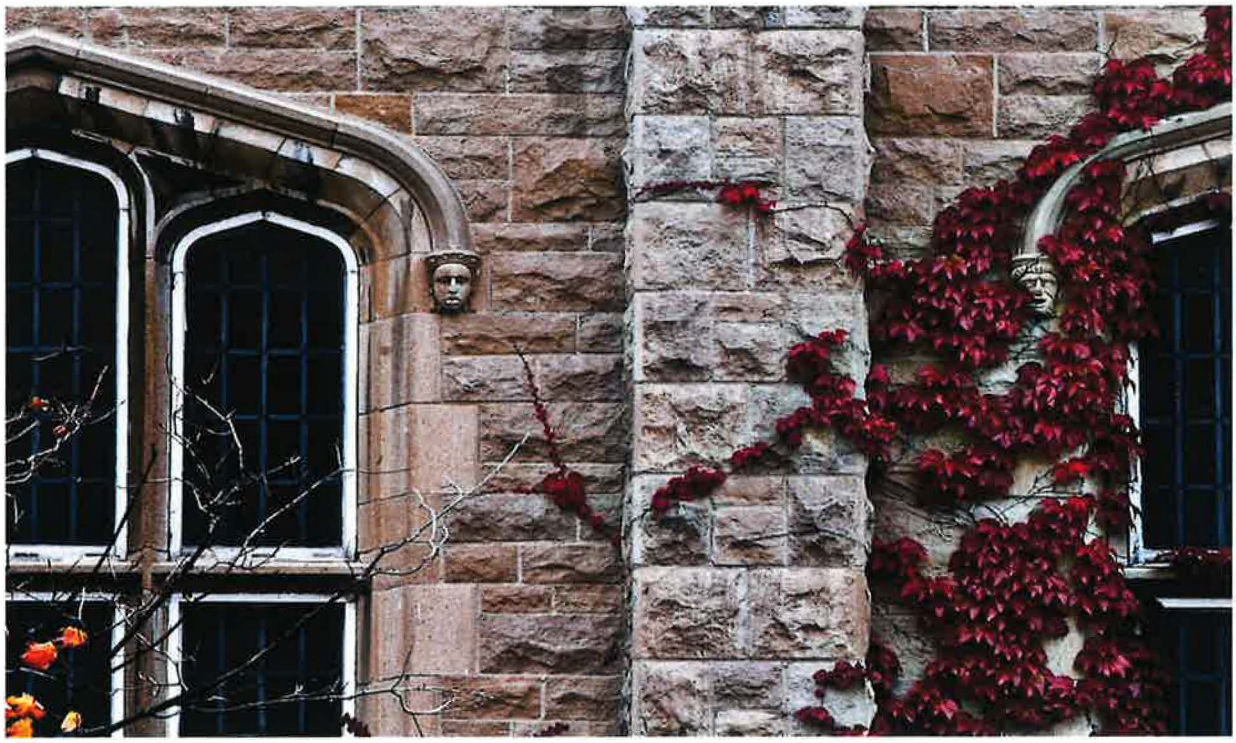
The Twenty-Fifth Annual Scientific Meeting for Health Science Research Trainees
Faculty of Health Sciences
Queen's University

Tuesday, June 27th, 2023
Queen's School of Medicine & Biosciences Atrium



Members of the Organizing Committee

Alan Lomax
Andrew Craig
Chandra Tayade
Katrina Gee
Maria Velez
Peter Greer
Prameet Seth
Sebastien Talbot
Xiaolong Yang

Session Chairs

Eva Kauffman
Faith Brenan
Mark Ormiston

Members of the Adjudication Team

Abbie Davis
Abhishek Shastry
Aleks Biorac
Alison McCallion
Bruce Masotti
Carla Gallardo
Chinmay Potdar
Chris Wells
Danielle Harper
Danielle Sisnett
Daria Ostroverkhova
Dilakshan Srikanthan
Donya Hayati
Doriana Taccardi
Dure Khan
Emma Kalin
Emma LeBlanc
Emmanuel Zangio
Ethan Thomas
Farzaneh Afzali
Gabriella Stefan
Hannah Doherty-Plummer
Hannah Wood
Harshavardhan Lingegowda
Isabella Delano
Jack Babulic
Jasleen Jagayat
Jasmine Khan
Julia Barilo
Kaitlyn Kobayashi
Kartik Sachdeva
Katie Zutautas
Keira Frosst
Lauren Brown
Megan Cull
Mia Wilkinson
Nasry Bouzeineddine
Natasha Iaboni
Richard Nauman
Ryan Kirkpatrick
Sadaf Rahimi
Safara Holder
Sakura Koner
Samantha Holmes
Shaina Smith

Oral Presentation Adjudicators

Jean-Francois Pare
Karine Roversi
Kshitiz Singh

Acknowledgments

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

The Twenty-Fifth Annual Scientific Meeting

for Health Science Research Trainees

Faculty of Health Sciences

Queen's University

Tuesday, June 27th, 2023

Queen's School of Medicine and Biosciences Atrium

8:00 – 8:40 am	Registration – School of Medicine: The Britton Smith Foundation Lecture Theatre (132A) & The David M C Walker Atrium
	Poster set-up for morning participants – BioSciences Atrium
	Oral Presentations – School of Medicine, Room 132A
8:40 – 8:50 am	**Welcome and Introduction** *Dr. Chandra Tayade, Associate Dean, Graduate and Postdoctoral Education, Queen's Health Sciences*
	Introductory Remarks *Dr. Jane Philpott, Dean, Queen's Health Sciences*
8:50 – 9:20 am	**Keynote Speaker** *Dr. Heidi Cramm, School of Rehabilitation Therapy, Queen's Health Sciences* **WHAT ABOUT THE FAMILIES? AN ECOLOGICAL PERSPECTIVE ON HEALTH AND WELLBEING FOR FAMILIES OF DEFENCE AND PUBLIC SAFETY PERSONNEL**

Oral Presentation – Session 1: SOM 132A

Chair: Dr. Mark Ormiston

9:20 – 9:32 am	Gillian Kupakuwana-Suk: **CHIP IN OLDER PATIENTS WITH LYMPHOMA: ITS IMPACT ON CHEMOTHERAPY RELATED OUTCOMES AND CLONAL EVOLUTION DURING TREATMENT**

9:32 – 9:44 am	Surbhi Gupta: **NOCICEPTOR NEURONS CONTROL VACCINE-INDUCED IMMUNITY**
9:45 – 9:57 am	Natasha Dmytryk: **ENHANCING ANTI-TUMOUR CYTOTOXIC LYMPHOCYTE RESPONSES BY TARGETING FES IN ANTIGEN PRESENTING CELLS**
9:57 – 10:09 am	Carla Gallardo Flores: **REGULATION OF PROTEIN KINASE R BY HEPATITIS C VIRUS NON-STRUCTURAL PROTEIN 5A**
10:10 am – 12:00 pm	Coffee Break & Poster Presentations – BioSciences Atrium
12:00 – 12:45 pm	Lunch/Poster Set-Up – BioSciences Atrium
12:45 – 2:20 pm	Poster Presentations – BioSciences Atrium

Oral Presentation – Session 2: SOM 132A

Chair: Dr. Faith Brenan

2:30 – 2:42 pm	Aurelie Brecier: **CONTRIBUTION OF CIRCADIAN RHYTHMS TO SENSORY NEURON ACTIVITY IN VITRO AND EX VIVO**
2:42 – 2:54 pm	Hannah Wood: **INVESTIGATING THE RELATIONSHIP BETWEEN LUMINAL MEDIATORS AND ABDOMINAL PAIN IN CROHN'S DISEASE PATIENTS**
2:54 – 3:06 pm	Theodore Aliyianis: **AN INVESTIGATION OF THE VALIDITY OF COGNITIVE ASSESSMENT VIA ROBOTICS IN PEOPLE WITH EPILEPSY**
3:06 – 3:18 pm	Courtney Bannerman: **THE ROLE OF THE GUT MICROBIOME IN THE MIGRATION OF GAMMA DELTA T CELLS AFTER SPINAL CORD INJURY**
3:18 – 3:30 pm	Priya Premranjitha: **MATERNAL EXPOSURE TO METALS AND TIME-TO-PREGNANCY: THE MIREC COHORT STUDY**
3:30 - 3:45 pm	Coffee Break – The David M C Walker Atrium - SOM

Oral Presentation – Session 3: SOM 132A

Chair: Dr. Eva Kauffman

3:45 – 3:57 pm	Jacob Kment: **COORDINATED BLOCKADE OF TGF-B AND PD-L1 BY BINTRAFUSP ALFA PROMOTES SURVIVAL IN PRECLINICAL OVARIAN CANCER MODELS BY PROMOTING T EFFECTOR MEMORY RESPONSES**
3:57 – 4:09 pm	Jina Nanayakkara: **YAP AND TEAD FORM A TRANSCRIPTIONAL COMPLEX REGULATING DIFFERENTIATION AND TUMORIGENESIS IN NEUROENDOCRINE TUMORS**
4:09 – 4:21 pm	Andrea Petkovic: **STRUCTURAL BASIS OF AMYLOID FORMATION BY *C. ALBICANS* ADHESINS**
4:21 – 4:33 pm	Lauren Brown: **INVESTIGATING THE IMPACT OF VALPROIC ACID ON PLACENTAL STRUCTURAL DEVELOPMENT IN CD-1 MICE.**
4:35 – 5:00 pm	Concluding Remarks and Awards – SOM 132A
5:00 – 7:00 pm	Reception with Cash Bar (small snack will be provided) – SOM: The David M C Walker Atrium

Oral Presentations

Session One

1. Gillian Kupakuwana-Suk (Field: Hematology) **CHIP IN OLDER PATIENTS WITH LYMPHOMA: ITS IMPACT ON CHEMOTHERAPY RELATED OUTCOMES AND CLONAL EVOLUTION DURING TREATMENT.**

O. Lopes[2], G. Kupakuwana-Suk[2,3], M. CHEUNG[1], L. Mozessohn[1], L. Chodirker[1], K. IMRIE[1], N. BERINSTEIN[1], S. CHOW[1], , A. Fu[4], , A. Parmentier[7], P. Sasitharakumar[7], H. Tsui[8], L. Zhang[9], A. McNaughton[5,6] R. Buckstein[1], M. Rauh[2]; [1]Odette Cancer Center, Hematology, Toronto, ON, Canada, [2]Queens University, Pathology and Molecular Medicine, Kingston, Canada, [3]Queens University, Medicine, Division of Hematology, Kingston, Canada, [4]Queens University, Bachelor of Health Sciences Program, Kingston, Canada, [5]Queens University, Pathology and Molecular Medicine, Kingston, AB, Canada, [6]Queens University, Pathology and Molecular Medicine, Kingston, ON, Canada, [7]Sunnybrook Health Sciences Center, Clinical Trials, Toronto, Canada, [8]Sunnybrook Health Sciences Center, Pathology and Molecular Medicine, Toronto, Canada, [9]MACROSTAT, Statistics, Toronto, Canada

Clonal hematopoiesis of indeterminate potential (CHIP), is a potentially premalignant condition that results from expansion of somatically mutated hematopoietic progenitor cells without the evidence of a hematologic malignancy. While CHIP is known to be associated with aging and can be detected in 10-

15% of healthy older adults, it is also known to have adverse health associations. We hypothesized that CHIP may be a risk factor for chemotherapy related complications in lymphoma patients aged ≥60. We performed targeted next-generation sequencing (57 genes that included canonical CHIP and DNA damage response (DDR) genes) on peripheral blood samples of 56 lymphoma patients prior to chemotherapy, after 3 cycles, and at 6 and 12 months post chemotherapy. Median age was 71, 45% male, 64% DLBCL, 88% treated first-line. CHIP (and DDR) mutations were detected in 22.6% (1.6%) patients at baseline, in 28.3% (8.7%) after cycle 3, in 50% (32.1%) and 38.5% (19.2%) at 6 and 12 months post chemotherapy respectively. Most DDR variants were present at <2% VAF at baseline and expanded with treatment. There were no differences in baseline clinical nor laboratory characteristics, dose attenuation or blood count decrements on treatment between those with and without DDR mutations, but patients with DDR mutations experienced more infections (43% versus 19% (p=.03), particularly respiratory (18% vs 0%, p=.01) and trended to have higher rates of hospitalization (57% vs. 40%).

2. Surbhi Gupta (Field: Neuroimmunology) **NOCICEPTOR NEURONS CONTROL VACCINE-INDUCED IMMUNITY.**

Surbhi Gupta[1], Francesco Borriello[2], Jo-Chiao Wang[1], Hannah Merrison[3], Abigail J Dutton[3], David Dowling[2], Clifford J. Woolf[4], Ofer Levy[2], Simmie L. Foster[3], Sebastien Talbot[1]

[1] Department of Biomedical and Molecular Sciences, Queen's University, Kingston, Canada
[2] Department of Medicine, Division of Infectious Diseases, Children's Hospital Boston, Boston, USA.
[3] Department of Psychiatry, Massachusetts General Hospital and Harvard Medical School, Boston, USA.
[4] FM Kirby Neurobiology Center, Boston Children's Hospital, Boston, USA.

Nociceptors, the sensory neurons that detect noxious stimuli and trigger pain, interact with immune cells to modulate immune responses. The nociceptor-released neuropeptide Substance P promotes B cell polarization, antibody class switching to IgE, and IgE release in models of allergic inflammation. In this study, we investigated whether nociceptors respond to vaccine adjuvants and control IgG production and clonal selection in the context of vaccination. We activated and sensitized sensory neurons from mice with vaccines and adjuvants against influenza virus and pneumococcal and meningococcal bacteria in vitro and evaluated influenza vaccine-specific IgG antibody levels in mice with ablated nociceptors. Our results showed that sensory neurons respond to vaccines and exhibit differential activation by various noxious ligands. In mice with ablated nociceptors, IgG2c titers were reduced, while capsaicin-treated mice showed increased IgG titers. These findings suggest a role for nociceptors in maintaining humoral immunity after vaccination. We will further explore how sensory neuron ablation or overactivation affects B-cell trafficking and antibody production in response to vaccination and pathogen challenges in mice. This research provides insights into the role of nociceptor neurons in humoral immune responses during vaccination and has implications for the development of more effective vaccines.

3. Natasha Dmytryk (Field: Pathology and Molecular Medicine) **ENHANCING ANTI-TUMOUR CYTOTOXIC LYMPHOCYTE RESPONSES BY TARGETING FES IN ANTIGEN PRESENTING CELLS**

Natasha Dmytryk[1], Brian Laight[1], and Dr. Peter Greer[1]

[1]Department of Pathology and Molecular Medicine, Queen's University, Kingston, ON, Canada

Antigen-presenting cells (APCs) can elicit immune responses through the release of inflammatory cytokines. The non-receptor tyrosine kinase FES has been implicated in the suppression of these inflammatory signalling pathways in APCs; giving reason to presume it may be dampening APC production of inflammatory cytokines in the tumour niche. These cytokines are required for the third and final signal of cytotoxic lymphocyte (CTL) activation – one of the main anti-tumour immune cells. We believe disrupting FES in APCs will increase signal 3 cytokine production, thereby enhancing the priming of anti-tumour CTLs. To examine the effect of FES disruption on inflammatory signalling cascades, immunoblotting was conducted on LPS-stimulated *fes*$^{-/-}$ and WT mouse macrophages, probing for components of the inflammatory signalling cascade. qRT-PCR was used to analyze signal 3 cytokine transcript levels in WT and *fes*$^{-/-}$ LPS-stimulated macrophages. Furthermore, OT-1 CTLs were co-cultured with OVA-presenting WT or *fes*$^{-/-}$macrophages to explore differences in CTL activation capabilities. Results show that *fes*$^{-/-}$macrophages display increased inflammatory signalling in response to LPS stimulation. These observations are consistent in unpolarized macrophages, M1 polarized macrophages and dendritic cells. *fes*$^{-/-}$ macrophages also demonstrate increased signal 3 cytokine mRNA production in response to LPS stimulation, and CTLs display increased activation marker expression post *fes*$^{-/-}$ macrophage co-culture. This immunosuppressive function of FES makes it an actionable target for improving the efficacy of cancer immunotherapies and anti-cancer adaptive immune responses. (Supported by the Canadian Cancer Society)

4. Carla Gallardo (Field: Microbes, Immunity and Inflammation) **REGULATION OF PROTEIN KINASE R BY HEPATITIS C VIRUS NON-STRUCTURAL PROTEIN 5A**

Carla E. Gallardo Flores[1], Jihyun Cho[1] and Che C. Colpitts[1]
[1]Department of Biomedical and Molecular Sciences, Queen's University

Chronic hepatitis C virus (HCV) infection affects 71 million people worldwide and increases the risk of developing hepatocellular carcinoma (HCC). HCV is genetically diverse, with 6 genotypes reported to date that differ in their severity and pathogenicity. Although direct-acting antivirals against HCV can cure infection, they do not eliminate the risk of developing HCC. Protein kinase R (PKR), a multifaceted kinase, is implicated in liver disease and cancer, but its regulation in the context of chronic HCV infection and HCC is poorly understood. During infection, PKR is activated by binding to viral double-stranded RNA (dsRNA), leading to protein translation shutdown and stress granule (SG) formation. Interestingly, PKR interacts with HCV non-structural protein 5A (NS5A), and mutations in the PKR-binding domain of NS5A are associated with HCC. However, the functional consequences of the NS5A-PKR interaction remain unclear. Here, we show that NS5A activates PKR, leading to translation shutdown and SG formation. We compared the effect of NS5A from HCV genotype (gt) 1b or 2a strains in PKR activation and SG formation. Our studies show that HCV-gt1b-NS5A and HCV-gt2a-NS5A differentially activate PKR, restrict protein translation, and induce SG formation. These differences may contribute to the higher pathogenicity of HCV gt1b relative to 2a. Overall, these findings contribute to understanding the regulation of PKR during HCV infection and may provide insight into new chemopreventive strategies for HCC.

