeR)). Differential expression analysis (DEA) between treatment groups was performed using DESeq2. Functional pathway enrichment analysis (GO, KEGG, HP) for top DEGs at 5% FDR was performed using g:Profiler and genes of interest were validated by RT-qPCR.

Results: Δ9-THC-exposed pups were growth restricted at GD19.5 with no change in placental weights. Markers of fetal myelosuppressive anemia (Alas2, Hbb, Cdc25b) were observed in Δ9-THC-exposed placentae. Upregulation of Tfrc and downregulation of Fpn in Δ9-THC-exposed placentae indicated presence of maternal anemia with prioritization of placental iron metabolism. Decreased Alas2 and Slc38a5 suggests decreased placental capacity for generation of fetal heme precursor molecules. Biological pathway analysis suggests that Δ9-THC exposure during pregnancy alters hemopoiesis and presents a phenotype similar to anemia in humans.

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28. Ojobile Innocent (Cancer Research)

Inflammatory breast cancer (IBC) is a rare and aggressive subtype of breast cancer (BC). It constitutes approximately 2% of all BC cases yet causes nearly 10% of all BC deaths. The median overall survival of IBC patients relative to non-IBC is 4 years at stage III and 2 years at stage IV. Therefore, this unique, highly lethal sub-type of BC requires a novel therapeutic strategy. Receptor interacting protein kinase 2 (RIPK2), a downstream signalling molecule of the nucleotide oligomerization domain 2 (NOD2) has been implicated in the aggressive nature of IBC. RIPK2 plays a key role as mediator of inflammatory responses through downstream activation of the NF-kB pathway resulting in transcription of pro-inflammatory cytokines. In our study, preliminary findings indicate that RIPK2 regulates the progression of IBC through NF-kB, which promotes the expression of pro-inflammatory cytokines such as IL-1b, IL-6 leading to the highly proliferative, metastatic and angiogenic phenotypes of IBC. Our in-vitro results using trypan blue exclusion assay indicates that RIPK2 potentially increases the viability of IBC cells. Using 2D trans-well invasion assay, knockout of RIPK2

slightly increases invasive ability of the IBC cells which was augmented by 3D culture which showed enhanced ability to form spheres in rescue cells compared to knockout cells. Our study has thus far demonstrated the potential role RIPK2 plays in progression of IBC therefore can be imperative therapeutic target in the treatment of IBC. Therefore, using other IBC cell lines and humanised mouse models, we will seek to identify and determine the potency of RIPK2 inhibitors in the treatment IBC.

29. Christina Ferazzutti (Reproduction and Developmental Sciences) Inflammation-induced fetal loss leads to innate immune reprogramming of uterine macrophages

Christina Ferazzutti, Nakeisha Lodge-Tulloch, Tiziana Cotechini, Charles H. Graham
Department of Biomedical and Molecular Sciences, Queen's University, Kingston ON

OBJECTIVES: Pregnancy complications such as pre-eclampsia, fetal growth restriction, and pregnancy loss are associated with dysregulation of the maternal immune system and recurrence in subsequent pregnancies. Recent evidence suggests innate immune cells may acquire non-specific memory, altering cytokine responsiveness upon secondary stimuli exposure. This innate immune cell reprogramming is mediated by alarmins, which are elevated in inflammatory pregnancy complications. We hypothesize that inflammation-induced pregnancy loss leads to innate immune reprogramming of uterine macrophages, contributing to the risk of subsequent pregnancy complications.

STUDY METHODS: We established a semi-allogeneic murine model of lipopolysaccharide (LPS)-induced fetal loss. Female BALB/c mice (mated with C57BL/6 males) were injected with 20 µg/kg LPS (or phosphate-buffered saline) on gestational day 10.5, resulting in fetal demise. Uterine tissue was collected and enzymatically dispersed at various postnatal experimental endpoints. To determine whether LPS-induced fetal loss leads to uterine macrophage reprogramming, isolated cells (F4/80-positive selection; STEMCELL Technologies) were exposed to a secondary stimulus (LPS: 100 ng/mL) *in vitro* for 24 hours. Cytokine levels in the cell culture supernatant were assessed using a multiplex assay (EveTechnologies).

RESULTS: In response to LPS as a secondary stimulus, pro-inflammatory and anti-inflammatory cytokine levels were reduced in the supernatant from macrophages isolated from dams with LPS-induced fetal loss compared with controls.

CONCLUSION: LPS-induced fetal loss is associated with innate immune reprogramming of uterine macrophages. Future research will explore a connection with recurrent pregnancy complications.