Supporting Agency: NSERC and the Canadian Network on Hepatitis C

Session 2

1. Aurelie Brecier (Field: Neuroscience) **CONTRIBUTION OF CIRCADIAN RHYTHMS TO SENSORY NEURON ACTIVITY *IN VITRO* AND *EX VIVO***

Aurélie Brécier [1] & Nader Ghasemlou [1,2,3,4]
1 Pain Chronobiology & Neuroimmunology Laboratory, Queen's University, Kingston, Ontario, Canada K7L 3N6
2 Department of Biomedical & Molecular Sciences, Queen's University, Kingston, Ontario, K7L 3N6, Canada.
3 Department of Anesthesiology & Perioperative Medicine, Queen's University, Kingston, Ontario, K7L 3N6, Canada.
4 Centre for Neuroscience Studies, Queen's University, Kingston, Ontario, K7L 3N6, Canada.

Recent studies have unravelled a daily rhythm of thermal and mechanical sensitivity in humans and mice, suggesting a circadian control of nociception. However, the mechanisms underlying this phenomenon remain unclear. At the molecular level, circadian rhythms operate in each mammalian cell owing to core clock genes. While nociceptive information is primarily transduced by the sensory neurons of the dorsal root ganglia (DRG), a link between the activity of DRG neurons and the circadian regulation of nociception has never been established. We propose that circadian rhythms control the excitability of DRG sensory neurons. RT-qPCR analysis revealed abnormal expression over time of the two main clock genes *Bmal1* and *Nr1d1* in the non-treated cultured DRG neurons, suggesting a disruption of the circadian clock *in vitro.* In contrast, dexamethasone-treated cultures successfully expressed *Bmal1* and *Nr1d1* in an anti-correlated manner. Interestingly, the excitability of dexamethasone-synchronized neurons remains identical 12h and 24h post-treatment, while recordings from whole-mount DRGs revealed a decreased excitability of sensory neurons at ZT14 compared to ZT2. Overall, we suggest that *in vitro* experiments are not a good model for studying circadian rhythm in DRG sensory neurons. More importantly, our study uncovered a daily fluctuation in the excitability level of sensory neurons *ex vivo* in healthy mice.

2. Hannah Wood (Field: Neuroscience Research) **INVESTIGATING THE RELATIONSHIP BETWEEN LUMINAL MEDIATORS AND ABDOMINAL PAIN IN CROHN'S DISEASE PATIENTS.**

Hannah M. Wood, Prameet M. Sheth, Stephen J. Vanner, David E. Reed, Alan E. Lomax

Gastrointestinal Diseases Research Unit, Queen's University, Kingston, ON, Canada

Background: Abdominal pain is a debilitating symptom of Crohn's disease (CD) and commonly persists when inflammation is absent. This suggests that factors other than inflammatory mediators contribute to pain reported in the remission phase of CD, thus warranting further investigation. **Methods:** The effects of fecal supernatant (FS) from 5 healthy volunteers (HV) and 19 CD patients on pain-sensing neurons were assessed using *ex-vivo* colonic afferent nerve recordings in mice. **Results:** FS from CD patients with active disease increased action potential discharge from colonic afferent nerves by 60%, with FS from patients reporting high pain being 40% more excitatory than FS from patients reporting low pain. FS from CD patients in remission, regardless of reported pain level, did not excite colonic afferent nerves, which mirrored the effects of HV FS. A protease inhibitor cocktail and protease-activated receptor (PAR)-2 antagonist reduced, but did not abolish, neuronal excitation in response to CD FS. A 3-

fold increase in proteolytic activity was detected in CD FS samples compared to HV FS. **Conclusion:** These findings suggest that active disease leads to the generation of luminal mediators, including proteases acting on PAR-2, that excite visceral nociceptive neurons. These luminal mediators do not appear to be present and/or driving nociception when inflammation is in remission. Supported by CIHR.

3. Theodore Aliyianis (Field: Neuroscience) **AN INVESTIGATION OF THE VALIDITY OF COGNITIVE ASSESSMENT VIA ROBOTICS IN PEOPLE WITH EPILEPSY.**

Theodore S. Aliyianis, Spencer Finn, Brooke Beattie, Lysa Boissé Lomax, Garima Shukla, Ada Mullett, Stephen H. Scott, Gavin P. Winston.

Center for Neuroscience Studies, Queen's University, Kingston, Ontario, Canada

Rationale: Cognitive impairment from epilepsy is well-recognized. Neuropsychological assessment can detect cognitive impairments through clinical criteria and quantitative measures. In this study, we test the validity of robotic assessment to measure cognitive ability beyond motor function in people with epilepsy by comparing it to a brief neurocognitive assessment.

Methods: Participants with temporal lobe epilepsy (TLE, n=33) and genetic generalized epilepsy (GGE, n=25) underwent a brief neurocognitive assessment and robotic assessment. Five cognitive and sensorimotor tasks were added to the Repeatable Battery for the Assessment of Neuropsychology Status (RBANS) to form a brief neuropsychological assessment across a range of cognitive domains. The Kinarm Endpoint robot was used for robotic assessment with 9 standardized tasks assessing domains integrating motor, cognitive, and sensory function. Robotic assessment measurements were converted to composite task scores which are adjusted for age, sex, and handedness.

Results: In the cognitive domains of complex attention (3/6 tests, $p<0.05$), executive function (3/7 tests, $p<0.05$), memory (2/4 tests, $p<0.05$), visual-motor coordination (5/12 tests, $p<0.05$), and visuospatial skill (1/7 tests, $p<0.05$) moderate ($r \sim .30$) to strong ($r \sim .50$) associations exist between our brief neurocognitive assessments and robotic assessments.

Significance: These results demonstrate that robotic assessment can measure cognitive ability beyond sensorimotor function, similar to a brief neurocognitive screening for people with both general and focal epilepsies.

Funding: Queen's University Faculty of Health Sciences, Queen's University Translational Institute of Medicine (TIME), University Hospitals Kingston Foundation (UHKF), PSI Foundation

4. Courtney Bannerman (Field: Microbes, Immunity and Inflammation) **THE ROLE OF THE GUT MICROBIOME IN THE MIGRATION OF GAMMA DELTA T CELLS AFTER SPINAL CORD INJURY**

Courtney A Bannerman[1], Katya Douchant[1,2], Prameet M Sheth[1,2,3,4], Nader Ghasemlou[1,5,6]

1. Department of Biomedical and Molecular Sciences, Queen's University, Kingston, Ontario, Canada.
2. Gastrointestinal Disease Research Unit, Kingston Health Sciences Center, Kingston, Ontario, Canada.
3. Department of Pathology and Molecular Medicine, Queen's University, Kingston, Ontario, Canada.
4. Division of Microbiology, Kingston Health Sciences Centre, Kingston, Ontario, Canada.

5. Department of Anesthesiology and Perioperative Medicine, Kingston Health Sciences Centre, Kingston, Ontario, Canada.
6. Centre for Neuroscience Studies, Queen's University, Kingston, Ontario, Canada.

Spinal cord injuries (SCI) affect more than 10,000 Canadians annually, with 60-80% developing chronic pain. Aside from opioids, which have multiple side effects, few effective treatment options exist. Recent work has shown that the collection of bacteria in the gut, called the gut microbiome, may play a role in pain processing. This collection of bacteria usually plays an essential role in helping us properly digest food and the maturation of our immune system. Disruptions to the gut microbiome have been well documented in SCI in both patients and laboratory models. When disruptions of the gut microbiome are exasperated or reduced after SCI, pain outcomes increase or decrease. γδ T cells, a barrier immune cell found in large quantities in the gastrointestinal tract, are found in greater numbers in the spinal cord when greater gut dysbiosis is present. Mice who lack γδ T cells also show reduced hypersensitivity. Using the KikGR33 mouse line, it was determined that γδ T cells migrate from the small intestine to the site of injury acutely after injury. When there is a depletion of sensory or sympathetic neurons, this migration no longer takes place. This suggests that interrupting the migration of γδ T cells from the small intestine to the injured spinal cord could offer valuable treatment options.

5. Priya Premranjith (Field: Epidemiology) **MATERNAL EXPOSURE TO METALS AND TIME-TO-PREGNANCY: THE MIREC COHORT STUDY**.

Priya Premranjith[a], Will King[a], Jillian Ashley-Martin[b], Maryse Bouchard[c], Warren Foster[d], Tye E. Arbuckle[b], Maria P. Velez[a,e]. Department of Public Health Sciences, Queen's University, Kingston, Ontario, Canada.

[a]Department of Public Health Sciences, Queen's University, Kingston, Ontario, Canada

[b]Population Studies Division, Environmental Health Science and Research Bureau, Health Canada, Ottawa, Ontario, Canada

[c]Department of Environmental and Occupational Health, Université de Montréal, Montreal, Quebec, Canada

[d]Department of Obstetrics and Gynecology, McMaster University, Hamilton, Ontario, Canada

[e]Department of Obstetrics and Gynecology, Queen's University, Kingston, Ontario, Canada

Introduction: Metals such as arsenic (As), cadmium (Cd), Lead (Pb), manganese (Mn), and mercury (Hg) have potential endocrine disruptive properties. We evaluated the association between metals and TTP in participants from the Canadian MIREC Study.

Methods: Concentrations of metals were measured in maternal blood during the first trimester of pregnancy. Cox proportional hazards models modified for discrete-time data generated fecundability odds ratios (FORs) for the association between metal concentrations and TTP. FORs < 1 denote a longer TTP and FORs > 1 denote a shorter TTP. The odds of infertility were estimated using logistic regression. Models were adjusted for maternal age, pre-pregnancy BMI, education, income, clinical site, and serum lipids.

Results: A total of 1784 women were eligible for the study. Mean maternal age at interview was 32.2 (5.0) years. Most participants had detectable levels of all five metals in serum. Exposure to As, Cd, Mn,

and Hg was not associated with TTP. While increments of one standard deviation of Pb concentrations resulted in increased fecundability (aFOR 1.08, CI 95% 1.01-1.16), the association was not linear when exposure was modeled in tertiles. None of the metals were associated with infertility.

Conclusion: Serum concentrations of metals at current levels of exposure in Canada are not associated with TTP or infertility. Further studies are needed to assess the role of lead, if any, on TTP.

Funding: The MIREC Study was funded by Health Canada's Chemicals Management Plan, the Canadian Institute of Health Research (grant # MOP - 81285) and the Ontario Ministry of the Environment.

Session 3

1. Jacob Kment (Field: Biochemistry and Cell Biology) **COORDINATED BLOCKADE OF TGF-B AND PD-L1 BY BINTRAFUSP ALFA PROMOTES SURVIVAL IN PRECLINICAL OVARIAN CANCER MODELS BY PROMOTING T EFFECTOR MEMORY RESPONSES**

Jacob Kment,[1] Daniel Newsted,[1] Stephanie Young,[1] Yan Lan,[2] & Andrew W Craig[1]

[1]Department of Biomedical and Molecular Sciences, Queen's University; Cancer Biology & Genetics division, Queen's Cancer Research Institute, Kingston, ON, CANADA

[2]EMD Serono Research and Development Institute Inc., Billerica, MA 01821, USA

Introduction: Previous studies have linked high levels of the immunosuppressive cytokine Transforming Growth Factor- β (TGF-β) to poor prognosis in high grade serous ovarian cancer (HGSC). Here, we test whether coordinated blockade of TGF-β and PD-L1 with bintrafusp alfa (BA) promotes anti-tumour immune responses in preclinical HGSC models that enhances survival outcomes.

Methods: Syngeneic murine HGSC models were used: BR5-Luc (LOF Trp53/Brca1 & GOF Myc/Akt) engrafted (i.p.) in female FVB mice, ID8-Trp53^{KO}/Brca2^{KO}-Luc cells in female B cell-deficient µMT- (C57BL/6) mice. For the FVB model, we used anti-CD20 B cell-depletion to avoid neutralization of BA. Treatments were administered biweekly with control IgG or BA and effects on survival, tumor burden, ascites volume, cytokines, and tumor immune profiles were analyzed.

Results: BA treatments caused depletion of TGF-β, reduced tumour burden and ascites development in the ID8/µMT- model, but there were no long-term survivors. This correlated with reduced VEGF, more activated T cells in the ascites and skewing towards M1 tumour-associated macrophages. Extended BA treatments in the BR5/FVB model resulted in long-term survivors. Compared to naïve FVB mice, re-challenged survivors rejected BR5-Luc cells without additional treatments and had increased peritoneal memory T and NK cells.

Conclusions: These results show BA treatment leads to acquired anti-tumour immunity in some HGSC

models and will deliver important advances for immunotherapy regimes for ovarian cancer patients soon.
This research was funded by the Canadian Institute of Health Research. Bintrafusp alfa was provided by EMD Serono.

2. Jina Nanayakkara (Field: Transcription Factors in Neuroendocrine Tumours) **YAP AND TEAD FORM A TRANSCRIPTIONAL COMPLEX REGULATING DIFFERENTIATION AND TUMORIGENESIS IN NEUROENDOCRINE TUMORS**

Jina Nanayakkara[1], Xiaojing Yang[1], Markus Hafner[2], Xiaolong Yang[3], Neil Renwick[1]
[1]Laboratory of Translational RNA Biology, Department of Pathology and Molecular Medicine, Queen's University, 88 Stuart St, Kingston, ON, K7L 3N6, Canada
[2]Laboratory of Muscle Stem Cells and Gene Regulation, NIAMS, 50 South Drive, Bethesda, MD, 20892, USA
[3]Cancer Research Laboratory, Department of Pathology and Molecular Medicine, Queen's University, 88 Stuart St, Kingston, ON, K7L 3N6, Canada

Introduction: Neuroendocrine tumors (NETs) have neural and secretory morphology and lose expression of Yes-associated protein (YAP), a transcriptional co-activator of the Hippo pathway and canonical oncogene. Although YAP typically binds TEAD transcription factors to drive gene expression, we demonstrate that YAP-TEAD complex formation represses NET differentiation and tumorigenesis through gene dysregulation.

Methods: Using lung and pancreatic NET cells, we compared neuroendocrine markers, cell proliferation, and anchorage-independent cell growth after overexpressing constitutively active YAP (YAP-S127A) or mutant YAP (YAP-S127A/S94A) disrupting YAP-TEAD binding. Subsequently, we mapped YAP-TEAD DNA-binding sites using ChIP-sequencing and evaluated gene expression using RNA-sequencing.

Results: Neuroendocrine marker expression, cell proliferation and anchorage-independent cell growth diminished with active YAP, but recovered with mutant YAP. ChIP-seq revealed 34,924 YAP-TEAD DNA-binding sites, and RNA-seq uncovered 206 upregulated and 137 downregulated genes following active YAP overexpression. Interestingly, ChIP-seq clusters correspond with modular roles for YAP/TEAD activation or repression of gene expression, including targeting histone components (cluster 1), classical signalling pathways like TGFβ and Notch (cluster 2 and 3), neuroendocrine differentiation (cluster 4) and general cell functions (cluster 5 and 6). In cluster 4, YAP/TEAD binds to distal enhancers regulating master neuroendocrine transcription factors (ASCL1, INSM1, NEUROD1, NKX2-2) and represses gene expression.

Discussion: YAP-TEAD complex formation represses NET differentiation and tumorigenesis through several potential mechanisms including upregulation of TGFβ and Notch signalling and downregulation of master neuroendocrine transcription factors.

3. Andrea Petkovic (Field: Protein Structure and Function) **STRUCTURAL BASIS OF AMYLOID FORMATION BY *C. ALBICANS* ADHESINS**.

Andrea Petkovic, Byron Hunter, John Allingham. Queen's University. Kingston, Ontario, Canada.

Candida albicans colonizes its host by adhering to epithelial cells or medical devices and by forming biofilms. Adhesion of *C. albicans* to these biotic and abiotic surfaces is primarily dictated by the agglutinin-like sequence (Als) adhesins, a family of cell-wall-associated glycoproteins that contain a highly-conserved protein-binding cavity at their N-terminus, a central 36-amino-acid tandem repeat region, and a Ser/Thr/Asn-rich C-terminal domain that attaches the protein to the fungal cell wall via the remnant of a GPI anchor. Recent studies have shown that adhesion of *C. albicans* to surfaces is enabled by shear force-induced formation of linear aggregates, or amyloids, of Als adhesins. A conserved amyloid forming region (AFR) in a few Als adhesins have been implicated in this aggregation and have been suggested to be responsible for the robustness of *Candida* biofilms. It is hypothesized that when Als proteins are not bound to a host surface, their AFR is concealed by its flanking regions. However, we do not yet know what structural changes need to occur in Als proteins to expose the AFR and produce amyloid formation. Using experimental and in silico structural biology approaches along with biophysical analyses of purified wild type and mutant *C. albicans* Als proteins, we will identify residues flanking the AFR that regulate its accessibility for Als protein amyloidogenesis.

4. Lauren Brown (Field: Therapeutics, Drug Development, and Human Toxicology) **INVESTIGATING THE IMPACT OF VALPROIC ACID ON PLACENTAL STRUCTURAL DEVELOPMENT IN CD-1 MICE.**

Authors: Lauren Brown, Lihua Xue, and Louise Winn.

Department of Biomedical and Molecular Sciences. Queen's University.

Valproic acid (VPA) is a potent teratogen that causes fetal growth restriction (FGR). FGR is mainly associated with placental insufficiency, characterized as inadequate nutrient transport to the fetus. Recent evidence suggests that gestational VPA exposure alters placental structure, impacting its ability to deliver nutrients and consequently disrupting fetal growth and development. As such, this study aims to investigate VPA's impact on placental structural development by measuring the relative size of each placental layer. The mouse placenta comprises three layers: the decidua, the junctional zone, and the labyrinth zone. Each layer is critical for proper fetal growth and development, and their disruption can lead to FGR. Pregnant CD-1 mice will receive an injection of either saline, 400 mg/kg, or 600 mg/kg VPA on gestation day (GD) 9. On GD 18, the dams will be sacrificed, and the placental and fetal tissues will be excised. Fetus, fetus head, and placental weights will be recorded to assess growth restriction. The placental tissue will undergo histological analysis using alkaline phosphatase (AP) staining counterstained with nuclear fast red. This technique will stain phosphatase molecules associated with the placental vasculature (AP) and the cellular nuclei (nuclear fast red) allowing for clear definition of each layer for a size analysis. These results will indicate which placental layers are affected by VPA exposure and provide insight into how VPA disrupts placental structural development.

Poster Presentations

Morning Session

1. Madison Robertson (Field: Health Quality/Long-Term Care/Geriatrics) - **EXPLORING THE RELATIONSHIP BETWEEN SPOUSAL SEPARATION AND FEELINGS OF LONELINESS AND DEPRESSION ON RESIDENTS IN LONG-TERM CARE**

Problem - Loneliness and depression have a pernicious influence on older adults' overall health and wellbeing. Social relationships, specifically spousal relationships, have a significant impact on the mental health of older adults. However, there is limited research on the experience or effect of spousal separation on long-term care (LTC) residents' feelings of loneliness and depression.

Objective – The overall objective of this dissertation is to explore spouses' feelings of loneliness and depression resulting from involuntary separation due to an admission into an LTC facility.

Methods –The design of this dissertation involves two phases. First, a qualitative systematic review using the Joanna Briggs Institute methodology will be completed to situate this work within a comprehensive review of evidence. Second, a qualitative multi-case study with 10 spouse dyads will be conducted using a participatory action research approach.

Results – Four synthesized findings were included from eleven papers for the systematic review, including: Lack of physical and social connection is a common cause for loneliness and depression, lack of support during spousal separation results in loneliness and depression, spouses developed strategies to prevent loneliness and depression, and spouses had diverse presentations of loneliness and depression over time during. Findings from the second phase are preliminary.

Conclusions - Results from these studies will inform theoretical and practical LTC policies, and future knowledge translation effort for researchers, policy makers, and interested parties.

2. Jo-Chiao Wang (Field: Inflammation, Infection and Immunity) - **BASOPHILIC ONCOSTATIN M FUELS NOCICEPTOR NEURON-INDUCED ASTHMA**

Severe asthma affects 4-8% of patients but accounts for 60% of healthcare costs due to the lack of response to, and consequently high demand for, corticosteroid treatments in these patients. Recently published single-cell RNA sequencing predicts that airway-innervating jugular vagal C-fiber neurons expressing oncostatin M (OSM) receptor (OSMR) respond to bronchoconstrictors, histamine, serotonin, and leukotriene C4 and that *Osm* expression is enriched mouse lung basophils. Thus far, we have confirmed the enriched *Osm* expression in fluorescent-sorted lung basophil by qPCR. More interestingly, such basophilic *Osm* expression was found higher during ovalbumin-induced allergic airway inflammation. In cultured neurons dissociated from jugular-nodose complex, we found increased calcium influx in responses to capsaicin, a compound from chili pepper that activates the ion channel transient

receptor potential vanilloid 1 (TRPV1), and chloroquine, an Mas-related G-protein coupled receptor member A3 (Mrgpra3)-activating pruritogen, when treated with recombinant OSM, assessed by real-time calcium imaging, suggesting a sensitizing role of OSM in nociceptor and pruritogenic activations in vagal sensory neurons. Moreover, OSM-treated neurons express higher *Trpv1*, but not *Mrgpra3* mRNA, suggesting that the heightened response to chloroquine is due to the increased TRPV1 expression, but not Mrgpra3 per se. The sensitizing mechanism and pathophysiological roles of this OSM-C-fiber axis in asthmatic context remain to be unveiled.

Jo-Chiao Wang[1], Theo Crosson[1], and Sebastien Talbot[1,2]

1. Département de pharmacologie et physiologie, Université de Montréal, Montréal, Québec, Canada
2. Department of Biomedical and Molecular Sciences, Queen's University, Kingston, Ontario, Canada

Supporting agencies
1. Fonds de recherche du Québec – Santé (FRQS)
2. Canadian Institutes of Health Research (CIHR)

3. Aleisha Fernandes (Theme: Health Policy, Population Health, and Epidemiology) **CANADIAN LONGITUDINAL STUDY ON AGING 62-ITEMS (CLSA-FI) CAN PREDICT POOR STROKE OUTCOMES AT 3 MONTHS IN A PROSPECTIVE STROKE REGISTRY**

Authors: Aleisha Fernandes[1], Kelly Estrada[2], Kiran Reehal[3], Hilal Al Hasni[3], Al-Waleed Al-Battashi[2], Aarti Vyas[4], Ravinder Jeet Singh Sidhu[5], Benjamin Ritsma[6], Shirin Jalini[2], Ramana Appireddy[4], Zihang Lu[1], Olga Theou[7];

[1]Queen's University, Public Health Sciences, Kingston, ON, Canada,
[2]Queen's University, Neurology, Department of Medicine, Kingston, AB, Canada,
[3]Queen's University, Centre for Neuroscience Studies/Department of Medicine, Kingston, AB, Canada,
[4]Queens University, Department of Medicine–Division of Neurology, Kingston, ON, Canada,
[5]Northern Ontario School of Medicine University, Department of Medicine, Sudbury, AB, Canada,
[6]Providence Care Hospital, Physical Medicine & Rehabilitation, Kingston, ON, Canada,
[7]Dalhousie University, School of Physiotherapy, Halifax, Canada

Background: Stroke is a major contributor to the burden of disability and morbidity globally. With an aging demographic and improved life expectancy, the prevalence of frailty is increasing. We sought to determine if pre-stroke frailty affects stroke outcomes.

Methods: Participants with an imaging confirmed stroke were identified from a prospective stroke registry based at Kingston Health Sciences Center (KHSC). Frailty was measured using the Canadian Longitudinal Study on Aging 62-items Frailty Index (CLSA-FI) and post-stroke disability status at 3 months using the modified Rankin Scale(mRS). We examined the association of frailty with post-stroke disability using logistic regression analysis adjusted for age, sex, chronic kidney disease, COPD, dementia, heart disease, peripheral arterial disease and stroke.

Results: 459 patients were enrolled (mean(SD) age:73.3(14.3)years. 50.1% males). The prevalence of pre-stroke frailty (Frailty index≥0.2) was 177(38.6%) and post-stroke disability (mRS:3-6) was 245(53.4%). Individuals with pre-stroke frailty were more likely to experience poor post-stroke outcomes with an odds ratio (OR) of 9.8*(95% CI 6.1-15.7), p=<.0001)* and the association remained strong after adjustment for covariates (adjusted OR of 6.4*(95% CI 3.7-11.2, p = <.001*)).

Conclusion: The CLSA-FI can be computed from electronic medical records and is an objective frailty measure. Understanding the association between pre-stroke frailty and post-stroke outcomes can improve the triaging of resources, tailoring care to maximize outcomes in frail individuals and can improve the quality of stroke care and health outcomes.

4. Noah James (Field: Translational Medicine) **COST-EFFECTIVENESS OF THE HUMAN PAPILLOMAVIRUS AUTOMATED VISUAL EVALUATION (HPV-AVE/PAVE) SCREEN-TRIAGE-TREAT STRATEGY IN TANZANIA**.

Noah W. James, Nicole G. Campos, Karen E. Yeates Department of Medicine, Queen's University, Kingston, Ontario, Canada.

In 2020, the WHO issued a call to accelerate the elimination of cervical cancer through HPV screening and vaccination, as a result of an estimated 341 831 cervical cancer deaths worldwide. Cervical cancer is the leading cause of female cancer death in Tanzania. HPV infection is associated with over 95% of cervical cancer cases, making it an effective target for screening. The PAVE strategy in Tanzania incorporates self-sampling, HPV DNA detection and risk genotyping, triage for HPV+ women, and automated visual evaluation of the cervix for pre-cancer/cancer, to create a comprehensive screen-triage-treat algorithm using novel technology. The PAVE strategy will follow a stepwise implementation across the Kilimanjaro region prior to being scaled-up to a national program. A cost-effectiveness study will estimate the value (i.e. dollars per year of life saved) of the PAVE strategy components (including health human resources, equipment, and timing) across initiation sites. Importantly, the results of this study will inform the national program expansion and provide detailed cost estimates stratified by site characteristics. The purpose of this project is to evaluate the cost-effectiveness of the PAVE strategy to create a toolkit and inform the scale-up of this strategy to improve the delivery of cervical cancer prevention services in Tanzania. It is hypothesized that the PAVE strategy will lead to a decreased cost per woman screened, enhanced patient autonomy, and reduced cervical cancer burden. (Grand Challenges Canada, Grand Challenges Africa and the National Cancer Institute (U.S))

5. Emma Kalin (Field: Inflammation, Infection and Immunity) **CHARACTERIZING ANTIVIRAL GENE INDUCTION BY IL-27 AND INFLUENZA A VIRUS INFECTION IN POLARIZED HUMAN MACROPHAGES**

Emma Kalin[1] and Katrina Gee[1]
[1]Department of Biomedical and Molecular Sciences, Queen's University, Kingston, ON, Canada.

Influenza A virus (IAV) is a global health threat and gaining further understanding of the molecular mechanisms of the human immune response to this virus is critical. Toll-like receptors (TLRs) likely play a role in switching from a beneficial inflammatory response to one that is detrimental. Of the TLRs activated by IAV infection, TLR7 signaling ultimately results in IL-27 production, a cytokine that has been

attributed to antiviral functions. Macrophages (MΦs) are sentinel immune cells targeted by IAV and are a producer of IL-27. Additionally, IL-27 regulates interferon-stimulated genes (ISGs) and type I interferon (IFN-I) expression. *TLR7*, which also regulates IFN-I expression, is located on the X chromosome and escapes X chromosome inactivation resulting in sex-based IFN-I variation. We hypothesize that IL-27-mediated upregulation of ISG expression influences MΦ responses to pandemic IAV infection, in a sex-dependent manner. Our preliminary data shows increased basal expression of IL-27 subunits in M1 MΦs compared to M0 and M2 cells and in response to IAV exposure, M1 cells secrete IL-27, but are not productively infected. When stimulated with IL-27, M0 and M2 cells express high levels of *CIITA,* a M1 marker, indicating IL-27 may promote a shift towards an M1 phenotype; suggesting IAV infection and IL-27-stimulation induce an M1-like phenotype in the M0 and M2 MΦs, indicating that IL-27 may play an important role in the MΦ antiviral response.

6. Brooke Beattie (Field: Neuroscience) **A PROTOCOL FOR MULTIMODAL PREDICTION OF SEIZURE RECURRENCE AFTER UNPROVOKED FIRST SEIZURE TO GUIDE CLINICAL DECISION-MAKING.**

Brooke C Beattie, Jason Gallivan, Donald Brien, Lysa Boisse Lomax, Garima Shukla, Ada Mullett, Kristin Ikeda, Matthias Schmidt, Steven Beyea, Krista Biggs, Christopher Bowen, Antonina Omisade*, and Gavin P Winston* (*joint senior authors). Centre for Neuroscience Studies, Queen's University, Kingston, Ontario, Canada (in collaboration with Nova Scotia Health Authority and Dalhousie University)

Rationale: Epilepsy is a common neurological disorder characterised by recurrent seizures. Almost half of patients that have an unprovoked first seizure (UFS) have additional seizures and develop epilepsy. No current predictive models exist to determine who has a higher risk of recurrence to guide treatment. Emerging evidence suggests alterations in cognition, mood, and brain connectivity exist with the UFS. Examining the baseline brain changes present following an UFS will enable the development of the first multimodal biomarker-based predictive model of seizure recurrence in adults with UFS.

Methods: 200 patients and 75 matched healthy controls (aged 18-65) from the Halifax and Kingston First Seizure Clinics will undergo neuropsychological assessments, structural and functional magnetic resonance imaging, and electroencephalography. Seizure recurrence will be assessed prospectively. Regular follow-ups will occur to monitor recurrence. Comparisons will be made between UFS and healthy control groups and those with and without seizure recurrence after UFS. A multimodal machine learning model will be trained to predict seizure recurrence at 12 months.

Significance: Initiation of anti-seizure medications (ASM) after UFS in people at risk for further seizures can help prevent seizure recurrence, whilst unnecessary treatment with ASM in people at low risk exposes patients to harmful side-effects. Early prognostic biomarkers will inform the first multimodal predictive model of seizure recurrence following UFS to significantly alter and optimize clinical decision-making about treatment in this population.

(Supported by the Canadian Institutes of Health Research.)

7. Aidan Bennett (Field: Experimental Medicine) **SEX DIFFERENCES IN THE EFFECT OF THE LUMINAL MEDIATORS FROM IRRITABLE BOWEL SYNDROME PATIENTS ON ABDOMINAL PAIN**

A.S.W. Bennett, C.C. Baker, T.A. Alward, S.J. Vanner, D.E. Reed, and A.E. Lomax. Department of Biomedical and Molecular Sciences, Queen's University, Kingston, Ontario.

Irritable bowel syndrome (IBS) is a chronic abdominal pain disorder that affects women twice as often as men. Luminal mediators of host and bacterial origin have been implicated in modulating abdominal pain in IBS patients. We hypothesized that the effects of pro-nociceptive mediators within the gut lumen are increased in females, contributing to the female predominance of IBS. Fecal supernatant (FS) from male and female IBS patients were perfused through murine colonic preparations while performing extracellular colonic afferent nerve recordings to measure changes in action potential frequency. Vaginal swabs determined the phase of female mice estrous cycle. FS from female IBS patients increased afferent nerve discharge, whereas FS from male IBS patients had no effect. Analysis from female IBS patients divided into abdominal pain score groups revealed that high abdominal pain IBS patients FS, but not moderate or low pain IBS patients, increased visceral afferent nerve discharge by 70%; this excitatory effect was abolished in ovariectomized mice. Analysis of nociceptive axons revealed that male IBS FS, previously shown not to affect nociceptor activity in male mice, increased nociceptor activity in proestrus/estrus female mice. This work suggests that the effects of luminal mediators that impact abdominal pain are increased in female IBS patients compared to males, and females appear to be more sensitive to their pro-nociceptive effects.
(Supported by CIHR.)

8. Timothy Walker (Field: Cancer Cell Biology) **LOSS OF TUMOUR SUPPRESSOR TMEM127 DRIVES CONSTITUTIVE RET-MEDIATED ACTIVITY THROUGH DISRUPTED MEMBRANE DYNAMICS.**

Timothy J. Walker, Eduardo Reyes-Alvarez, Brandy D. Hyndman, Michael G. Sugiyama, Larissa C.B. Oliveira, Douglas S. Richardson, Costin N. Antonescu, and Lois M. Mulligan. Department of Pathology and Molecular Medicine, Queen's University, Kingston, Ontario, Canada

Internalization from the cell membrane and endosomal trafficking of receptor tyrosine kinases (RTK) are important regulators of signaling in normal cells that can frequently be disrupted in cancer. The adrenal tumour pheochromocytoma (PCC) can be caused by activating mutations of the RET receptor tyrosine kinase, or inactivation of TMEM127, a transmembrane tumour suppressor implicated in trafficking of endosomal cargos. However, the role of aberrant receptor trafficking in PCC is not well understood. Here, we show that loss of TMEM127 causes wildtype RET protein accumulation on the cell surface, where increased receptor density facilitates constitutive ligand-independent activity and downstream signaling, driving cell proliferation. Loss of TMEM127 altered normal cell membrane organization and recruitment and stabilization of membrane protein complexes, impaired assembly, and maturation of clathrin coated pits, and reduced internalization and degradation of cell surface RET. In addition to RTKs, TMEM127 depletion also promoted surface accumulation of several other transmembrane proteins, suggesting it may cause global defects in surface protein activity and function. Together, our data identify TMEM127 as an important determinant of membrane organization, including membrane protein diffusability and protein complex assembly, and provide a novel paradigm for oncogenesis in PCC where altered membrane dynamics promotes cell surface accumulation and constitutive activity of growth factor receptors to drive aberrant signaling and promote transformation.

(Supported by the Cancer Research Society of Canada and Canadian Institutes of Health Research)

9. Marco Buttigieg (Field: Pathology and Molecular Medicine) **MOLECULAR SUBTYPING OF CLONAL HEMATOPOIESIS IN SOLID CANCERS REVEALS A NOVEL PATTERN OF THERAPEUTIC SELECTION.**

Marco M. Buttigieg, Caitlyn Vlasschaert, and Michael J. Rauh. Department of Pathology and Molecular Medicine, Queen's University, Kingston, Ontario, Canada.

Background: Clonal hematopoiesis (CH) describes the pre-malignant clonal expansion of hematopoietic stem cells with somatic mutations in drivers of myeloid (M-CH) or lymphoid (L-CH) malignancies. The role of CH-derived inflammation in tumourigenesis remains unclear. M-CH is common in cancer patients and generally associated with poorer survival, though cancer-specific cohorts show varying findings and L-CH has never been evaluated. Here, we surveyed the distributions of M-CH and L-CH in solid cancer patients to determine how CH is influencing tumourigenesis.

Methodology: Blood genomic sequences were obtained from 896 cancer patients from the International Cancer Genome Consortium. The GATK-Mutect2 algorithm was used to detect somatic mutations in CH-associated genes.

Results: CH prevalence was 23.0%, evenly split between M-CH and L-CH and significantly higher than expected from a healthy cohort. CH prevalence varied greatly between cancer types, with thyroid cancers seeing the highest relative rates of L-CH. L-CH was unexpectedly driven by mutations in *KMT2C*, an epigenetic regulator associated with the DNA damage response and therapy-related hematologic neoplasms.

Conclusions: M-CH and L-CH occur frequently in cancer patients and variations in prevalence may be dictated by tumour characteristics and therapeutic pressures. The strong presence of *KMT2C* mutations is likely indicative of exposure to cancer therapy. Future work involving transcriptomic and histological analysis using The Cancer Genome Atlas will be conducted to determine the implications of these mutations for cancer patients.

10. Andrea Ellsay (Field: Neuroscience - Epilepsy) **CLINICAL UTILITY OF ADVANCED NEUROIMAGING TECHNIQUES IN PREOPERATIVE WORKUP OF EPILEPSY**

Andrea Ellsay, Madeline Hopkins, Dr. Lysa Boissé Lomax, Dr. Garima Shukla, Donald Brien, Ada Mullett, Dr. Ron Levy, Dr. Gavin P. Winston

Center for Neuroscience Studies, Queen's University, Kingston, Ontario, Canada

Approximately 30% of people with focal epilepsy are medically refractory; for these patients, neurosurgery may be a viable treatment option. However, identifying suitable candidates and localizing the epileptogenic zone remain significant challenges. We evaluate the clinical utility of three advanced neuroimaging techniques developed at established epilepsy surgery centers in the pre-surgical assessment at the recently designated District Epilepsy Center, Kingston Health Sciences Center.