30. Richard Nauman (Immunology; Cancer) Bacillus Calmette-Guérin (BCG)-Mediated Innate Immune Reprogramming Enhances T Cell Activation in Bladder Cancer

Richard W. Nauman[1], Brian Laight[1], Peter Greer[1], Charles H. Graham[1,2]

[1]*Department of Biomedical and Molecular Sciences, Queen's University, Kingston, Ontario, Canada*

[2]*Department of Urology, Queen's University, Kingston, Ontario, Canada*

Funding: Canadian Institutes of Health Research (CIHR)

Introduction: Bacillus Calmette-Guérin (BCG) immunotherapy remains the standard of care for higher-risk non-muscle invasive bladder cancer (NMIBC), though its mechanism is not fully understood. Trained immunity (TI), a heterologous form of memory acquired by innate immune cells, is thought to be involved. We previously found that TI acquisition in peripheral immune compartments is linked to recurrence-free survival after BCG therapy, enhancing antigen presenting cell (APC) functions and increasing tumour-specific cytotoxic T cell (CTL) populations. Notably, macrophages without the FES protein-tyrosine kinase show TI characteristics, including higher proinflammatory cytokine production. We aim to elucidate FES's role in TI and how BCG-induced TI contributes to anti-tumour immunity in bladder cancer.

Methods: TI was induced *in vivo* by administering BCG to wild-type or FES-knockout mice, training bone marrow progenitor cells. Trained and untrained APCs were co-cultured with CTLs to assess T cell activation.

Results: BCG-trained APCs activated $CD8^+$ T cells to a greater extent compared to untrained APCs, as indicated by increased IFN-γ production. Training and lacking FES protein had an additive effect in increasing the ability of APCs to induce T cell activation.

Conclusions: TI enhances tumour-specific adaptive immune responses, potentially increasing BCG's therapeutic benefit. FES inactivation may further boost this effect. BCG failure in NMIBC could be due to inadequate TI acquisition. Strategies to optimize TI, possibly via FES inhibition, might improve outcomes for NMIBC patients.

31. Anna Willmott (Reproduction and Cancer) Exploring innate immune reprogramming of mammary gland macrophages in parous mice: implications for postpartum breast cancer

Anna Willmott, Christina Ferazzutti, Nakeisha Lodge-Tulloch, Charles Graham, Tiziana Cotechini. Department of Biomedical and Molecular Sciences, Queen's University, Kingston, ON, Canada.

Uncomplicated childbirth is associated with an increased risk of developing breast cancer (BC) five-to-ten years postpartum (postpartum breast cancer; PPBC) compared with age-matched nulliparous individuals. PPBC is also associated with a poor prognosis relative to nulliparous individuals with BC. Contrastingly, preeclampsia (PE), an inflammatory pregnancy complication, protects against development of PPBC. We hypothesize that both parity-induced increased risk of PPBC and PE-associated protection against PPBC are conferred through differential parity-induced innate immune reprogramming of mammary gland macrophages. Mammary gland macrophages isolated on postpartum day 10 from allogeneic pairings of female BALB/c and male C57Bl/6 mice treated with either saline or lipopolysaccharide (LPS; 20 µg/kg) to induce pregnancy complications were plated

($4x10^4$ cells/well) and exposed to a secondary challenge (LPS; 100 ng/ml; Pam3Cys; 10 ng/ml) for 23 hours. Cytokine concentrations in cell culture supernatant were assessed using a multiplex assay (Eve Technologies) and analyzed by two-way ANOVA. Compared with supernatant from mammary gland macrophages of nulliparous saline-treated mice, supernatant from macrophages from parous saline-treated mice exhibited significantly increased interleukin (IL)-1β and tumour necrosis factor-α (TNFα), and significantly decreased IL-6 following secondary challenge. TNFα, IL-6 and IL-10 in supernatant from macrophages from parous LPS-treated mice was significantly decreased compared with parous saline-treated controls. These results demonstrate differential reprogramming in mammary gland macrophages relative to parity and inflammatory pregnancy complication status. Further research will temporally evaluate reprogramming to better understand contributions to PPBC.

32. Seily Shrestha (Experimental Medicine – Immunology) Stromal cell-derived CSF1 is important in macrophage differentiation and proliferation during tissue injury

    Macrophages are pivotal in tissue repair, playing crucial roles in resolving inflammation and promoting tissue regeneration. While the requirement of colony-stimulating factor 1 (CSF1) for macrophage survival, proliferation, and differentiation is well-documented, the specific cellular sources of CSF1 in vivo for these functions is not well understood. This study aims to elucidate the role of stromal-derived CSF1 in the survival and self-renewal of tissue-resident macrophages (TRMs) essential for tissue homeostasis, and in the expansion of monocytes and macrophages in the setting of injury. Surprisingly, global CSF1 knockout experiments revealed significant disruptions in TRM numbers but did not impair their self-renewal in steady-state conditions. However, in a mouse model of muscle regeneration, we observed CSF1 was essential to the differentiation of monocytes to macrophages, as previously shown. Further investigation into stromal-specific CSF1 knockouts, including endothelial cells, pericytes, and fibroblasts, highlighted fibroblasts as the critical drivers of macrophage differentiation during muscle injury. Additionally, CSF1 deletion in fibroblasts significantly reduced macrophage proliferation during injury, underscoring their importance in macrophage regulation. These findings confirm the essential function of fibroblast-derived CSF1 in governing macrophage population dynamics during muscle repair. Understanding the CSF1-mediated crosstalk between fibroblasts and macrophages offers new insights into tissue healing processes and identifies potential therapeutic targets for enhancing tissue regeneration and reducing fibrosis in muscle injuries.