Patients in the pre-surgical pathway were discussed in multidisciplinary team (MDT) meetings (n=11). Patients who had already undergone resections(n=3) and those deemed unsuitable for surgery(n=4) were excluded. Patients meeting the criteria for surgical candidacy with inconclusive clinical data, such as a negative MRI or discordant data(n=4), were recruited for a comprehensive MRI evaluation. This

evaluation included high-resolution 3D T1-weighted scans for hippocampal/amygdala quantitative volumetry, high-resolution 3D FLAIR to facilitate lesion detection, and functional MRI for language lateralization. The benefits of this additional data were documented in a follow-up questionnaire during the reevaluation of the patients.

Following our multimodal assessment, previously discussed surgery candidates were deemed suitable or could proceed to next assessment steps. The protocol has improved localization of epileptic tissue, identified imaging abnormalities, and lateralized language (Figure 2), as acknowledged by MDT clinicians.

Our findings demonstrate that these advanced neuroimaging modalities can be introduced into clinical practice at a newly established epilepsy surgery center and provide valuable information benefiting patient care.

Patient	Clinical diagnosis	Imaging	Outcome	Overall
1	Left mesial temporal lobe epilepsy	Language fMRI	Demonstrated left language dominance (right-handed)	Patient suitable for left hippocampal and anterior temporal lobe resection, surgery will be done awake with language mapping given resection on ipsilateral side of language dominance
2	Right temporal lobe epilepsy	Language fMRI	Demonstrated left language dominance (right-handed)	Patient suitable for right temporal lobe resection, language fMRI confirms the low risk of post-operative language impairment
3	Suspected right mesial temporal lobe epilepsy	Language fMRI	Demonstrated right language dominance (left-handed) consistent with seizure semiology (post-ictal aphasia)	Patient previously not suitable for surgery with available data now considered suitable for right temporal lobe resection if a repeat EMU recording can confirm seizure onset
		Volumetry	Demonstrated right hippocampal atrophy (not previously noted)	

		High-resolution FLAIR	Demonstrated right hippocampal sclerosis (not seen on prior clinical imaging)	
4	Suspected left mesial temporal lobe epilepsy	Language fMRI	Demonstrated left language dominance (right-handed), consistent with seizure semiology (post-ictal aphasia)	Patient previously not suitable for surgery, with available data now considered suitable to proceed to SEEG with the hypothesis of left amygdala (or hippocampal) onset
		Volumetry	Symmetrical, no additional information	
		High-resolution FLAIR	Demonstrated left amygdala blurring and increased signal intensity (not seen on prior clinical imaging)	

11. Flourish Adebayo (Field: Computer Science) **SINGLE-CELL TRANSCRIPTOME ANALYSIS OF HUMAN SKIN IDENTIFIES NOVEL FIBROBLAST SUBPOPULATION AND ENRICHMENT OF IMMUNE SUBSETS IN ATOPIC DERMATITIS.**

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

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

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

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

Atopic dermatitis (AD) is a common and chronic inflammatory skin condition that has a significant impact on individuals' quality of life and healthcare costs. Understanding of AD has relied on analyzing gene expression patterns in bulk tissue samples using techniques like RNA sequencing and microarrays. This approach has limitations when it comes to studying the cellular diversity within AD. With the advent of single-cell RNA sequencing, we can explore the complexities of tissues at the individual cell level. In this study, I worked with the dataset from Helen et al., 2020 (Single-cell transcriptome analysis of human skin identifies novel fibroblast subpopulation and enrichment of immune subsets in atopic dermatitis), which focused on the single-cell transcriptomics of human skin, specifically examining the fibroblast and keratinocyte subpopulations in AD. My goal was to unravel the heterogeneity of AD, cellular subpopulations, and their unique characteristics within AD. Through careful data preprocessing,

clustering, and classification of the transcriptomic data, I validated the findings reported by the authors but also discovered additional genes that may be relevant in the context of AD. These genes, including AIBG, AIBG AS1, A2M, A2M AS1, A2ML1, A2MP1, A4GALT, AAAS, AACS, and AACSP, could potentially play crucial roles in the development and progression of AD. Statistical tests show significant associations and correlations between different cell types and gene expression patterns, providing valuable insights into the heterogeneity of AD. My next steps involve further exploration of the dataset, focusing on other subgroups of cells to gain a deeper understanding of the molecular heterogeneity of AD.

Keywords: atopic dermatitis, single-cell RNA sequencing, cellular heterogeneity, single-cell transcriptome analysis

12. Yuandong Xing (Field: Prostate Cancer) **COMPARISON OF GENE SET ENRICHMENT BETWEEN PROSTATE TISSUE AND URINE**

Yuandong Xing[1,2], Hamid Ghaedi[1,2], Anna Y. Lee[3], Dan Dion[3], Vanessa F. Bratti[1,2], D. Robert Siemens[2,4,5], Palak G. Patel[1,2], Ania A. Namin[2,6], Robert J. Gooding[1,7], Jane Bayani[3,8], David M. Berman[1,2,4*]

1 Department of Pathology and Molecular Medicine, Queen's University, Kingston, Ontario, Canada

2 Queen's Cancer Research Institute, Queen's University, Kingston, Ontario, Canada

3 Ontario Institute for Cancer Research, Toronto, Ontario, Canada

4 Kingston General Hospital Research Institute, Kingston, Ontario, Canada

5 Departments of Urology, Oncology and Biomedical and Molecular Sciences, Queen's University, Kingston, Ontario, Canada

6 Currently in Canadian College of Naturopathic Medicine, Toronto, Ontario, Canada

7 Department of Physics, Engineering Physics, and Astronomy, Queen's University, Kingston, Ontario, Canada

8 Department of Laboratory Medicine and Pathology, University of Toronto, Toronto, Ontario, Canada

* Corresponding author. Email address: bermand@queensu.ca

Background

Urine prostate cancer biomarkers are typically translated from tissue genes. As a prerequisite, genes must be enriched enough in urine for detection. Therefore, comparison of gene enrichment in prostate tissue vs urine may help in selecting genes highly enriched in urine as urine biomarker candidates.

Methods

Gene expression was profiled on 9 prostate tissue samples and 9 urine samples. We proposed a Tissue-Urine Comparability Index (*TUCI*) to evaluate different data processing methods in terms of rendering tissue and urine profile comparable. The method achieving the highest *TUCI* (*i.e.*, performing best) was employed to process the data. Next, enrichment of pre-defined gene sets was analyzed by Gene Set Enrichment Analysis and Gene Set Variation Analysis.

Results

Removing batch effect followed by normalizing by *GNAS* and *TAF6L* genes achieved the highest *TUCI*. After processing, distance between matched tissue and urine samples were much less than in the

original data. Both prostate epithelial gene set and stromal gene set were significantly enriched in tissue compared to urine, while stromal gene set had larger difference of enrichment scores between tissue and urine. Moreover, compared to tissue-enriched gene sets, urine-enriched ones comprised more immunity-related gene sets, less miRNA-targeted gene sets, and less gene sets involved in Biological Process in Gene Ontology.

Conclusion

Prostate tissue-enriched gene sets were different from urine-enriched ones. The differences were significant biologically.

13. Adriana Farcas (Field: Clinical Neuroscience) **IMPACT OF COGNITIVE BEHAVIORAL THERAPY FOR PSYCHOSIS ON QUALITY OF LIFE IN INDIVIDUALS WITH SCHIZOPHRENIA**

Adriana Farcas and Dr. Felicia Iftene, Centre for Neuroscience Studies, Queen's University

Background: With its complex etiology and presenting symptoms, schizophrenia continues to challenge clinicians and researchers alike in finding the most effective approach to alleviating symptoms and enhancing the quality of life of those affected by it. Here we set to explore the impact Cognitive Behavioral Therapy for psychosis(CBTp) has on the quality of life of individuals with schizophrenia as reported in recent randomized controlled trials.
Methods: We performed a systematic search on four databases: Web of Science, Pubmed, Embase, and Google Scholar. The Boolean operator "AND" was used between each of the keywords and a 10-year limit was applied. Criteria set for inclusion: CBT intervention, the population represented by individuals with schizophrenia, the study had to be a randomized controlled trial (RCT).
Results: The search yielded 244 articles of which 7 studies met the criteria for this scoping review. Improvement in quality of life as a result of a CBTp intervention was found in four of these studies, no improvement was found in two, while one of the studies did not discuss their quality of life assessment results.
Conclusions: A small number of RCTs (7) reported mixed findings. Although quality of life is known to be considerably affected in individuals with schizophrenia, studies assessing the effectiveness of CBT interventions tend to focus on clinical symptoms and cognitive deficits.

14. Tashifa Imtiaz (Field: Pathology and Molecular Medicine) **USER-DRIVEN MACHINE LEARNING APPROACH FOR RNA-BASED SAMPLE DISCRIMINATION AND CLASSIFICATION**

Tashifa Imtiaz BSc[1], Alexis Fang[1], Jina Nanayakkara BSc[1], Neil Renwick MD PhD[1], Kathrin Tyryshkin PhD[1]

[1]Laboratory of Translational RNA Biology, Department of Pathology and Molecular Medicine, 88 Stuart St, Queen's University, Kingston, ON K7L 3N6, Canada

RNA-sequencing data provides valuable insights into biological processes underlying health and disease. Machine learning-based classification can identify patterns in high-throughput, multidimensional omics data to generate predictive models. These models can be used for diverse applications including disease diagnosis, prognosis, and/or discovery of molecular targets for investigation[1-4]. However, to create

accurate and generalizable classification models, we must consider multiple factors such as the quality of data, the classification scheme, quality of the features used, and the training and validation process.

We propose a protocol for hierarchical classification of biological samples using RNA expression data. This protocol outlines data preprocessing, data exploration, feature selection, and building a hierarchical classification model. Through open-source code and a user-friendly ensemble feature selection app, we encourage users to explore their data and interpret results from a biological perspective. Our hierarchical method also reflects the inherent hierarchies of biological systems, and provides increased accuracy and interpretability compared to other classification methods. Additionally, the protocol is modular and can be easily adapted for different omics data in diverse applications.

As an example, we applied this protocol to design a classifier for gastroenteropancreatic neuroendocrine tumors using microRNA sequencing profiles, with a validation accuracy of 96.9%. Our versatile protocol will support scientists in identifying important markers from biomedical data, creating prediction models with clinical relevance, and guide hypothesis generation for further experimental work.

References:

1. Nikiforova, M.N., et al., *Analytical performance of the ThyroSeq v3 genomic classifier for cancer diagnosis in thyroid nodules.* Cancer, 2018. **124**(8): p. 1682-1690.
2. Lopez, R., R. Wang, and G. Seelig, *A molecular multi-gene classifier for disease diagnostics.* Nature Chemistry, 2018. **10**(7): p. 746-754.
3. Lindahl, L.M., et al., *Prognostic miRNA classifier in early-stage mycosis fungoides: development and validation in a Danish nationwide study.* Blood, 2018. **131**(7): p. 759-770.
4. Xu, J. and Y. Li, *Discovering disease-genes by topological features in human protein–protein interaction network.* Bioinformatics, 2006. **22**(22): p. 2800-2805.

15. Julia Barilo (Field: Microbes, Immunity and Inflammation) **CHARACTERIZING THE PHENOTYPIC DIFFERENCES OF IL-4 TREATED SPLEEN AND BONE MARROW-DERIVED MACROPHAGES**

Julia Barilo[1] and Sam Basta[1]

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

Macrophages (Mϕ) are a heterogeneous population of immune cells responsible for recognizing and responding to foreign pathogens, making them necessary for homeostasis. Mϕ can be polarized into an anti-inflammatory state classified as M2 which, when stimulated with IL-4, promotes an M2a phenotype involved in cell growth and tissue repair. Two common sources of Mϕ used in research are spleen-derived macrophages (SpM) and bone marrow-derived macrophages (BMDM). While the characteristics of each type of Mϕ have been studied separately, comparison of phenotypic differences between the two populations after polarization needs better characterization, especially when comparing the phenotypic differences in the two populations following prolonged IL-4 stimulation. This study aims to characterize the phenotypic differences of IL-4 treated SpM and BMDM through functional assays, gene expression profiles, and flow cytometry staining of surface and intracellular phenotypic markers. The data from this

study will contribute to better characterization of diverse macrophage population involvement in innate immunity.

(Research is supported by NSERC funding)

16. Megan MacRae (Field: Reproduction and Developmental Studies) **THE SCA-1/HYPOXIA RELATIONSHIP IN MOUSE TROPHOBLAST STEM (MTS) CELLS**

MacRae M[1], Natale BV[2], Natale DRC[1,2]

Departments of [1]Biomedical and Molecular Sciences and [2]Obstetrics and Gynaecology, Queen's University, Kingston, ON, Canada

Mouse (m), cell surface marker, stem cell antigen-1 (Sca-1) is expressed in many stem cell populations, including the trophoblast stem (TS) cells that give rise to the placenta.[1] This population and HIF-1α (hypoxia marker) increase concurrently in a mouse preeclampsia model.[2] However, their differentiation potential in a hypoxic environment is unknown. As such, using different oxygen concentrations (20% or 1% O_2), I wanted to assess the difference between mTS cells with high (Sca-1^{HI}) and no Sca-1 expression (Sca-1^{NEG}) by 1) using transcriptomic analysis to see how their transcriptomes differed and 2) using differentiation analysis to determine their lineage potential. Finally, I wanted to assess mTS differentiation in a physiologically relevant oxygen concentration (8%). Following this rationale, I hypothesized that Sca-1^{HI} and Sca-1^{NEG} mTS cells would have different gene expression, differentiation potential and response to oxygen concentration. I found that neither Sca-1^{HI} nor Sca-1^{NEG} populations were lineage-restricted. However, in 1% O_2 *Aldh1a3* (glycogen trophoblast marker) was differentially expressed. Similarly, 20%, 8%, and 1% O_2 did not lineage-restrict mTS cells; however, levels of expression of trophoblast markers were altered. The results suggest that Sca-1^{HI} mTS cells respond uniquely to 1% O_2, reinforcing a potential functional difference in their contribution to the placenta.

17. Kieran Pace (Field: Sex Differences in Bladder Cancer) **SEX DIFFERENCES IN B-CELL EXHAUSTION STATES DURING BLADDER CANCER PROGRESSION**.

Keiran Pace, Priyanka Yolmo, Kartik Sachdeva, Sadaf Rahimi, Keshav Sharma, Gwenaelle Conseil, Madhuri Koti. Department of Biomedical and Molecular Sciences, Queen's University, Kingston, Ontario, Canada.

Age and sex are two prominent risk factors of non-muscle invasive bladder cancer (NMIBC). While over 80% of cases are diagnosed in aging adults and the disease is three times more incident in men than women, aging women experience poorer disease outcomes. Literature has associated high-grade female tumours with increased B-cell recruitment to the bladder mucosa where they can form ectopic lymphoid follicles known as tertiary lymphoid structures (TLSs) at sites of chronic inflammation. Furthermore, both chronic inflammation and biological aging may lead to the systemic expansion of an exhausted B-cell subset known as the atypical B-cell (ABC) that dampens antitumor immunity. However, a gap in knowledge remains with respect to whether ABC expansion occurs in a sex-dependant manner with

NMIBC progression. To test this, young and aging murine mice were exposed to the carcinogen N-butyl-N-(4-hydroxybutyl) nitrosamine (BBN). Systemic and local immune changes were assessed using hematoxylin & eosin staining and flow cytometry at baseline and post-exposure to BBN. The results indicated that female mice had a higher systemic ABC profile before and after exposure to BBN and that healthy female mice exhibited more inflammation in the bladder mucosa. The findings of this study highlight the importance of researching sex and age immune differences in bladder cancer and may inform future work on targeted therapies along the ABC axis in humans.

This study was supported by funding from Bladder Cancer Canada and the Cancer Research Society.

18. Yun Jiang (Field: Biochemistry and Cell Biology) **CANCER CELLS TREATED WITH ACTIN TOXIN MYCALOLIDE B ACQUIRE DNA DAMAGE AND ABORTED CELL DIVISION LEADING TO ANEUPLOIDY IN A P53-DEPENDENT MANNER**

Yun Jiang, Daria Trofimova, Andrew Evans, John Allingham, and Andrew Craig

Department of Biomedical and Molecular Sciences, Queen's University; Department of Chemistry, Queen's University; Cancer Biology & Genetics Division, Queen's Cancer Research Institute

Mycalolide B (Myc B) is a marine natural product that potently disrupts the actin cytoskeleton, leading to loss of cell shape and motility. These effects of MycB, which are also mirrored in simplified synthetic MycB analogs, have recently been recognized as a potentially useful means to block cancer metastasis. However, the effects of actin disruption by Myc B and the analogs on cancer cell division has not been studied. In our studies of A549 lung cancer cells treated with Myc B or our lead analog, we observed the formation of binucleated cells. Further investigation into the cell division process revealed that both Myc B and lead analog treatments caused dose-dependent cytokinesis defects leading to asymmetric cell division and aneuploid cells with DNA damage foci. These aneuploid cells failed to form an actomyosin ring during mitosis due to actin disruption. However, other mitotic processes, such as septin ring formation, remained unaffected. Interestingly, the accumulation of aneuploidy cells and cell death was dependent on the mutation status or levels of the p53. Stabilization of p53 in A549 cells treated with Nutlin-3 led to increased killing by MycB and our lead analog. Together, our findings show that disruption of actin dynamics in cancer cells can trigger failed cell division in cancer cells, but that overt killing may require an intact p53 tumor suppressor pathway.

19. Kartik Sachdeva (Field: Bladder Cancer, T Cells, Immunology) **INVESTIGATING CIRCULATING T FOLLICULAR HELPER CELL PROFILES IN NON-MUSCLE INVASIVE BLADDER CANCER**

Kartik Sachdeva[1], Priyanka Yolmo[1], Sadaf Rahimi[1], Gwenaelle Conseil[1], Nick Vanin[2], D. Robert Siemens[2] and Madhuri Koti[1]

[1]Queen's Cancer Research Institute, [2]Department of Urology, Queen's University, Kingston, ON, Canada

Background: Our recent study found a strong association between high density of intra-tumoral B cells and poor clinical outcomes in patients with non-muscle invasive bladder cancer (NMIBC). Specifically, we identified a subset of B cells called 'atypical B cells (ABCs)' located within tumor associated tertiary lymphoid structures. Given the critical role of T follicular helper (TFH) cells in the regulation of B cell

responses at mucosal surfaces, we hypothesized that the circulating profiles of cTFH cells could inform B cell exhaustion states associated with tumor stage and response to treatment.

Methods: Bulk-RNAseq based tumor transcriptome profiles from pre-treatment NMIBC tumors from a of 535 patients (UROMOL) were analyzed to determine the association between TFH associated transcript abundance and clinical outcomes. Profiles of cTFH and ABCs were determined in the peripheral blood of patients with NMIBC, undergoing transurethral bladder tumor resection (TURBT) at the Kingston Health Sciences Center, using multi-parametric flow cytometry. The N-butyl-N-(4-hydroxybutyl) nitrosamine (BBN) carcinogen induced murine model of bladder cancer was employed to investigate the role of TFH cells in disease progression.

Results: Preliminary results showed an inverse correlation between cTFH and circulating ABCs in patients undergoing TURBT (n=26). A similar trend was observed in the BBN carcinogen exposed mice.

Conclusion and significance: Preliminary results from this study demonstrate a possible link between cTFH and ABCs in NMIBC disease progression. Further investigations are warranted to establish the precise role of cTFH in bladder tumor progression and treatment response. Findings from this study will allow development of immune monitoring biomarkers and identification therapeutic targets within the cTFH pathways.

Research funded by Bladder Cancer Canada and the Cancer Research Society

20. Emma LeBlanc (Field: Microbes, Immunity and Inflammation) **INVESTIGATING THE ROLES OF SARS-COV-2 SPIKE GLYCOSYLATION IN TLR4-INDUCED CYTOKINE STORM**

Emmanuelle V. LeBlanc[1] and Che C. Colpitts[1]

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

The COVID-19 pandemic underscores the threat posed by respiratory viruses. The high pathogenicity of some respiratory viruses is attributed to a detrimental hyperinflammatory response, known as cytokine storm. Toll-like receptor 4 (TLR4) is an immune protein critical for the induction of a cytokine storm during severe respiratory infections, including COVID-19. TLR4 can be activated by viral glycoproteins, such as SARS-CoV-2 spike. 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, and that this response is abrogated when TLR4 is knocked out by CRISPR/Cas9. To investigate the role of spike glycans, we produced spike pseudoparticles in cells deficient in MGAT1, an enzyme required for the elaboration of complex N-glycans. Interestingly, we observed 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 to investigate which glycosylation sites may contribute to TLR4 activation. We are expanding this work to other respiratory viruses to further our understanding of the role of TLR4 in the pathogenesis of severe respiratory virus infections.

21. Avery McGinnis (Field: Reproduction and Developmental Studies) **INVESTIGATING THE DIFFERENTIATION POTENTIAL OF EOMES^{POS} MOUSE TROPHOBLAST CELLS**.

Avery McGinnis[1], Megan Cull[1], Nichole Peterson[2], David Natale[1,2], Departments of Biomedical and Molecular Sciences[1], Obstetrics and Gynaecology[2], Queen's University, Kingston, ON, Canada

Mouse trophoblast stem (TS) cells can be derived from the blastocyst or trophoblasts of the extraembryonic ectoderm (ExE), until embryonic day (E) 6.5. Eomesodermin (Eomes) is a transcription factor that identifies TS cells. During early development, *Eomes* is restricted to the ExE and by E7.5, to the chorion after which its expression declines. The junctional zone and labyrinth layers of the placenta are thought to develop from layer-specific progenitor cells of the ectoplacental cone and chorion, respectively. Eomes-expressing TS cells *in vitro,* differentiate to all trophoblast sub-types of the placenta; however, our objective was to assess their development *in vivo*. Lineage tracing was used to evaluate the *in vivo* differentiation of Eomes^{POS} trophoblast, using a tamoxifen-inducible, Eomes-Cre mouse crossed with an Ai6 reporter mouse. Cre was activated at E7.5-9.5, permanently marking all placental Eomes^{POS} trophoblast and daughter cells. This was combined with immunostaining to assess differentiation in healthy E17.5 placentae. Daughters of Eomes^{POS} trophoblast contributed to both placental layers. Specifically, Eomes^{POS} cells gave rise to glycogen trophoblast and spongiotrophoblast within the junctional zone, and sinusoidal trophoblast giant cells and layer II syncytiotrophoblast within the labyrinth but not Epcam^{POS} labyrinth progenitors. Eomes^{POS} trophoblast have the capacity to contribute to both placental layers *in vivo* after E6.5. Future studies will use lineage tracing this to assess Eomes^{POS} trophoblast throughout gestation and their role in placental pathology.

This work is supported by a CIHR MSc Graduate Scholarship (CGS-M; AM) and NIH/NICHD Operating Grant (DRCN)

22. Amanda Zacharias (Field: Experimental Medicine) **ANALYZING TRANSCRIPTOMICS TO DISCOVER CIRCADIAN PATHWAYS AND NETWORKS IN THE NAÏVE CENTRAL NERVOUS SYSTEM**

Amanda Zacharias[1], Hanlin Chen[1,2], Danai Topouza[1], Qingling Duan[1,2*], and Nader Ghasemlou[1,3,4*]
[*]These authors contributed equally to this work.

[1]Dept. of Biomedical & Molecular Sciences, [2]School of Computing, [3]Dept. of Anesthesiology & Perioperative Medicine, [4]Centre for Neuroscience Studies, Queen's University, Kingston, Ontario, Canada. *co-senior authors

Introduction: Circadian rhythms are near 24-hour internal cycles of biological processes associated with the earth's daily rotation cycle. These cycles are controlled by a central clock in the suprachiasmatic nucleus of the hypothalamus and many peripheral clocks throughout the body. At the molecular level, circadian clocks are regulated by transcriptional-feedback loops, whose components modify the expression of downstream clock-controlled genes (CCGs), of which there are many. Various tools can be used to investigate these CCGs and their interactions, including *Metacycle*, *DynOmics*, and weighted gene co-expression analysis (*WGCNA*).

Methods: To investigate these genes and mRNA-microRNA co-expression, we used mRNA and microRNA-sequencing samples taken from the cortex, striatum, hypothalamus, and liver of naïve male mice every 3 hours for 36 hours. For each tissue, gene counts were normalized with *edgeR* and *arrayQualityMetrics* was used to remove outliers. We removed genes whose mean expression was < 1. We used *Metacycle* to identify rhythmically expressed genes, *dynOmics* and *WGCNA* to identify microRNA-mRNA pairs, and *g: Profiler* to perform pathway analysis on the results.
Results: The decreasing number of cycling genes in each tissue is as follows: liver, cortex, hypothalamus, and striatum. Most appear circadian, though some genes' periods are around 8 or 12 hours. mRNA genes tend to have peaks of expression during the transition between light and dark or vice versa. Though core circadian genes are rhythmically expressed across most tissues, few genes are shared across all tissues. Most of the cyclic mRNA-microRNA co-expression pairs are novel; between 27.18% and 46.28% of these pairs may involve microRNAs directly regulating mRNA expression. Finally, we found that most groups of rhythmically expressed mRNA genes contain markers of the immune and vasculature systems.
Discussion: Our results from Metacycle are overall supported by the literature, though we are surprised that the cortex had more cycling genes than the striatum. The vast number of novel mRNA-microRNA pairs suggests that microRNAs may be key for regulating CCGs, though further validation is needed. We hope researchers can use our results to inform their own research. To this end, we have made our results explorable at https://www.ghasemloulab.ca/. (Supported by NSERC and the Craig H. Neilsen Foundation)

23. Jennifer Veeneman (Field: Life Science) **MITOCHONDRIAL PHENOTYPING IN OPTIC NERVES OF Wild-Type vs. OCTN1 KO MICE.**

Jennifer Veeneman[1], Miranda Mathieson[2], Dr. Jacob Rullo[1,2], Dr. Kimberly Dunham-Snary[1,3].
[1]Department of Biomedical and Molecular Sciences, Queen's University, Kingston, Ontario, Canada.
[2]Department of Ophthalmology, Queen's University, Kingston, ON
[3]Department of Medicine, Queen's University, Kingston, ON

Background: Glaucoma, a leading cause of worldwide blindness, is largely untreatable. Retinal ganglion cell (RGC) loss in the optic nerve is characteristic of glaucoma. RGCs have high mitochondrial density due to the eye's energetic demands. During mitochondrial energy production via the electron transport chain (ETC), reactive oxygen species (ROS) are produced. Mitochondrial dysfunction and ROS production are potential targets for glaucoma therapy, leading to investigation of antioxidants like ergothioneine (ERG). ERG requires Organic Cation Transporter Novel 1 (OCTN1) for transport, yet the consequences of preventing ERG absorption via OCTN1 knockout (KO) are unclear.
Methods: Mitochondria from n=6 pooled optic nerves of wild-type (WT) and KO mice were isolated using homogenization and centrifugation. Immunoblot analysis measured OCTN1 and ETC subunit abundance, and dipstick assays measured NADH Dehydrogenase (Complex I) activity.
Results: OCTN1 KO was confirmed in optic nerve mitochondria (-90% vs. control). OCTN1 KO decreased expression of ETC Complex I (88.2%), III (27.2%), IV (44.2%), and V (10.1%) subunits compared to the control. Complex I activity remained unaffected.

Conclusions: These promising results demonstrate the feasibility of investigating mitochondrial composition and function in the murine optic nerve. They also support further exploration of OCTN1, mitochondrial antioxidant capacity, and overall mitochondrial integrity in glaucoma pathogenesis. Enhancing sample size and abundance is crucial to optimize future experiments and adequately power statistical analysis.

(Supported by the grants for Dr. Kimberly Dunham-Snary: Tier 2 Canada Research Chair 2020-00192, CFI Innovation Fund 41511, Banting Research Foundation – Mitacs Discovery Award.)

24. Logan Germain (Field: Toxicology) **EXPOSURE TO THE ENDOCRINE DISURPTING CHEMICAL TRIPHENYL PHOSPHATE ALTERS THE EPIGENOME OF EMBRYONIC CELLS IN AN AQUATIC IN VITRO MODEL**

Logan Germain, Lihua Xue, Louise Winn.

Department of Biomedical and Molecular Sciences, Queen's University, Kingston, Ontario.

Triphenyl phosphate (TPP) is a widely used flame retardant, plasticizer and ubiquitous environmental pollutant that humans and ecosystems are exposed to on a daily basis. Exposure to TPP has been shown to alter gene expression in metabolic and estrogenic signaling pathways, and as such, is considered to be an endocrine disrupting chemical (EDC). EDC exposure is increasingly being associated with changes to the epigenome. The objective of this study was to determine if exposure to TPP causes changes to the epigenome in two immortal cell lines derived from trout: STE-137 is derived from embryonic tissue and RTGill-W1 is derived from epithelial tissue. Results show that following TPP exposure, post-translational modification profiles of histone H3 are altered in the embryonic cells. Histone H3 acetylation is decreased with increasing TPP concentration and histone H3 mono- and tri-methylation at lysine 9 is decreased at 80 µM of TPP. Global DNA methylation is also reduced with increasing TPP concentration in the embryonic cells. No changes to the epigenome have been observed in the epithelial cells. These results indicate that TPP exposure is altering the epigenome of embryonic cells. Given that the specific epigenetic modifications investigated in this study play a role in regulating gene expression in a variety of pathways, exposure to TPP during critical developmental windows could have profoundly detrimental effects on human and ecosystem health.

(Supported by NSERC and Queen's University)

25. Ashwaq Alqahtani (Field: Rehabilitation) **UNDERSTANDING THE STATE OF RESEARCH EVIDENCE INVOLVING FAMILY CAREGIVERS OF CHILDREN WITH CEREBRAL PALSY IN THE ARAB CONTEXTS: WORK IN PROGRESS.**

Ashwaq Alqahtani, MSc[1,2], Sumaya Mehelay[3], Siona Phadke[4,5], Danielle Macdonald, PhD[6], Heather Aldersey, PhD[1], Amanda Ross-White, MLIS [7], Afolasade Fakolade, PhD[1]

[1]School of Rehabilitation Therapy, Queen's University, Kingston, Canada; [2] Department of Physical Therapy, College of Medical Rehabilitation, Qassim University, Buraydah 52645, Saudi Arabia; [3]Faculty of Health Sciences, Queen's University, Kingston, Canada; [4]Department of Psychology, Queen's University, Kingston, Canada; [5]Department of Biology, Queen's University, Kingston, Canada; [6]School of Nursing , Queen's Health sciences, Queen's University , Kingston, Canada; [7]Bracken Health Sciences Library, Queen's University, Kingston, Canada.

Background: Raising a child with cerebral palsy (CP) can be incredibly rewarding and challenging. Caregivers, typically family, play an important role in supporting and caring for children with CP. The research in CP family caregiving is growing, but Arab family caregivers appear to be absent from this body of work. **Objective:** This scoping review aims to explore the existing literature on family caregivers of children with CP in the Arab contexts and identify gaps in knowledge to guide future research. **Methods:** The scoping review was conducted following the JBI methodology for scoping reviews. Six databases were searched from until February 2023. We selected peer-reviewed articles reporting primary studies of family caregivers of children with CP in Arab countries, regardless of time or research design. **Results:** We identified ten studies that met our criteria published from 2013 onward. The data analysis is ongoing and is expected to be completed by the June 2023. Data will be analyzed quantitively and qualitatively. We will present preliminary findings on the range and nature of CP family caregiving research in Arab contexts and the knowledge gaps in the existing literature. **Conclusion:** We anticipate that the findings from this study will guide future research and inform the development of culturally sensitive and contextually appropriate interventions, programs and services that support family caregivers of children with CP in the Arab world.

26. Sahil Sachdev (Field: Experimental Medicine) **MICROBIALLY-MEDIATED IMPAIRMENT OF VAGAL AFFERENT NEURONAL EXCITABILITY IS OREXIN RECEPTOR DEPENDENT.**

Sahil Sachdev, Ayssar Tashtush, Taylor A. Alward, David E. Reed, Alan E. Lomax. Department of Biomedical and Molecular Sciences, Queen's University, Kingston, Ontario, Canada

Background: An impairment of vagally-mediated satiety signaling has been implicated in the caloric imbalance that leads to weight gain during obesity. Previous studies have suggested that a reduction in the excitability of vagal afferent neurons with cell bodies in nodose ganglia (NG) is responsible, but the cellular mechanisms are unclear. We hypothesized that the microbiota of obese individuals produce mediators that impair NG neuron excitability and satiety in mice.

Methods: Perforated patch clamp was used to measure the excitability of NG neurons following exposure to fecal supernatants (FS) from > 5 healthy human donors or FS from > 5 obese donors.

Results: NG neurons incubated in FS from obese individuals were significantly less excitable (i.e. rheobase was 30% higher and action potential discharge at 2x rheobase was 50% lower) than NG neurons exposed to FS from non-obese donors. We next attempted to identify potential mediators in FS that may account for this inhibitory effect. These candidate mediators that inhibit vagal afferent neurons were tested using receptor antagonists that block GABA, ghrelin and orexin signaling. Ghrelin and GABA receptor antagonists did not block the inhibitory effect of obese patients' FS on NG neurons however orexin receptor antagonists did. These findings suggest that obese patient microbiota produces an orexin receptor agonist that inhibits satiety and may contribute to caloric imbalance.

27. Madeline Hopkins (Field: Neuroscience) **COMPARING LANGUAGE LATERALIZATION FROM FMRI AND DICHOTIC LISTENING IN PATIENTS WITH EPILEPSY**

Authors: Madeline Hopkins[1], Andrea Ellsay[1], Donald Brien[1,] Lysa Boissé Lomax[1,2], Garima Shukla[1,2], Ada Mullett[1,2], Gavin P. Winston[1,2]

[1]Queen's University, Centre for Neuroscience Studies, Kingston, ON, Canada

[2]Queen's University, Medicine - Neurology, Kingston, ON, Canada

Background: Language lateralization is the specialization of language functions in the brain, which can help in identifying epileptic foci and assessing postoperative language risks before epilepsy surgery. Functional magnetic resonance imaging (fMRI) and dichotic listening (DL) are non-invasive techniques used in clinical practice, but their concordance in lateralizing language in adult patients with epilepsy remains unclear. We compared the results of fMRI and DL in patients with epilepsy.

Methods: Five patients under assessment for epilepsy surgery and seven healthy controls underwent fMRI and DL evaluation. Sentence completion and word generation fMRI paradigms were used to activate receptive and expressive language areas, respectively. The "iDichotic" DL task was used for comparison with fMRI. fMRI data was preprocessed, and laterality was analyzed using SPM12 software in MATLAB. Laterality indices from both methods were compared.

Results: Language paradigms were effective in determining interhemispheric lateralization of receptive and expressive language areas in epilepsy patients. However, the laterality indices between methods were inconsistent in all five patients and four controls, particularly in cases of atypical lateralization.

Significance: These findings invalidate the reliability of the iDichotic task and necessitate the inclusion of fMRI language lateralization in clinical settings. This study has the potential to impact the improvement of language assessment prior to surgery and guide the development of clinical guidelines on utilizing language fMRI to evaluate patients with epilepsy.

28. Golnar Taheri (Field: Microbes, Immunity and Inflammation) **IDENTIFYING A NOVEL ANTAGONIST FOR THE CCR4 RECEPTOR**

Golnar Taheri[1], Nader Ghasemlou[1,2,3,4]
[1]Pain Chronobiology & Neuroimmunology Laboratory, [2]Department of Biomedical & Molecular Sciences, [3]Department of Anesthesiology & Perioperative Medicine, and [4]Centre for Neuroscience Studies

Inadequate pain management strategies are an influential factor in developing chronic post-surgical pain. Struggling through CPSP reduces the quality of life and increases the potential for opioid abuse. The current opioid epidemic results from overprescribing pharmaceuticals to treat CPSP, among other diseases. Therefore, identifying potent analgesics, not relying on the opioid system, is necessary. To develop better analgesic options, it is essential to identify underlying physiological pathways contributing to the development of post-surgical pain. Previous work from our group uncovered that skin-resident dendritic cells mediate post-incisional pain through the release of CCL22 and CCL17, two chemokines acting on the same receptor, CCR4, which we found is expressed in a specific subset of skin-innervating sensory neurons. Moreover, our group found that pain hypersensitivity was inhibited by using the CCR4 inhibitor (C021). Based on this, *I aim to identify novel CCR4 antagonists using an AI-generated chemical library.* Since CCR4 is suggested to be a G-protein coupled receptor potentially responsible for activating

various calcium-related signalling pathways and altering cellular migration, I have focused on using *in vitro* transmigration assays to identify new CCR4 antagonists.