33. Julia Barilo (Microbes, immunity, & inflammation)

    PLX5622 is a colony stimulating factor 1 receptor (CSF-1R) inhibitor that is known to deplete microglial cells in vivo. Recently its effects on macrophages (Mϕ) were also observed in vivo. Therefore, we performed this study to assess its in vitro effects on the differentiation and functions of polarized Mϕ derived from different tissues. Our findings show that addition of PLX5622 early on after ex vivo isolation hinders Mϕ differentiation and survival. However, its addition post Mϕ

differentiation did not significantly affect the viability. Furthermore, PLX5622 affects certain functions and degree of polarization of IL-4 (M2a) Mϕ but not polarization of M1-like Mϕ. Our study provides novel aspects on the application of PLX5622 to study Mϕ functions in vitro, where polarization is affected by CSF-1R signalling and provides distinctive evidence to its ability to affect certain populations of Mϕ during in vitro differentiation and maturation.

34. Kaila Gabriel (Cancer) Investigating the Impact of Hypoxia on Immune Checkpoint Expression in Bladder Cancer Cells

Kaila Gabriel, Dr. Jean-François Paré, Dr. Charles Graham

Department of Biomedical and Molecular Sciences

Bladder cancer is highly prevalent in Canada. Non-muscle invasive bladder cancer (NMIBC) patients experience a recurrence rate of 40%, highlighting the need to explore how changes in the tumour microenvironment might drive disease progression. Tumour hypoxia, characterized by low oxygen levels, is common in solid cancers and contributes to progression. Hypoxic cancer cells may evade the immune system by altering the expression of immune checkpoint molecules, which inhibit immune responses. Tumours exploit these inhibitory pathways to escape immune detection. Although hypoxia is known to promote immune escape, its effect on immune checkpoint expression in bladder cancer is unclear. I hypothesize that hypoxia changes the expression of several immune checkpoint molecules in bladder cancer cells.

To test this, human and mouse NMIBC cell lines were incubated at 0.5% and 20% oxygen for 24-48 hours. Several immune checkpoint genes, including LAG3, TIM3, and CD80, demonstrated altered expression. Future studies will expand this data by observing the impact of hypoxia on the surface expression of immune checkpoint proteins in human bladder cancer cells. This data will guide experiments to stain bladder cancer tumours to see if hypoxic areas correlate with altered immune checkpoint expression. My research assesses how hypoxia impacts immune checkpoint molecules and aids cancer cells in evading adaptive immune responses. Understanding how hypoxia influences immune checkpoints is essential for addressing immune evasion in NMIBC.

35. Agnes Chan (Immunology) Characterizing Murine M2d Cells: Alternatively Activated Anti-Inflammatory Macrophages

Agnes Chan, Julia Barilo, and Sam Basta
Department of Biomedical and Molecular Sciences, Queen's University

Macrophages (Mϕ) are a highly heterogeneous population within the innate immune system. Mϕ are highly plastic and polarize into a range of subtypes. Classical activation of Mϕ results in an M1 pro-inflammatory phenotype elicited in response to pathogen invasion and tissue damage. Alternative activation into an M2 anti-inflammatory phenotype supports tissue healing and homeostasis after pathogen clearance. M2 macrophages can be further classified into subsets (M2a, M2b, M2c, and M2d) based on activation pathways, biomarkers, and functional activity. M2d cells

are anti-inflammatory, pro-tumorigenic, and pro-angiogenic. Differentiation into M2d from resting macrophages (M0) can occur by one of two pathways: (1) interleukin (IL)-6, or (2) toll-like receptor (TLR) agonist and A2A adenosine receptor agonist. This study investigates the characterization of murine-derived M2d cells using lipopolysaccharide (LPS) and 5'-N-ethylcarboxamido adenosine (NECA) with a distinctive set of biomarker expression, and functional assays.

36. Andrew Butterfield (Immunology) Effects of Beta-Glucan Administration against Non-Muscle Invasive Bladder Cancer

    Non-muscle invasive bladder cancer (NMIBC) is the 8th most common form of cancer in Canada. The gold standard treatment, intravesical BCG administration, has seen wide success, but is also associated with significant limitations such as a high overall recurrence rate and extremely high rate of adverse effects. Beta-glucan is an immunogenic stimulus associated with induction of a trained immune response. It was proposed that prophylactic beta-glucan administration could have anti-tumorigenic effects against NMIBC. 21 mice were split into two groups, control (PBS; 10 mice) and experimental (beta-glucan; 11 mice). Two doses of each treatment were administered before instillation of MB49 tumour cells containing the luciferase enzyme. Mice were analyzed several times through IVIS, before euthanasia and analysis of bladder and tumour-draining lymph node tissue using flow cytometry. While limited differences were seen in bladder tissue, several populations of immune cells were significantly different in lymph node tissue. A significant increase in B cells was observed in lymph node tissue, as was a significant decrease in macrophages and both cDC-1/cDC-2 cells. In the bladder, only a significant increase in M1 macrophages was observed, which could point to a more anti-tumorigenic immune microenvironment after beta-glucan prophylaxis. Activity of trained immunity was not assessed in this experiment, but is an evident next step in uncovering the effects of beta-glucan against NMIBC.