Keywords: *Dendritic cells, Cellular migration, Pain, Antagonist*

29. David Bunsick (Field: Experimental Medicine) **CANNABINOIDS TRANSMOGRIFY A NOVEL CBD BIASED G PROTEIN-COUPLED RECEPTOR SIGNALING PLATFORM.**

David Bunsick, Jenna Matsubuko, and Dr. Myron Szewczuk. Department of Biomedical and Molecular Sciences, Queen's University, Kingston, Ontario, Canada

Rationale: With the proposed Canadian July 2018 legalization of marijuana through the Cannabis Act, a thorough critical analysis of the current research on the efficacy as a treatment option is necessary. Although additional studies have filled in some of the gaps since 2013, the specific mechanisms and details of the effects of cannabis on host metabolism and cancer remain to be elucidated, as many promote cannabis as an option for Canadians to improve their health. However, cannabis consumption may increase risk factors for metabolic syndrome and disease. This project will investigate the impact of cannabinoid agonists and their subsequent combined effects on epigenetic modifications, such as cancer metabolism, through a biased G protein-coupled receptor signaling paradigm regulating several hallmarks of cancer. Our group aims to examine the role of cannabinoid receptors CB1 and CB2 signaling in activating numerous receptor tyrosine kinases to confirm our **hypothesis** that GPCR cannabinoid-biased CBD-induced receptor transactivation is mediated by NMBR-Neu1-MMP9 crosstalk on the cell surface in the induction of epigenetic landscape alterations in cancer cells.

Methods: Immunocytochemistry (ICC), coimmunoprecipitation (coIP), siRNA knockdown, live cell sialidase activity, cancer cell metabolic activity, cancer stem cell markers, and epigenetic markers

Significance: This research has the potential to elucidate an entirely new signaling process for CBD receptors and cell responses, which could represent a common metabolic target with metabolic provenances. This research also has the potential to uncover novel mechanism(s) of cannabinoid-induced metabolic changes and cancer metastases.

30. Quentin Tsang (Field: Neuroscience) **CANNABINOID AND OPIOID RECEPTOR SIGNALING IN COLONIC NOCICEPTORS IS MARKEDLY ALTERED BY ACUTE COLITIS**

Quentin K. Tsang, Hailey M. Schincariol, Claudius E. Degro, Alan E. Lomax, Stephen J. Vanner, & David E. Reed.

Gastrointestinal Diseases Research Unit, Queen's University, Kingston, Ontario, Canada

Abdominal pain is a debilitating symptom of inflammatory bowel disease. Combining sub-analgesic concentrations of cannabinoid 1 receptor (CB1R) and mu-opioid receptor (MOR) agonists inhibit colonic pain signalling in non-inflamed mice and is devoid of side effects. This study investigated the effects of cannabinoid and MOR agonists on colonic nociception during acute colitis. **Methods:** Dextran sulfate sodium administration induced colitis in mice. Colonic nociception was measured by *in vivo* visceromotor response (VMR) to colorectal distension and mechanosensitivity of single colonic afferent axons in *ex vivo* recordings. **Results:** ACEA (CB1R agonist; 3 mg/kg), effective in healthy mice, did not inhibit VMR during acute colitis ($p=0.55$). Conversely, while HU-308 (CB2R agonist; 3 mg/kg) had no effect in healthy

mice, it inhibited VMR during acute colitis ($p<0.05$). Morphine (0.3 mg/kg) had no effect in healthy mice, but inhibited VMR during colitis ($p<0.01$). Interestingly, the effect of combining low dose ACEA (0.3 mg/kg) and morphine (0.3mg/kg) was larger than morphine alone ($p=0.06$). While ACEA (1μM) inhibited colonic mechanosensitivity in healthy mice, 10 μM was required in colitis ($p<0.05$). Combining sub-analgesic concentrations of ACEA (100 nM) and DAMGO (MOR agonist;1 nM) reduced mechanosensitivity ($p<0.05$). **Conclusions:** During colitis, the effectiveness of CB1R, CB2 and MOR agonists on pain signaling is altered. However, combining sub-analgesic CB1R and MOR agonists is still more effective than the MOR agonist alone. (Supported by NSERC and OGS).

31. Ciara O'Connor (Field: Neuroimmunology) **CIRCADIAN RHYTHMS OF MICROGLIAL ACTIVATION IN NEUROPATHIC PAIN MODEL**

Ciara O'Connor, Nader Ghasemlou

Department of Biomedical and Molecular Sciences

Microglia have been shown to be drivers of pain hypersensitivity in the spared nerve injury model of neuropathic pain in mice. Following spared nerve injury, microglia in the dorsal horn of the spinal cord proliferate and transition from a homeostatic phenotype, characterized by ramified cell morphology, to a pro-inflammatory phenotype, characterized by amoeboid cell morphology. In the spared nerve injury model, male mice experience circadian fluctuations in sensitivity to cold and mechanical stimuli. However, the role of microglial circadian rhythms in the development of rhythms in pain sensitivity following spared nerve injury has yet to be ascertained. To investigate this gap in knowledge, male C57Bl/6 mice were given a spared nerve injury, then at 3, 7, and 14 days following injury mice were sacrificed at 9am and 9pm. The spinal cord was removed, sectioned, immunofluorescence stained for markers of homeostatic (P2RY12) and pro-inflammatory (Iba1/CD68) microglia, and imaged on a fluorescence microscope. At both 7 and 14 days following injury, microglia in the dorsal horn took on a more pro-inflammatory phenotype at 9am, and a more homeostatic phenotype at 9pm. The experiment was repeated in CX3CR1Cre; Bmal1flox mice, in which clock gene Bmal1 is specifically ablated in microglia. At 7 and 14 days following injury microglia retained a pro-inflammatory phenotype at both timepoints.

Funding source: NSERC (The Natural Sciences and Engineering Research Council of Canada)

32. Megan Cull (Field: Therapeutics, Drug Development, and Human Toxicology) **MECHANISMS OF *IN-UTERO*-INITIATED BENZENE TOXICITY IN THE PLACENTA (STUDY PROPOSAL)**

Megan Cull[1], Lihua Xue[1], Louise M. Winn[1]. [1]Dept. of Biomedical and Molecular Sciences, Queen's University.

Benzene is an environmental chemical and a known carcinogen, with benzene metabolism increasing reactive oxygen species (ROS) production. The placenta is critical for fetal development, as it establishes the maternal-fetal vascular interface. Benzene can cross the placenta, making exposure during pregnancy of concern. However, the interaction between these factors in the placenta and their relevance to the *in-utero* carcinogenicity of benzene is unknown. I hypothesize that *in-utero* benzene exposure increases ROS and placental DNA damage, resulting in fetal growth restriction (FGR). To study *in-utero*-initiated benzene toxicity, pregnant CD-1 dams will be exposed to benzene (200 mg/kg) or corn oil (control) by

intraperitoneal injection on gestational days (GDs) 8, 10, 12, and 14. Dams will be sacrificed 2, 6, and 24 hours following final exposure. Fetus and placenta weights will be measured to assess whether benzene exposure alters their growth. Placenta immunohistochemical analysis will be used to identify the fetal and maternal blood spaces to determine if benzene alters placenta morphology and vasculature structure. Whether benzene alters placenta levels of ROS, markers of ROS damage, global DNA damage and/or the activity of important enzymes for benzene metabolism (i.e., myeloperoxidase) will be assessed to investigate if benzene toxicity results in placental dysregulation and DNA damage. This work will help to develop a fundamental understanding regarding the consequences of *in-utero* benzene exposure and its toxicities on the placenta.

33. Megan Morrison (Field: Reproductive and Developmental Sciences) **UNDERSTANDING THE ROLE OF HYPOXIA IN ANGIOGENIC SIGNALLING AND PERICYTE DROPOUT IN THE PLACENTA.**

Megan J. Morrison, Bryony V. Natale, Avery J. McGinnis, T. Nichole Peterson, David R.C. Natale, Department of Biomedical and Molecular Sciences, Queen's University, Kingston, ON, Canada.

Placental pericyte cells are an understudied population that wrap fetal capillaries in the placental villi: the sites of maternal-fetal exchange. In other organs, pericytes interact with endothelial and fibroblast cells to regulate vascular growth, and they may similarly contribute to fetal vessel expansion in the placenta. Interestingly, reduced fetal vessel branching and pericyte coverage have been reported in the context of placental pathologies associated with fetal growth restriction (FGR). Given that FGR is associated with defective placentation and placental hypoxia, we hypothesized that hypoxia would alter the *in vitro* angiogenic secretion of placental pericyte cells and models of other villous cell populations, such that the factors that promote vessel regression would be increased in hypoxia. Human placental pericytes, human umbilical vein endothelial cells (HUVECs), and human skin fibroblasts were cultured in normoxic and hypoxic (1% O_2) conditions for 48 hours, and cell culture supernatant was collected to assess the secretion of angiogenic factors by enzyme-linked immunosorbent assays (ELISAs). Our results indicate that, while the fibroblast population remains resilient to changes in oxygen tension, hypoxia increases the secretion of pro-angiogenic factors in placental pericyte cells and a model of villous endothelial cells. Considering that diminished pericyte presence has been reported in pathological placentas, our findings suggest that the loss of pericyte-derived pro-angiogenic factors may contribute to compromised fetal vessel branching in placental pathologies associated with FGR.

This work is supported by the National Institutes of Health (National Institutes of Child Health and Development).

34. Doriana Taccardi (Field: Microbes, Immunity and Inflammation) **CIRCADIAN RHYTHMICITY AFFECTS PAIN INTENSITY AND OPIOID CONSUMPTION**

Introduction: Chronic pain contributes to most years lived with disability worldwide. Previous work suggests that chronic pain fluctuates throughout the day. Investigating circadian rhythmicity in pain intensity and gene expression is crucial to predict health outcomes and medication use.

Methodology: Gene Ontology was used to identify whether circadian rhythms contribute to pain and biopsychosocial outcomes using the UK Biobank (UKBB), particularly in a cohort of people with chronic

low back pain (CLBP) and with musculoskeletal pain (CMSP) opioid users vs non-users. Gene- and pathway-level summary results were obtained from SNP-level summary results using MAGMA. Concurrently, 74 participants with CLBP in our Kingston, ON-based cohort study completed e-diary assessments tracking daily pain symptoms over 7 days at 3 times per day (8 am/2 pm/8 pm). Based on self-reported pain intensity (adjusted for low and high SD), participants were divided into four clusters of pain rhythmicity (i.e., constant low, constant high, rhythmic↑, and mixed). Blood was collected within a 12-h period (8 am/8 pm) to quantify changes in immune cell populations; RNA from PBMCs was used to assess changes in core circadian genes.

Results: Circadian rhythmicity, on a molecular and biopsychosocial level, influences pain intensity, gene expression, and opioid use. In our sample, only people in rhythmic↑ and constant low clusters were associated with characteristic rhythms of core clock genes, which were observed to be dysregulated in constant high and mixed clusters.
Circadian pathways were significantly associated with opioid usage in UKBB participants with chronic back or musculoskeletal pain.

Conclusions: The experience of pain varies across different configurations of pain rhythmicity: mixed, constant (high and low), rhythmic↑. Circadian rhythmicity, on a molecular and biopsychosocial level, influences pain intensity, gene expression, and opioid use. Genetic circadian pathways might have a role in opioid consumption in CLBP and CMS.
Future studies will build on these findings, exploring whether individualized interventions targeting circadian rhythmicity improve prognosis and reduce the global health cost of chronic pain.

Funding: CIHR-SPOR Chronic Pain Network

Doriana Taccardi1, Hailey Gowdy1, Mitra Knezic1, Amanda Zacharias1, Etienne J. Bisson2,5, Marc Parisien3 Lesley Norris Singer4,Jennifer Daly-Cyr4, Daenis Camiré5, Rosemary Wilson5, Elizabeth Brown5, Zihang Lu6 Manon Choinière7, Luda Diatchenko3, M. Gabrielle Pagé7,8, and Nader Ghasemlou1,2,9.

1Department of Biomedical and Molecular Science, Queen's University, Canada. 2Department of Anesthesiology and Perioperative, Medicine, Queen's University, Canada. 3Department of Anesthesia, Alan Edwards Centre for Research on Pain, McGill University, Canada. 4Chronic Pain Network, Canada. 5Kingston Health Sciences Centre, Canada. 6Department of Public Health Sciences, Queen's University, Canada. 7Département d'anesthésiologie et médecine de la douleur, Université de Montréal, Canada. 8Département de psychologie, Université de Montréal, Canada. 9Centre for Neuroscience Studies, Queen's University, Canada

35. Georgia Kersche (Field: Cardiovascular Imaging) **CORONARY ARTERY CALCIUM SCORE IS ASSOCIATED WITH CAROTID PLAQUE BURDEN IN LOW-INTERMEDIATE RISK PATIENTS**

GEORGIA KERSCHE BScH, MARIE-FRANCE HETU MSc PhD, RAVEEN PAL MPH MD, MURRAY F. MATANGI MD FRCPC, and AMER M. JOHRI MD MSc FRCPC FACE

Queen's University, Kingston ON, Canada.

Background: Carotid ultrasound (CUS) quantifies atherosclerotic plaque and is associated with cardiovascular disease and events. The coronary artery calcium score (CACS) uses computed tomography and is associated with adverse cardiovascular events. However, the CACS requires radiation and is more costly than ultrasound. This study investigated the association between CACS and carotid plaque quantity and composition.

Methods: Adult participants (n=35) with no history of cardiovascular disease were recruited to undergo CUS. Maximum plaque height, total plaque area, and plaque score (Rotterdam method) was measured. Grayscale pixel distribution analysis determined tissue composition within plaques. Participants then underwent CT to determine CACS (Agatston method) and scores were categorized as absent (0), mild (1-99), moderate (100-399), and severe (400+).

Results: Total plaque area, maximum plaque height, and plaque score were significantly associated with CACS (r=0.71, p<0.0001; r=0.44, p=0081; and r=0.60, p=0.0002). There was a significant difference in the mean total plaque area of those with a severe CACS category compared to lesser categories (Figure). Echogenic plaque composition features were not associated with CACS (Table).

Conclusions: While carotid plaque burden was associated with CACS, plaque composition was not. CUS gives information on both burden and composition, but CACS only identifies calcification. CUS is easily incorporated into clinic visits. Its use in conjunction with traditional risk factors could improve prediction of cardiovascular events in the moderate risk population.

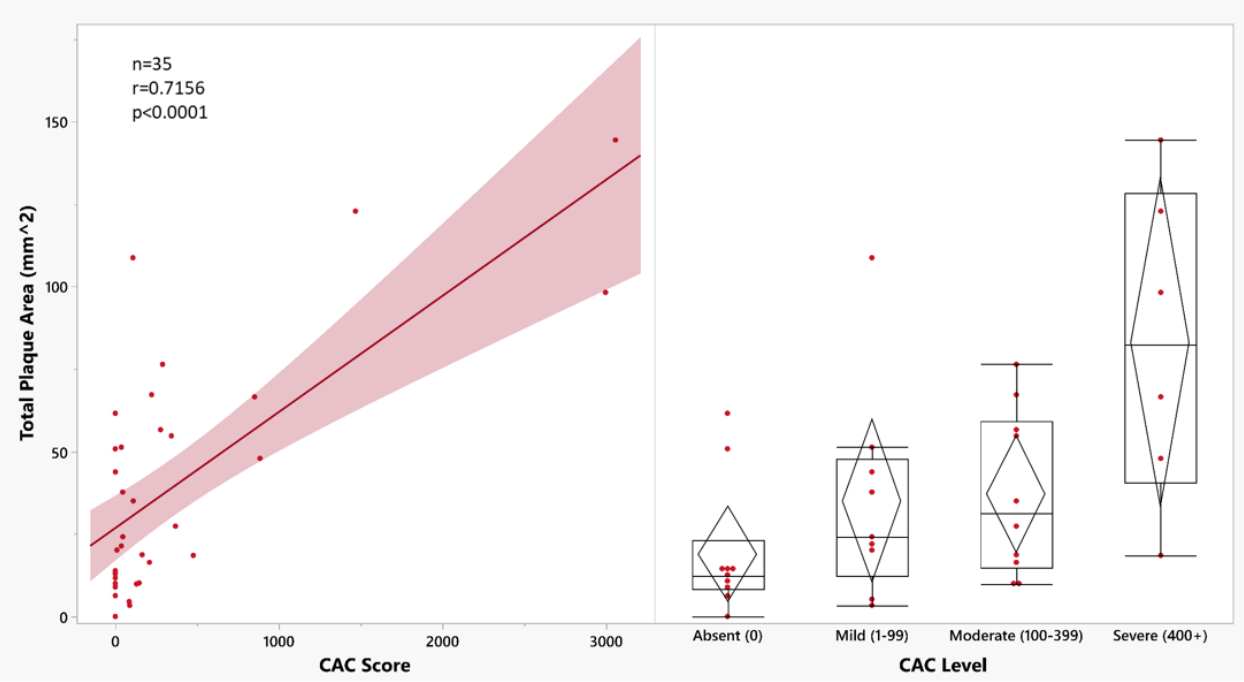


Figure. Total plaque area and its association with CAC score and CAC score level.

Table. Correlations of carotid artery plaque features and CAC score.

Carotid Plaque Feature	Pairwise Correlation	p-value
Total Plaque Area (mm^2)	0.72	**<0.0001**
Maximum Plaque Height (mm)	0.44	**0.0081**
Plaque Score (0-6)	0.60	**0.0002**
Total % Calcium	0.12	0.51
Total % Fibrous Tissue	0.15	0.38

36. Julia Hellas (Field: Neuroscience) **INVESTIGATING THE MECHANISM AND IDENTITY OF AN ACTIVATOR OF SPREADING DEPOLARIZATION.**

Julia A. Hellas, Dr. Peter Gagolewicz, Dr. Chole Lowry, and Dr. R. David Andrew. Centre for Neuroscience Studies, Queen's University, Kingston, Ontario, Canada.

Stroke is the second leading cause of death globally[1]. During stroke, the brain loses oxygen/glucose-rich blood, preventing neuronal adenosine triphosphate (ATP) generation, function of ATP-dependent pumps and neurons within minutes[2,3]. Specifically, Na^+/K^+ transporters fail, promoting spreading depolarization (SD). SD is a wave of inactivation traversing the higher brain which can recur, largening the ischemic core and leaving patients with neurological deficits, yet there are no pharmacological treatments[4-9]. Fundamentally, we don't understand what drives the spread *or* depolarization.

Marine poison, palytoxin (PLTX), opens Na^+/K^+ transporters into channels, inducing SD in brain slices, and swelling/hemolysis in red blood cells (RBCs). Here, we investigated a proposed SD activator (SDa) that we suspect is released by stressed grey matter, initiating, and driving SD in a PLTX-like manner. We first collected SDa by inducing SD in slices with oxygen/glucose-deprivation and collecting artificial cerebrospinal fluid (aCSF) surrounding them. This 'Post-SD aCSF' evoked SD in naïve slices with 78-82% frequency. We then ruled out the role of pH or K^+. Finally, we continued previous findings where small [PLTX] seemed to *prime* RBC Na^+/K^+ transporters to open, replicating this in slices.

Post-SD aCSF should be a reasonably purified SDa solution, given the absence of cellular disruption. A stronger understanding of the activity and identity of an SDa and mechanisms driving SD will help elucidate novel targets to reduce/stop recurrent SDs in clinical populations.

1. Feigin VL, Stark BA, Johnson CO, et al. Global, regional, and national burden of stroke and its risk factors, 1990–2019: a systematic analysis for the Global Burden of Disease Study 2019. The Lancet Neurology 2021;20:795-820.

2. Sakoh M, Østergaard L, Røhl L, et al. Relationship between residual cerebral blood flow and oxygen metabolism as predictive of ischemic tissue viability: sequential multitracer positron emission tomography scanning of middle cerebral artery occlusion during the critical first 6 hours after stroke in pigs. J Neurosurg 2000;93:647-657.

3. Kim D. Does ischemia cause neuronal damage by converting the Na+/K+ pump into a channel? Kingston, ON: Queen's University, 2014.

4. Ayata C, Lauritzen M. Spreading Depression, Spreading Depolarizations, and the Cerebral Vasculature. Physiol Rev 2015;95:953-993.

5. Brisson CD, Hsieh Y-T, Kim D, Jin AY, Andrew RD. Brainstem Neurons Survive the Identical Ischemic Stress That Kills Higher Neurons: Insight to the Persistent Vegetative State. PLoS One 2014;9:e96585.

6. Dreier JP, Reiffurth C. Exploitation of the spreading depolarization-induced cytotoxic edema for high-resolution, 3D mapping of its heterogeneous propagation paths. Proceedings of the National Academy of Sciences 2017;114:2112-2114.

7. Petrin D, Gagolewicz PJ, Mehder RH, Bennett BM, Jin AY, Andrew RD. Spreading depolarization and neuronal damage or survival in mouse neocortical brain slices immediately and 12 hours following middle cerebral artery occlusion. J Neurophysiol 2019;121:1650-1663.

8. Hellas JA, Andrew RD. Neuronal Swelling: A Non-osmotic Consequence of Spreading Depolarization. Neurocrit Care 2021;35:112-134.

9. Hartings JA, Shuttleworth CW, Kirov SA, et al. The continuum of spreading depolarizations in acute cortical lesion development: Examining Leão's legacy. J Cereb Blood Flow Metab 2017;37:1571-1594.

37. Caitlin Piccone (Field: Rehabilitation Science) **EXPLORING THE ROLE OF MENTAL HEALTH SUPPORTS IN COMMUNITY-BASED REHABILITATION PROGRAMMING IN GHANA.**

Caitlin Piccone and Vida Kasore. Department of Rehabilitation Science, Queen's University, Kingston

In Ghana, more than 13% of the population are estimated to have a mental health disability; however, access to mental health resources is poor. Lack of access to mental health supports negatively impacts overall wellbeing of individuals and families and represents a breach in the rights laid out in the United Nations Convention on the Rights of Persons with Disabilities (UNCRPD). Community Based Rehabilitation (CBR) represents a promising access point to provide services. However, there is limited research on the inclusion of mental health supports within CBR programming. This research will explore how mental health supports can be integrated into CBR programming by understanding what people with mental health disabilities view as the most important components of support to provide and how these supports be integrated into CBR programming in a culturally sensitive manner. I am using an Participatory Action Research Approach and African Feminist lens to understand how social determinants of health and power inequities influence mental health outcomes. We will conduct qualitative interviews with CBR attendees with mental health disabilities and family members to understand what important elements of support are, then host workshops to understand how these supports can be integrated into programming. This research will support the creation of interventions to integrate mental health supports into CBR programming in Ghana. It is a vital first step to improving access to mental health supports in the country to improve general wellbeing, support the attainment of the rights established in the UNCRPD, and reduce existing power inequities between people with and without disabilities.

Funding source: This work was supported, in part, by CIHR

38. Eric Fernandes (Field: Cardiac, Circulatory, and Respiratory Sciences) **HIGH DIETARY PHOSPHATE ALTERS ACUTE FETUIN-A MEDIATED PHOSPHATE BUFFERING IN EXPERIMENTAL CHRONIC KIDNEY DISEASE**.

Eric B P Fernandes BHScH,[1] Olivia M Novosel BScH,[1] Tyler S Rowsell BScH,[1] Rachel M Holden MD,[1,2] Michael A Adams PhD[1]

[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

Dysregulated phosphate (PO_4) homeostasis is associated with cardiovascular morbidity in chronic kidney disease (CKD). Fetuin-based calciprotein particles (CPPs) may represent a key homeostatic buffering system for circulating minerals, however, the effect of high-dietary PO_4 on this system is unclear. This study determined whether CPP-mediated PO_4 buffering was decreased in experimental CKD following high-dietary PO_4.

Cross-sectional assessments of acute protein-mediated PO_4 buffering were performed on serum samples collected from male Sprague-Dawley rats fed either a control or CKD-inducing diet (0.25% adenine, moderate-4wks, severe-6wks), which was exacerbated in a subset of severe-CKD animals by 2wks of a high PO_4 (1.0%) diet. PO_4 buffering was measured by T50 transition times alongside free and protein-bound [PO_4] following an *in vitro* PO_4 challenge. OsteoSense-detectable CPP levels were collected to characterize overall CPP profiles.

Compared to controls, elevations in protein-bound [PO_4] (1.6x, ***p*<0.01**) and Osteosense-detected CPPs (2.7x, ***p*<0.01**) occurred by wk6 of CKD, with no significant added effect attributable to high-dietary PO_4. However, only under high-dietary PO_4 were iPTH (17.5x-increased, ***p*<0.0001**) and serum PO_4:calcium (2.0x-increased, ***p*<0.0001**) elevated. This resulted in a similar pattern of perturbations in acute PO_4 buffering, evidenced by elevated supernatant/free [PO_4] (1.1x-increased, ***p*<0.05**) and reduced T50 time (2.1x-decreased, ***p*<0.005**).

In experimental CKD, high-dietary PO_4 reduced the acute PO_4 buffering capacity of CPPs. These findings validate CPPs as biomarkers for PO_4 dysregulation and have implications for dietary CKD management.

(Supported by a TIME grant from Department of Medicine, Queen's University, and a grant form OPKO Health Inc Renal Division)

39. Kody Klupt and Matthew Fishman (Field: Protein Function) **NOVEL APPLICATIONS OF ISOTHERMAL TITRATION CALORIMETRY (ITC) IN THERAPEUTIC DEVELOPMENT AND BIOMOLECULAR CHARACTERIZATION**

Matthew Fishman*, Kody Klupt*, and the Protein Function Discovery Facility

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

The proper functioning of many proteins is intimately associated with their ability to recognize and bind to a specific target molecule at the right time and with the right affinity. These target molecules can consist of ions, co-factors, and other proteins, and their interactions play crucial roles in various cellular functions such as cell motility, muscle contraction, apoptosis, enzyme catalysis, and regulation. Isothermal titration calorimetry (ITC), traditionally used to study bimolecular interactions and thermodynamics of biological systems, has expanded to support a number of medical applications. We share herein how ITC can enable the label-free quantification of drug-target binding and provide a platform to optimize existing therapeutics. Our group demonstrates how ITC aids in the characterization of biomolecular interactions including monitoring protein folding, enzyme kinetics, and the stability of biological systems. We provide evidence for how insight into these intrinsic biophysical properties provides a rich understanding of biological pathways and the derivation of innovative therapies. ITC systems at the Protein Function Discovery Facility provide a robust biophysical methodology that can directly measure these parameters, including a range of affinities extending from nanomolar to millimolar, in solution. With limited cost and time, researchers can generate thermodynamic information for their system beyond conventional methods with hazardous labels or contaminants.

(Supported by the Natural Sciences and Engineering Research Council of Canada and the Ontario Graduate Scholarship Program.)

40. Jasleen Jagayat (Mental Health Care – Online Therapy) **INCORPORATING A STEPPED CARE APPROACH INTO ELECTRONIC COGNITIVE BEHAVIOURAL THERAPY FOR DEPRESSION: A RANDOMIZED CONTROLLED TRIAL**

Jasleen Jagayat BScH*; Anchan Kumar MD; Anastasia Shao MA; Amrita Pannu MD; Charmy Patel MSc; Amirhossein Shirazi MD, PhD; Mohsen Omrani MD, PhD; Nazanin Alavi MD FRCPC

*Centre for Neuroscience Studies, Queen's University, Kingston, Ontario, Canada.

Depression is a leading cause of disability, annually affecting up to 300 million people worldwide, yet fewer than one third of patients receive care. Electronic cognitive behavioural therapy (e-CBT) is an effective treatment for depression. A stepped care model is a care model that begins treatment with the least resource intensive, yet effective, method while adapting care based on patients' needs. This study investigated the efficacy of a stepped-care e-CBT model for depression through the reduction of depressive symptoms. In this single-blinded randomized controlled trial, participants were randomized to either the e-CBT group (n = 28) or the e-CBT with stepped care group (n = 28). Both groups received a 13-weeks e-CBT program for depression. Participants in the experimental group received additional interventions including messages, phone calls, video calls, or a video call with a psychiatrist. The results of this study indicate that the e-CBT program was effective in significantly reducing depressive symptoms, as measured by the PHQ-9 ((F(4, 80) = 9.95, p < .001)) and QIDS (F(2, 28) = 5.73, p = .008); however, there were no significant differences in the reduction of depressive symptoms between the two groups (PHQ-9: (F(4, 80) = .43, p = .785); QIDS: (F(2, 28) = 3.05, p = .063)). The stepped care group did not show to be significantly better in reducing depressive symptoms than the e-CBT group.

41. Daniella Gilmour (Field: Neuroscience Research) **IRRITABLE BOWEL SYNDROME: A ROLE FOR HISTAMINE AND PROTEASE SYNERGY**

Daniella Gilmour, Nestor Jimenez-Vargas, David E. Reed, Alan E. Lomax, Stephen J. Vanner
Gastrointestinal Diseases Research Unit, Queen's University, Kingston General Hospital, Kingston, Ontario

Irritable bowel syndrome (IBS) is one of the most prevalent disorders of gut-brain interactions, with the most debilitating symptom being abdominal pain. Previous work has shown that IBS patient fecal supernatant (FS) increases the excitability of nociceptive neurons compared to healthy volunteer FS. Altered production of intestinal mediators, specifically histamine and proteases, have been implicated in these effects. It is hypothesized synergy between these mediators may exist. **Methods:** Colonic afferent nerve recordings, as well as perforated patch clamp experiments, were used to examine the potential that synergy exists between histamine and protease effects on nociceptive neurons. **Results:** Luminal administration of histamine and trypsin increased afferent excitability. Luminal administration of histamine or protease antagonists inhibited the excitatory effect previously seen upon administration of IBS patient FS. The application of subthreshold concentrations of histamine and trypsin had no effect on neuronal activity when applied individually, but induced a 38% increase in excitability in the afferent nerve recording experiments, and a 57% reduction in rheobase in the perforated patch clamp experiments when applied in combination. **Conclusion:** The results suggest that histamine and proteases play a critical role in the hypersensitivity induced by IBS patient FS, and that synergy between these mediators exacerbates their excitatory effects on nociceptive neurons. These findings could be pivotal in the development of treatments for those suffering with IBS. Supported by CIHR.

42. Paola Dantonio (Field: Cancer Research and Therapy) **EMBRYONIC MORPHOGEN NODAL AS AN INDUCER OF METABOLIC PLASTICITY**

Paola M Dantonio1, Hannah Plummer-Doherty1, David Papadopoli2,3, Jiahui Liu4, Guihua Zhang4, Edna T. Kimura5, Lynne-Marie
Postovit1,4
1Department of Biomedical and Molecular Sciences, Queen's University, Kingston ON, Canada; 2Lady Davis Institute for Medical
Research, Jewish General Hospital, Montreal QC, Canada; 3Gerald Bronfman Department of Oncology, McGill University, Montreal
QC, Canada; 4Department of Oncology, University of Alberta, Edmonton AB, Canada; 5Institute of Biomedical Sciences, University
of Sao Paulo, Sao Paulo, Brazil

Cancer cells rely on plasticity and adaptation to the microenvironment to ensure their survival. Metabolic plasticity refers to the ability of cells to use alternative sources of energy to fuel activities, such as aerobic glycolysis and glutaminolysis. The ability of cells to switch between aerobic glycolysis and oxidative phosphorylation confers higher resistance to therapeutic approaches targeting metabolic processes. NODAL, an embryonic morphogen belonging to the TGF-β superfamily, exerts highly context-dependent effects during embryo patterning. NODAL re-expression in certain adult cancers (e.g., breast, ovarian, endometrial, and melanoma) can be induced by stressors such as hypoxia and chemotherapy,

and promotes cancer cell survival to such stressors by conferring plasticity to cancer cells. In most cancer types, NODAL is associated with aggressive phenotypes and advanced clinical stage, increased cell migration and invasion, angiogenesis, and stem cell-like characteristics. However, we have observed an opposing role for NODAL in thyroid cancer (TC), whereby dedifferentiated tumours lack NODAL expression, and *NODAL*-overexpressing (NOE) cells present decreased capacity to form tumour spheres and produce significantly smaller tumours in NSG mice in our preliminary animal studies. We also observed that TC cells are highly dependent on glucose, and that NOE spheres produce more lactate than their respective controls. Sphere formation in stem cell medium is known to enrich for tumour-initiating cells, which harness high plasticity, including metabolic, to support their survival under different microenvironmental conditions. Recent literature suggest that NODAL indirectly induces glucose uptake in embryonic stem cells, and that Nodal establishes a bipotential state that allows cells to switch their fate during embryonic development. Together with this evidence, our preliminary results suggest that NODAL could be inducing a metabolic shift in thyroid cells that induce glucose-dependency, and might therefore be a potential avenue to overcome metabolic plasticity and target glucose metabolism for therapeutic purposes. Overall, we have been consistently observing that NODAL exerts anti-tumourigenic and likely pro-glycolytic effects in thyroid cancer cells, and will continue to investigate its role in shaping metabolic plasticity.
Methods: NODAL expression in human thyroid tumours was assessed by immunohistochemistry with anti-Nodal antibody (PA5-28486, Invitrogen, 1:100). Thyroid cancer cell lines were stably transfected with *NODAL*-overexpressing plasmid and kept under geneticin selection. Cells were grown in ultra-low attachment plates in DMEM/F12 medium supplemented with B27, FGF and EGF for sphere formation. Glucose consumption and lactate production were measured in conditioned media with Nova BioProfiler 400. Preliminary glucose dependency assessment was done by culturing control and NOE cells in low-serum media containing 25mM glucose or galactose, followed by cell fixation, DAPI staining, and imaging and counting on Cytation 5. Preliminary animal studies consisted of injections of 1.5 million cells into both flanks of NSG mice (n=10/group).

43. Ahmad J.H. Albaghdadi and Danqi Liu (Field: Reproduction and Sexual Function) **INVESTIGATING A PROGESTERONE RECEPTOR-DEPENDENT MODE OF ACTION OF TACROLIMUS IN PROMOTING THE MIGRATION AND INVASION OF HUMAN-DERIVED FIRST-TRIMESTER PRIMARY TROPHOBLAST CELLS CULTURED IN-VITRO**

Danqi Liu1, Ahmad J.H. Albaghdadi1, Wei Xu1, Ashley Waddington2 and Frederick W. K. Kan1. 1 Dept. of Biomedical and Molecular Sciences and 2 Dept. of Obstetrics and Gynecology, Queen's University, Kingston, Ontario

Objectives: To test the hypothesis that, independent of its immunosuppressive properties, the macrolide immunosuppressant tacrolimus can positively influence embryo implantation through the promotion of migration and invasion of human firsttrimester trophoblast cells and the modulation of their progesterone receptor expression and activation.

Study methods: Primary human-derived first-trimester trophoblast cell cultures were established using a novel HLA-G nanobeads-based isolation protocol and samples of human first trimester termination

placental villi. The immortalized human-derived first-trimester extravillous trophoblasts (EVTs) HTR8/SVneo cells were also used for a comparison. Cells were treated with low-dose tacrolimus (0.05 ng/ml) in the presence and absence of the progesterone receptor inhibitor Mifepristone (50 mM). The protein expression of PGR-A, PGR-B and their phosphorylated isoforms were analyzed by Western blot analysis after 48-hours posttreatment. A transwell migration and invasion assay was performed using the xCELLigence real-time cell migration and invasion monitoring system.

Results: Low-dose tacrolimus significantly ($p < 0.01$) stimulated the migration and invasion of the human first-trimester trophoblast cells and abrogated the suppressive effect of Mifepristone on their PGR protein expression and phosphorylation invitro.