    Authors: Andrew Butterfield (presenting), Richard Nauman, Charles Graham

    This work was carried out in the Department of Biomedical and Molecular Sciences, Queen's University

37. Nasry Zane Bouzeineddine (Microbes, Immunity and Inflammation) Granulocyte Macrophage Colony Stimulating Factor exerts dominant effects over Macrophage Colony Stimulating Factor during bone marrow-derived Macrophage differentiation

    Maria Petrina, Torki Alothaimeen, Nasry Zane Bouzeineddine, Evan Trus, Andra Banete, Katrina Gee, and Sameh Basta
    Department of Biomedical and Molecular Sciences, Queen's University, Kingston, ON, Canada, K7L 3N6

    Macrophages carry out a variety of immune functions and can be influenced by diverse cytokines during their differentiation, which in turn can influence their polarization status. Macrophages can exist along a spectrum of different activation states when their polarization phenotype is committed to the pro-inflammatory (M1) state especially after exposure to LPS and IFNγ. Granulocyte

macrophage colony stimulating factor (GM-CSF) and macrophage colony stimulating factor (M-CSF) are important cytokines in hematopoiesis and can differentiate macrophages into a pro-inflammatory M1-like or neutral/anti-inflammatory phenotype, respectively. Limited evidence suggest that these two cytokines may act in a way that antagonize each other. To gain a greater understanding of the relationship between GM-CSF and M-CSF, an in vitro model of differentiation was used to elucidate how the antagonistic properties of these CSFs influence macrophage function and polarization. Our data implies that GM-CSF exerts dominance over M-CSF in mixed cultures by inducing surface marker expression and cytokine production in macrophages akin that of the M1-like state. These findings provide insight into the antagonism between M-CSF and GM-CSF during macrophage differentiation when both are present in the environment.

38. Aryaman Sharma (Neuro-oncology) p57 Defines a Treatment-Resistant Quiescent Cell Population in Sonic Hedgehog Medulloblastoma

Authors: Sharma, Aryaman (BSc - Presenting author)1; Purzner, James (MD/PhD)2; Scott, Matthew (PhD)3; Purzner, Teresa (MD/PhD)2

Affiliations:
1. Department of Medicine, Queen's University School of Medicine, Kingston, Ontario
2. Division of Neurosurgery, Queen's University School of Medicine, Kingston, Ontario
3. Department of Developmental Biology, Stanford School of Medicine, Stanford, CA

Tumour recurrence is the primary cause of death in medulloblastoma (MB), highlighting a pressing need for novel therapies that treat or prevent recurrence. A promising start to addressing this challenge has been the identification of quiescent tumour cells within MB that have been shown to resist standard chemotherapy and drive recurrence. While defined by markers, the mechanisms contributing to quiescence, chemoresistance, and recurrence remain unclear. We have identified high levels of the cell cycle inhibitor p57 (Cdkn1c) at the border of murine Sonic Hedgehog (SHH) MB tumours and normal brain tissue. Given p57's role in maintaining quiescence in neural stem cells, we characterized its expression and assessed the consequences of its overexpression in SHH MB cells. In culture, nuclear p57 was enriched in Nestin+ and Sox2+ quiescent, multipotent MB progenitor cells compared to rapidly-cycling Atoh1+ cells. High throughput single-cell imaging demonstrated a 6-fold increase in G0 phase with p57 induction (60% vs. 10%). When treated with vincristine, a frontline SHH MB chemotherapy agent, control cells exhibited dose-dependent cell death, while cells with high p57 showed robust survival at the highest doses (10μM). Our next step is to identify regulators of p57 in SHH MB to prevent its accumulation and the resulting quiescence, offering potential novel therapies. This approach aims to reduce tumour recurrence and improve long-term survival outcomes for patients diagnosed with SHH MB.

40. Marina Korovina (Chronic Cough and Asthma)

Introduction: The pathophysiology and clinical significance of cough in cough variant asthma (CVA) is incompletely understood. Methacholine (MCh)-induced cough with normal sensitivity (COUGH) is a phenotype distinct from asthma and healthy individuals, characterized by reduced bronchodilating effect of a deep inspiration (DI). We hypothesize that CVA and COUGH represent clinically relevant airway disease phenotypes, distinguishable by their responses to indirect challenges and DI.

Methods: Individuals 18-65 years of age performed MCh, eucapneic voluntary hyperventilation (EVH), and mannitol challenges on separate visits in random order 2-14 days apart. Dyspnea, cough counts, spirometry, plethysmography, and impulse oscillometry were assessed.

Results: 10 healthy participants (5 females; 33.1 ± 15.1 years [Mean ± SD]; FEV1 111.1 ±15.3 % pr; FeNO 28.1 ± 28.9 ppm) and 8 participants with CVA (6 females; 38.4 ± 18.6 years; FEV1 102.6 ± 17.4 % pr; FeNO 30.7 ± 33.0 ppm) have been studied to date. Compared to baseline, controls coughed significantly more during the mannitol challenge. The DI index increased significantly compared to baseline during the MCh challenge in controls but not CVA. There were no significant differences from baseline in the DI index during EVH or mannitol challenges in either group.

Conclusion: Sensory-mechanical responses to the challenges differed between the controls and participants with CVA studied to date. During the challenges, the controls developed comparable bronchoconstriction while the CVA group showed variability. The bronchodilating effect of a DI is preserved in controls and CVA during MCh, but the groups showed opposite effect during the indirect challenges. Comparison to participants with COUGH may be informative.

41. Danielle Harper (Cancer) Targeting Calpain Proteases to Impede Triple-Negative Breast Cancer Metastasis

Danielle Harper, Samantha Cockburn , Yan Gao, Peter A. Greer (1) (1) Department of Pathology & Molecular Medicine, Queen's University

Triple-negative breast cancer (TNBC) is characterized by a lack of estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor (HER2) overexpression. The absence of these targets limits treatment options and puts patients at risk of developing drug-resistant metastatic disease. My research explores the effects of genetic calpain disruption on TNBC tumorigenesis, metastasis, and drug sensitivity. Calpain proteases are involved in both pro- and anti-apoptotic signalling, and these opposing roles may be exploited to improve treatment response while protecting healthy cells from off-target cytotoxic effects. Using CRISPR-Cas9 knockout of capns1 in AC2M2 mouse mammary cancer cells, I have shown that calpain reduces the metastatic potential of TNBC cells in a mouse orthotopic engraftment model. To explore how calpain disruption in TNBC cells or hematopoietic cells affects anti-cancer immune response, I established calpain knockout and rescue E0771 cell lines and a transgenic C57BL/6J mouse strain with conditional deletion of CAPNS1 in the hematopoietic lineage. Both models will be used in syngeneic engraftment experiments to assess how loss of calpain in cancer cells or immune cells effects tumour growth and metastasis.

This work is supported by a CIHR grant to PAG, and DH is supported by a Dean's Doctoral Award.

42. Flourish Adebayo (Computational Biology) Enhancing Single-Cell RNA Sequencing: A Novel Approach for RNA Biomarker Discovery.

Flourish Adebayo[1,2], Parvin Mousavi[2], Neil Renwick[2] Gabor Fichtinger[2] Kathrin Tyryshkin[1,2]

1 Department of Pathology and Molecular Medicine, Queen's University, General and Hotel Dieu Hospitals, 88 Stuart Streets, Richardson Laboratory, Kingston, ON K7L 3N6

2 School of Computing, Queen's University, 557 Goodwin Hall, Kingston, K7L 2N8

Single-cell RNA sequencing (scRNA-seq) is a powerful method for understanding gene expression changes at a single cell level. However, these complex data are subject to doublet formation, batch effects, and dropout events that may affect biomarker detection. Current methods in single-cell data analysis, for doublet detection, dropout event identification, and batch effect removal, face significant limitations that demand the development of more robust techniques. For doublet detection, existing algorithms often struggle with scalability and accuracy, particularly in datasets with high cell counts or diverse populations, leading to either missed doublets or false positives. In dropout detection, the primary challenge lies in distinguishing between true biological zero expressions and technical dropouts, a distinction that current methods cannot consistently make, leading to skewed gene expression profiles. Lastly, the removal of batch effects remains problematic as many methods require some assumptions about the similarity between batches or rely heavily on manual tuning of parameters. These limitations can significantly impact the downstream analysis, such as clustering and differential expression analysis, thus underscoring the need for adaptable methods in single-cell data processing. In my project, I am developing a preprocessing pipeline to overcome these issues. I propose a three-tiered preprocessing strategy to enhance doublet detection, dropout imputation and batch effect detection and removal. Currently, I am exploring a range of statistical, and machine learning methods to identify duplicate cells (doublets) in single-cell RNA sequencing data. Results indicate that doublets frequently exhibit similar expression profiles and distribution patterns, suggesting that detecting all of them may be an impossible task. I also utilize Conditional Flow Matching (SCFMI) for the imputation of missing gene expression values (dropout imputation). Results show that SCFMI performs well in imputation accuracy, enhances clustering, and effectively preserves zero values, with analyses confirming its performance on differentially expressed and marker genes. Future work will focus on addressing batch effect detections using machine learning and visualization approaches.

This integrated approach aims to enhance data accuracy and reliability, enabling more precise biomedical analyses. We expect our approach to significantly reduce noise and increase the reliability of detecting novel RNA biomarkers. By improving the precision of biomarker discovery, our approach underscores the role of thorough preprocessing for genomic data, potentially facilitating more accurate clinical insights into complex disease.

43. Samantha Cockburn (Triple Negative Breast Cancer)

Triple-negative breast Cancer (TNBC) is an aggressive subtype of breast cancer (BC). TNBC distinctly lacks three receptors commonly targeted in therapies. As a result, efficacious treatment options remain limited, putting patients at risk of drug resistance, relapse, and metastasis. An intracellular protease, calpain-1/2, has emerged as a promising novel therapeutic target to improve TNBC outcomes due to its correlative clinical and functional implications in cancer progression. Calpains are aberrantly expressed in numerous cancer types, including breast cancer. Calpain-1 and calpain-2 are the most well-characterised isoforms and are known to cleave substrates mediating various pro-metastatic cell signalling pathways such as cytoskeletal remodelling, cell survival, and apoptosis. Based on these established roles for calpain, I aim to further characterise the effects of calpain knockout (KO) on migration, invasion, tumour growth, and paclitaxel-induced metastasis in a mouse model of TNBC. I will also explore the role of collapsin response mediator protein 2 (CRMP2), a calpain substrate and microtubule-binding protein, in paclitaxel-microtubule targeting therapy. By understanding calpain's role in metastasis, we aim to improve the efficacy of existing chemotherapeutics by combining them with pharmacological calpain inhibitors, thus expediting the development of much-needed therapies for TNBC.

44. Isabella Thomas (Epidemiology, Military-connected youth) Peer Connections and Social Media Use of Military Connected Youth in Canada: A Cross Sectional Study

Isabella Thomas, Dr. Wendy Craig, Dr. Heidi Cramm, Dr. Alyson Mahar

Introduction: Strong peer relationships are crucial for adolescent development, providing stress relief and promoting well-being. Military-connected students face unique stressors like frequent relocations, which can disrupt peer connections. Social media use may mitigate these disruptions but also poses a risk of problematic use and negative mental health outcomes.

Purpose: This study investigates the impact of Canadian military family membership on peer connections and problematic social media use compared to non-military-connected youth.

Methods: Data from the 2017/2018 Canadian Health Behaviour in School-aged Children (HBSC) survey, including students aged 11-15, will be used. Military connection is defined by parent, guardian, or family member involvement in the Canadian Armed Forces. Peer connections are measured using the Multidimensional Scale of Perceived Social Support (MSPSS), and problematic social media use is assessed with the nine-item Social Media Disorder scale. Analyses will control for sex, grade, socioeconomic status, and family structure using log binomial regression and generalized estimating equation (GEE) models.

Expected results: It is hypothesized that military-connected youth will report lower peer support and higher rates of problematic social media use than their non-military peers.

Conclusion: This study aims to fill a gap in Canadian research on the social support and online behaviours of military-connected youth, to understand ways to enhance their social and emotional well-being.

45. Amoon Jamzad (Diagnosis mass spectrometry imaging, point of care pathology) An end-to-end software solution for analysis of mass spectrometry imaging in point of care histopathology

Amoon Jamzad1,2, Jade Warren1, Martin Kaufmann2, Ayesha Syeda1, Natasha Iaboni3, David Hurlbut3, Kevin Y. M. Ren3, John Rudan2, Gabor Fichtinger1, Christopher J. B. Nicol3,4, Parvin Mousavi1

Departments
1School of Computing, Queen's University 2Department of Surgery, Queen's University 3Department of Pathology and Molecular Medicine, Queen's University, 4Queen's Cancer Research Institute, Division of Cancer Biology and Genetics,

Keywords
Mass spectrometry imaging, point of care histopathology, software development.

Mass Spectrometry Imaging (MSI) is a powerful tool capable of capturing metabolomic signatures to identify disease markers, with the potential to be used for point-of-care histopathological assessment. However, data analysis is computationally heavy, time-consuming, and requires coding knowledge. The purpose of this work is to develop a free software solution capable of performing the entire exploration and analysis pipeline of MSI end-to-end. Our software is a module in 3D Slicer, an open-source platform for the development of custom analytical solutions for medical and biomedical data. The implemented functionalities include data exploration via various targeted and non-targeted visualizations, co-localization to spatial labels (histopathology annotations), dataset generation with spatial- and spectral-guidance, multi-slide data aggregation via feature alignment, denoising via spatial aggregation, machine learning model training, and whole-slide model deployment. Our software has a modular design which adds flexibility for adding and customizing features upon user request. The software was tested using sample DESI-MSI data to evaluate the computational pipeline – from data visualization to whole-slide model deployment – with the results showing successful implementation of its functionalities and end-to-end usage. A preliminary test was also performed to assess user experience, with findings showing significant improvement. Our software aspires to satisfy the need for a single-stop comprehensive interface for MSI data analysis.

46. Abbey L. Politeski (Immunology) Investigating the role of tissue resident macrophages in skeletal muscle regeneration

Abbey L. Politeski1, Jasleen Kaur2, Jiarui Che2, Sarah A. Dick2
1 Department of Medicine, Queen's University, Kingston, CA.
2 Department of Biomedical and Molecular Sciences, Queen's University, Kingston, CA.

Skeletal muscle is critical for locomotion, force generation, energy metabolism, and thermoregulation. Disruptions in homeostasis caused by traumatic muscle injury and muscular dystrophies lead to compromised muscle regeneration, function, and poor quality of life. Inflammation has a dual role in muscle regeneration, as both chronic inflammation and the elimination of inflammatory immune cells correlate with worse regeneration. The types of immune

cells present during regeneration and their function have yet to be fully understood. Tissue resident macrophages (TRMs) have been identified in healthy muscle tissue, existing in three major subsets. TRMs are ontogenetically and functionally distinct from monocyte-derived macrophages recruited to tissue at different time points post-injury and participate in both inflammation and healing. To study the role of TRMs in muscle regeneration we utilized the Cx3cr1CreER:RosaTd fate mapping mouse model following a cardiotoxin induced muscle injury in the gastrocnemius muscle. Our initial studies have demonstrated TRMs expand in injured muscle via local proliferation near satellite cells. Through in silico analysis of publicly available single-cell RNA sequencing datasets collected at various time points following cardiotoxin-induced injury, we identified several macrophage subsets in healthy and injured muscles beyond the classical M1/M2 paradigm. Future work will include a TRM depletion model to specifically assess their functional role in muscle regeneration. Our goal is to identify novel targets for therapies to promote healthy muscle regeneration caused by muscular dystrophy and traumatic injury. Funding: NSERC

47. Matheson McFarlane (Asthma Clinical Research/ Knowledge Translation Research) Promoting Evidence-based Asthma Care using Digital Knowledge Translation Tools – Impact of the Provider Asthma Assessment Form

Matheson McFarlane1, Alison Morra1, Delanya Podgers2, David Barber3, and M. Diane Lougheed1
1Division of Respirology, Department of Medicine, Queen's University, Kingston, ON, Canada
2Kingston Health Sciences Centre, Kingston, ON, Canada
3Department of Family Medicine, Queen's University, Kingston, ON, Canada

RATIONALE: Despite national asthma care guidelines, care gaps persist between best practice and clinical practice which contributes to poor health outcomes. The Provider Asthma Assessment Form (PAAF) is an electronic asthma management and knowledge translation (KT) tool with an embedded decision support algorithm identifying severe and/or uncontrolled asthma, designed to support evidence-based asthma management (Morra et al. Can J Respir Crit Care Med 2022). We aimed to document baseline asthma practice patterns, and to determine whether PAAF integration into a primary care electronic medical record (EMR) improves evidence-based asthma diagnosis and management.

METHODS: We performed a single-centre pre-post observational study at a tertiary care family medicine centre in Kingston, Ontario. Retrospective baseline data were collected for two years prior to PAAF implementation, from Jan 1, 2018 - Dec 31, 2019. Prospective, post-intervention data were collected from Jan – Dec 2023. A validated adult asthma EMR case definition (Moloney et al., Eur Respir J, 2022) was applied to EMR data to identify suspected or objectively confirmed asthma cases for both data sets, on which detailed manual chart abstractions were performed. Descriptive analysis was performed on baseline data and preliminary post-intervention data.

RESULTS: 145 patients were included in the retrospective baseline cohort (61.4% female; 46.2% with =>4 comorbidities; BMI 30.1 ± 9.2 kg/m2) and 85 patients were included in the post-implementation

cohort (64.7% female; 43.5% with =>4 comorbidities; BMI 30.7 ± 7.1 kg/m2). In the baseline cohort, 71.7% of patient were classified as suspected asthma, compared to 80% in the post-implementation cohort. Recent emergency department visits for asthma decreased by 5.6% and exacerbations <1 year decreased by 10.2% in the post-implementation cohort. Significant increases in ICS and second controller use were found in the post-implementation period, as well as a non-significant increase in reliever use and a non-significant decrease in systemic corticosteroid use in the last year. With the use of the PAAF (n = 11), diagnosis status was documented in 100% of PAAFs used. An average of 4.7 ± 1.8 Canadian Thoracic Society symptom-based asthma control parameters were documented with use of the PAAF, compared to 2.4 ± 1.3 average parameters per visit from chart abstractions.

CONCLUSIONS: Asthma care gaps such as underutilization of PFTs for diagnosis and monitoring, asthma education, and addressing reasons for poor asthma control were common in this primary care study. Use of the PAAF increased asthma visit specific documentation for diagnosis status and quantity of asthma control parameters documented. Further post-implementation analysis will reveal the extent to which point-of-care KT tools with decision support improve documentation, quality of care and patient outcomes.

FUNDING: Queen's Health Sciences Internal Grant - William M. Spear Endowment Fund in Pulmonary Research/Richard K. Start Memorial Fund.

48. Karam Alosta (Respirology) The effects of acute bronchodilator administration on small airway function, neuromechanical coupling, and exertional dyspnea in hyperinflated patients with COPD: A study proposal

Karam A. Alosta1, Matthew D. James1,2, Sandra G. Vincent1, John M. McCleary1, Nicolle J. Domnik1,2, J. Alberto Neder1,2
1Respiratory Investigation Unit, Department of Medicine, Queen's University and Kingston Health Sciences Centre, Kingston, ON, Canada
2Department of Biomedical and Molecular Sciences, Queen's University, Kingston, ON, Canada

Background: Exertional dyspnea in COPD arises from mismatching between high inspiratory neural drive (IND) and low inspiratory capacity (IC), i.e. neuromechanical dissociation (NMD). Small airways dysfunction (SAD; e.g., mucus occlusion, bronchoconstriction) contributes to air trapping, upward shifting of operating lung volumes, and poor dynamic compliance. It is unclear whether the positive effects of pharmacologic deflation during rest and exercise are mechanistically linked to improved SAD and NMD.

Aim: To investigate the effect of bronchodilation versus placebo on SAD, operating lung volumes, NMD and exertional dyspnea at rest and during exercise in hyperinflated patients with COPD.

Approach: A double-blind, randomized, placebo-controlled study on 25 patients(>40 y/o) with COPD (FEV1/FVC<0.7, FEV1 30-70% predicted, and FRC>120% predicted or >ULN). Participants will

complete three visits. Visit 1: PFTs, impulse oscillometry [IOS], and a symptom-limited cycle cardiopulmonary exercise test [CPET] to determine maximum work rate. Visits 2&3: constant work rate cycle CPET (75% of maximum work rate) after either placebo or bronchodilator (salbutamol + ipratropium). During exercise an esophageal catheter will measure IND and mechanics, IOS will assess SAD, IC maneuvers will assess operating lung volumes, and Borg 0-10 scale will assess dyspnea.

Impact: Our study will link small airway function with exertional dyspnea and NMD during exercise for the first time. It will also establish a novel platform for assessing the efficacy of therapeutic interventions on SAD in COPD.

Funding: Spear-Start Endowment Fund, Queen's University

49. Sofia Skebo (Cardiopulmonary/cancer research) Designing a fluorescent reporter for the live-cell assessment of endothelial BMP9 signaling kinetics

S.I. Skebo1, M.M.VandenBroek1, D. Quilty2, M.L. Ormiston1,2
1Department of Medicine, Queen's University, Kingston ON
2Department of Biomedical and Molecular Sciences, Queen's University, Kingston ON

Rationale: Bone morphogenetic protein-9 (BMP9) regulates angiogenesis in oscillatory waves of downstream signaling via the Smad family of transcriptional mediators. Changes in the magnitude or frequency of these oscillations alters the endothelial proliferative response to BMP9, contributing to diseases of vascular obstruction like pulmonary arterial hypertension. We aim to design a reporter of BMP9 signaling kinetics to monitor Smad1 nuclear translocation in live cells.

Methods and Results: Constructs encoding N-terminally GFP-tagged Smad1 were optimized for linker length and flexibility using HiFi assembly and were expressed in HEK-293 and immortalized endothelial cells (Telo-HAECs) under the control of either an HSV (low expression) or CMV (high expression) promoter. NucBlue live cell nuclear stain was used to visualize the nucleus. Physiological (HSV) and supraphysiological (CMV) expression of GFP-Smad1 was confirmed by qPCR and fluorescent microscopy in both live and fixed cells. Nuclear and cytoplasmic cell fractions were collected, with and without BMP9 stimulation (1ng/mL), to assess the phosphorylation and localization of GFP-Smad1 by immunoblotting. Live cell imaging of transiently transfected Telo-HAECs demonstrated nuclear translocation of the construct 25 minutes post-BMP9 stimulation.

Conclusions: We have produced a functional GFP-Smad1 reporter for tracking BMP9 signaling kinetics. Ongoing studies involve inserting the construct into a lentiviral vector, for the purposes of improving on low rates of transient transfection in endothelial cells and creating a tool for screening drugs targeting BMP9-linked angiogenesis.

50. Lindsay Jefferson (Pulmonary Arterial Hypertension, Translational Medicine) Metabolic and Phenotypic Investigation of Macrophages in Pulmonary Arterial Hypertension (PAH)

Lindsay Jefferson1, Ashley Y. Martin1, Rachel E.T. Bentley1, Mia S. Wilkinson1, Kimberly J. Dunham-Snary1,2, Asish DasGupta1, Patricia D.A. Lima1,2, Stephen L. Archer1,2,3

1. Department of Medicine, Queen's University, Kingston ON
2. Department of Biomedical and Molecular Sciences, Queen's University, Kingston ON
3. Queen's CardioPulmonary Unit, Queen's University, Kingston ON

Pulmonary arterial hypertension (PAH) is an obliterative pulmonary vasculopathy, often resulting in RV failure. In males, inflammatory RV-macrophages play a role in mediating decompensated RV remodelling and failure. The metabolic programming of macrophages involving the suppression of mitochondrial oxidative function and an increased glycolytic capacity is associated with inflammation. We aim to understand the relationship between the inflammatory phenotype of macrophages in PAH and their glucose metabolic status. We established the culture of bone-marrow derived macrophages (BMDM) from control and monocrotaline (MCT; a PAH model) male rats. Flow cytometry confirmed these cells were macrophages (>95% CD45+CD68+CD11b+cells). We used the Seahorse Mito Stress Test to compare the oxygen consumption rate (OCR) in control and MCT BMDMs, while also measuring expression of inflammatory (NOS2) and anti-inflammatory (CD163) markers. Neither OCR levels nor the phenotype of cells differed between groups; however, western blot showed that MCT BMDMs trend towards increased expression of key factors of the NLRP3 inflammasome pathway (IL1B and NLRP3) and the pro-glycolytic enzyme, PDK1. In situ quantification of PDK1 in RV sections confirmed higher expression in RV-macrophages from MCT rats compared to controls. In conclusion, BMDMs from PAH-rats can be cultured and used for functional assays. A larger sample size is required to confirm our findings which suggest a link between uncoupled glycolysis and the inflammatory phenotype of macrophages in the bone marrow and RV.

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