Conclusions: Consistent with our previous findings of the stimulatory effects of low-dose tacrolimus in promoting the migration and invasion of the immortalized human-derived first-trimester EVTs, our present data confirm the progesteronereceptor dependent mechanism of action of tacrolimus using human first-trimester trophoblast cells and suggest an immuneindependent mode of action of tacrolimus in positively influencing embryo implantation , at least in part, through promoting the migration and invasion, and progesterone-receptor expression in the human-derived first trimester trophoblast cell cultures.

44. Ujjwal Sangwan (Field: Microbes, Immunity and Inflammation) **CYCLOSPORINE A, AN FDA-APPROVED DRUG, INHIBITS DENGUE VIRUS REPLICATION**

Ujjwal Sangwan and Dr. Che Colpitts

Department of Biomedical and Molecular Sciences, Queens' University, Kingston, Canada

Dengue virus (DENV) infects around 400 million people every year in more than 100 endemic countries, putting half of the world's population at risk. As the planet warms up, this mosquito-transmitted disease that was once restricted to tropical areas is now spreading worldwide, including Europe and North America. The best way to prevent dengue infection is vaccination; however, the approved vaccine is only for children aged 9-16 years with previous dengue infection. Unfortunately, there are currently no approved antivirals for dengue infection, with treatment limited to supportive care. Drug repurposing might be a useful strategy to identify antiviral candidates for dengue. Here, we found that cyclosporine A (CsA), a clinically approved immunosuppressive drug that inhibits T cell proliferation, dose-dependently inhibits DENV replication in Huh7 hepatoma cells, without affecting the cell viability. Interestingly, CsA treatment induces the expression of interferon-regulatory factor-1 (*IRF1*) and IRF1-dependent antiviral genes, such as *RSAD2*, which may contribute to restricting DENV replication. Our preliminary data also show that the classical CsA target proteins, cyclophilin A or B, are not required for DENV replication, indicating the involvement of other factors. Hence, further characterization of the antiviral mechanisms of the antiviral mechanisms of CsA is ongoing. Understanding the underlying mechanisms of IRF1-dependent antiviral activity of CsA will aid in the design and synthesis of non-immunosuppressive cyclosporine-like molecules for further development as antiviral drugs.

45. Danielle Harper (Field: Pathology and Molecular Medicine) **BREAKING DOWN THE ROLE OF CALPAIN PROTEASES IN TRIPLE-NEGATIVE BREAST CANCER**

Danielle Harper, Yan Gao, Ivan Shapovalov and Peter A. Greer. Department of Pathology and Molecular Medicine, Queen's University, Kingston, Ontario.

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 therapeutic targets limits treatment options and puts patients at risk of developing drug-resistant metastatic disease. My research explores the role of

calpain proteases in TNBC tumorigenesis, metastasis, and drug sensitivity. Using CRISPR-Cas9 knockout, I have demonstrated that genetic calpain disruption impedes cell migration and invasion *in vitro* and reduces the metastatic potential of AC2M2 murine mammary carcinoma cells in an orthotopic engraftment mouse model. We and others have shown that loss of calpain prevents phosphorylation and subsequent activation of the pro-survival kinase, AKT. I am continuing to explore calpain-mediated AKT regulation, focusing on three phosphatases reported as calpain substrates: PHLPP, PTEN and PP2A. Furthermore, using shRNA knockdown, I engineered calpain-1/2 deficient MDA-MB-231 cells for use in a repurposing screen of clinically approved drugs. Calpains are involved in both pro- and anti-apoptotic signalling, and these opposing roles may be exploited to improve treatment responses while protecting healthy cells from off-target cytotoxic effects. Together, these models will improve our understanding of calpain's role in TNBC and may lead to the development of novel combination therapies to improve patient outcomes.

46. Aminreza Nikpoor (Field: Cancer Research and Therapy) **NEUROMEDIN U-MEDIATED IMMUNE MODULATION: EFFECTS ON NATURAL KILLER CELL ACTIVITY.**

Amin Reza Nikpoor [1], Ali Ahmadi [2], Sebastien Talbot [1,3]

1. Department of biomedical and molecular sciences, Queen's University, Kingston, Ontario, Canada.
2. Département de Pharmacologie et Physiologie, Université de Montréal, Montréal, Quebec, Canada
3. Department of pharmacology and physiology, Karolinska Institutet, Stockholm, Sweden.

Introduction: This study investigates the role of neuromedin U (NMU), which is hypothesized to originate from dorsal root ganglia (DRGs), in modulating natural killer (NK) cells, an essential component of the innate immune system.

Methods: Our experimental strategy consisted of measuring NMU release from DRGs extracted from two mouse models: TRPV1-ablated mice and their littermate controls. Using Western blot and Real-Time PCR, the expression of neuromedin U receptor 1 (NMUR1) on NK cells was determined. Moreover, we examined the effect of NMU on the NK cell markers CD107 and XCL1 using flow cytometry.

Results: While different NMU release levels were observed (5.9±2.9 ng/mL and 8.5±4.7 ng/mL for TRPV1 ablated mice, and 6.3±3.9 ng/mL and 2.3±0.54 ng/mL for littermate controls, with and without capsaicin, respectively), these differences were not statistically significant. Flow cytometry revealed CD107 and XCL1

expression levels of 9.1±0.75%, 10.6±3.3%, and 7±1% and 9.8±4.5%, 16±10%, and 6±0.4%, respectively, in untreated, NMU-treated (500nM), and DRG supernatant culture media-treated NK cells.

Conclusion: Our findings indicate that the NMU released by stimulated littermate control mice and the DRG supernatant appeared to suppress NK cell activity. This novel's insight into NMU's potential role in NK cell suppression paves the way for further study in this area.

47. Matti McFarlane (Field: Translational Medicine) **A REVIEW OF THE KNOWLEDGE-TO-ACTION FRAMEWORK FOR IMPLEMENTING ASTHMA CARE GUIDELINES INTO CANADIAN PRIMARY CARE PRACTICE: A FOCUS ON ELECTRONIC KNOWLEDGE TRANSLATION TOOLS**

Matheson McFarlane[1,2], Alison Morra[1,2], and M. Diane Lougheed[1,2]

[1]Queen's University, Kingston, ON, Canada; [2]Asthma Research Unit, Kingston General Hospital Research Institute, Kingston, ON, Canada

Background

Asthma is one of the most common chronic respiratory diseases in Canada. Despite national asthma care guidelines, gaps persist in primary care. Several knowledge translation (KT) tools exist aiming to address these gaps.

Objective

To review the literature for key asthma care gaps and the limitations and identify future directions of Canadian electronic KT tools optimized for use in electronic medical records (EMRs).

Materials and Methods

The database OVID Medline was searched (1999-2022) using keywords such as asthma, knowledge translation, primary healthcare, EMRs, and Canada. Primary research articles, systematic reviews, and published international/national guidelines were included.

Results

Key asthma care gaps in primary care include: underrecognition of suboptimal control, underutilization of PFTs, barriers to care delivery, and limited access/referral to asthma education. Facilitators to electronic KT tools within EMRs have been a recent focus, including asthma management systems, physician/patient portals, decision support algorithms, data standards initiatives, and asthma case definition validation for EMRs.

Conclusions

The knowledge-to-action cycle has been adopted in Canada as a valuable framework for developing and implementing novel KT tools. Future research should integrate end-users into the process of KT tool development to improve the perceived utility of these tools. Additionally, the priorities of primary care physicians should be considered in future KT tool research to improve end-user uptake and overall primary care asthma management practices in Canada.

48. Ethan Thomas (Field: Microbes, Immunity and Inflammation) **HERPES SIMPLEX VIRUS (HSV) TEGUMENT PROTEINS PUL21 AND PUL16 PREVENT NASCENT CAPSIDS FROM DOCKING AT NUCLEAR PORE COMPLEXES DURING VIRION ASSEMBLY**

Ethan Thomas[1], Renée Finnen[1], Jeffrey Mewburn[2], Stephen Archer[2], and Bruce Banfield[1]

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

[2]Department of Medicine, Queen's University, Kingston, Ontario, Canada.

After entry into cells, herpes simplex virus (HSV) nucleocapsids dock at nuclear pore complexes (NPCs) through which viral genomes are released into the nucleoplasm where viral gene expression, genome replication, and early steps in virion assembly take place. Later in infection, nucleocapsids produced in the nucleoplasm are translocated to the cytoplasm for final virion assembly. Nascent cytoplasmic nucleocapsids are prevented from docking to NPCs and delivering their genomes to the infected nucleus from which they emerged, but how this is accomplished is not understood. While investigating HSV strains with mutations in the multifunctional viral proteins, pUL16 and pUL21 we noticed that, unlike parental virus strains, they accumulated empty capsids at the cytoplasmic face of NPCs at late times after infection. These findings suggested that pUL16 and pUL21, which form a heterodimer, prevent cytoplasmic nucleocapsids from docking at NPCs. In support of this idea, ectopic expression of pUL16 and pUL21 in cells prior to infection prevented incoming nucleocapsids from docking at NPCs, delivering their genomes to the nucleus, and initiating viral gene expression. Stimulated emission depletion super-resolution microscopy experiments demonstrated that both pUL16 and pUL21 localize adjacent to, and with, NPC components on the cytoplasmic face of nuclei placing them in an appropriate location to interfere with nucleocapsid/NPC interactions. We conclude that pUL16 and pUL21 interact with NPCs to prevent capsid docking, thereby abetting virion assembly.

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

49. Safara Holder (Microbes, Immunity and Inflammation) **THE IDENTIFICATION OF PROTEINS IN PROXIMITY TO VIRION-ASSOCIATED HSV-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, leading 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)

50. Andrew Garven (Field: Cancer Research and Therapy) **THE MOLECULAR AND PROGNOSTIC IMPACT OF TRANSPOSABLE ELEMENT EXPRESSION IN UROTHELIAL CARCINOMA**

Andrew Garven, Dr. Hamid Ghaedi, Dr. David Berman.

Department of Pathology and Molecular Medicine, Division of Cancer Biology and Genetics, Queen's University Cancer Research Institute, Kingston, Ontario, Canada

Transposable elements (TE) are mobile genetic sequences derived from endogenous retroviruses that constitute approximately 45% of the human genome. Previous studies in other cancers suggest that TE expression enhances tumour immunity.

Immunotherapy is an important therapeutic modality for urothelial carcinoma (UC); however, few have examined the utility of TE transcripts as prognostic biomarkers for UC. We investigated relationships between TE expression and immune response in UC using transcriptional profiling and immunohistochemistry.

We quantified the transcriptional expression level of both gene and TE sequences from early-stage (non-muscle invasive, n=535) and later-stage (muscle invasive, n=412). TE expression was subjected to unsupervised clustering, and clusters were correlated with molecular subtype 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 transcription. To validate these findings, immunohistochemical assessment of a TE surrogate marker (LINE-1's protein ORF-1) was performed on a local cohort (n=371).

Heightened TE expression correlated with the activation of an integrated stress response, including suppression of genes involved in antigen presentation. This constellation of findings was more prevalent in late-stage UC patients than in their early-stage counterparts. Contrary to previous work, these findings indicate that TE expression correlates with immune evasion and inferior treatment outcomes. Further work will investigate ways to leverage these findings for prognostic and therapeutic purposes.

51. Hannah Barnes (Field: Cancer Research and Therapy) **THE CANCER-RELATED EXPERIENCES OF VISIBLE MINORITIES IN CANADA: A SCOPING REVIEW PROTOCOL**

Hannah Barnes, Jacqueline Galica

Background: Despite novel advances in cancer research, the global burden of cancer remains high, putting strain on healthcare systems and their patients. Available research has focused on global visible minorities' cancer experiences; however, there is a gap in literature regarding this population in a Canadian context.

Objective: The purpose of this project is to conduct a scoping review to explore what is known of the cancer-related experiences of visible minority peoples in Canada.
Inclusion criteria: The population of interest is adult (≥18-years-old) members of visible minority groups, meaning those who belong to groups that are non-white in colour or non-Caucasian in race. Primary research with a Canadian participant population with human subjects and a cancer-related focus will be included.
Methods: An electronic search will be conducted in Spring of 2023 in MEDLINE, Embase, Epistemonikos, and CINAHL with screening and data extraction to be completed in Summer 2023 and results writeup in Fall 2023. Included studies will describe the cancer-related experiences of visible minorities in Canada with a date range from 2005 to present.
Discussion: By providing a broad overview of what is known about the cancer-related experiences of visible minorities in Canada, this scoping review may identify areas to guide future research, and aid clinicians and policy makers to address gaps in the care and access of care for these individuals.

52. Gwenaelle Conseil (Field: Neuroscience) **IDENTIFICATION AND CHARACTERIZATION OF GAP JUNCTION-FORMING INNEXINS FROM THE SEA SNAIL *APLYSIA CALIFORNICA***

Gwenaëlle Conseil, Christopher J. Carter, Christopher C. Beekharry, Alex B. Prosserman, Yueling Gu, and Neil S. Magoski

Department of Biomedical and Molecular Sciences, Queen's University, Kingston, Ontario

Gap junctions are intercellular channels found in all animals, allowing for the passage of ions, small molecules, and electrical signals between adjacent/connected cells. In neurons, gap junctions are the basis of electrical coupling and are formed by transmembrane subunits of connexins (in vertebrates) and innexins (in invertebrates). In the sea snail, *Aplysia californica,* an electrically coupled network of neuroendocrine bag cell neurons releases hormone during a synchronous afterdischarge to cause egg laying. Twenty innexins (ApInx-1, 2, 3, 4, 5, 6, 7, 8a, 8b, 8c, 9, 10, 11a, 11b, 12, 13, 14, 15, 16, 17) identified *in silico* were cloned by PCR from bag cell neurons, and qRT-PCR ranked ApInx-2, 3, 4, 5, 6, 7, "8", 9, and 10 as predominant. ApInx-2, 3, 5, 8b, and 8c were heterologously expressed in N2A cells to record junctional current, and in HEK293T cells for biochemistry. ApInxs presented a junctional current to that observed at bag cell neuron electrical synapses, i.e., voltage-independent and sensitive to the gap junction blockers niflumic acid and 5-nitro-2-(3-phenylpropylamino) benzoic acid. ApInxs were localized to cellular membranes, including in the contact zone between adjacent cells; ApInx-8 is glycosylated. Our data suggest *Aplysia* innexins mediate bag cell neuron electrical transmission and provide a molecular basis of the synchronous firing required for reproduction.

This work was supported by NSERC and CIHR.

53. Leah Sookhoo (Field: Nursing) **THE POSTPARTUM EXPERIENCES OF IMMIGRANT FIRST-TIME MOTHERS DURING THE COVID-19 PANDEMIC IN ONTARIO, CANADA.**

Leah Sookhoo, RN. School of Nursing, Queen's University, Kingston, Ontario, Canada.

Background: In many cultures the postpartum period is a scared time where mothers and birthing people can rest, recover, and connect with their newborns. While there is ample literature to demonstrate the unique experiences and challenges faced by immigrant women when accessing healthcare and postpartum supports in Canada, there is limited literature available about the experiences of first-time immigrant mothers. In 2020, the COVID-19 pandemic drastically impacted healthcare service delivery, creating challenges for postpartum patients and their newborns. Little is known about how the pandemic impacted the experiences of immigrant first time mothers during the postpartum period.

Purpose: The purpose of this study is to understand the postpartum experiences of first-time immigrant mothers during the COVID-19 pandemic in Ontario, Canada.

Methodology: This qualitative study is currently in process and will be guided by Feminist Poststructuralism to explore the concepts of power, gender, and discourses as they relate to immigrant first-time mother's experiences during the pandemic. Individual, one on one interviews will be conducted, and audio-recordings will be transcribed verbatim. Feminist Poststructuralist Discourse Analysis will be used to analyze the data.

Implications: Improved understandings of immigrant first-time mother's experiences during the COVID-19 pandemic in Ontario, can inform nursing practice and postpartum health care service delivery. Understanding immigrant first time mothers' postpartum experiences during the COVID-19 pandemic can promote cultural safety and cultural humility across interdisciplinary professions.

54. Emily Halajian (Microbes, Immunity and Inflammation) **ANALYZING FUNCTIONAL INTERACTIONS BETWEEN DENGUE VIRUS NON-STRUCTURAL PROTEIN 1 AND TLR4 IN A MACROPHAGE MODEL**

Emily A. Halajian[1], Katrina Gee[1], Che C. Colpitts[1]. [1] Department of Biomedical and Molecular Sciences, Queen's University, Kingston, Ontario

Research funded by support from CIHR, Canada Foundation for Innovation, NSERC, and Queen's University Faculty of Health Sciences.

Interactions between dengue virus (DENV) non-structural 1 (NS1) protein and Toll-like receptor 4 (TLR4) have been implicated in dengue pathogenesis, including proinflammatory cytokine induction. It is unclear how DENV NS1 and TLR4 interact in the context of DENV infection, and which features of NS1 and TLR4 mediate their interactions. In this study, we aimed to determine which downstream pathways respond to NS1 in the context of DENV infection. We performed RT-qPCR and ELISA to look at activation of the IRF3 and NF-κB pathways and induction of proinflammatory mediators in response to DENV infection. Transfection of DENV NS1 in 293T cells induced IRF3 and NF-κB activity upon exposure of the supernatant to THP-1 Dual reporter cells. DENV infection in the presence of a TLR4 inhibitor induced greater secretion of IL-6 and CXCL10 at later time points. Initial experiments in TLR4 knockout THP-1 cells

suggest that activation of IRF3 is partially dependent on TLR4 during DENV infection. To assess the functional role of NS1 during DENV infection, we deleted NS1 from the DENV genome using a reverse genetics system and site-directed mutagenesis. Experiments with the NS1-deletion mutant are ongoing to determine the role of DENV NS1 in activating TLR4 signaling in the context of viral infection. Overall, these findings enhance our understanding of how the immune response to NS1 is regulated during DENV infection.

55. Manav Jain (Field: Cancer Biology and Genetics) **INVESTIGATING THE TRANSCRIPTIONAL REGULATION OF TRANSPOSABLE ELEMENTS IN BLADDER CANCER.**

Manav Jain, Andrew Garven, Dr. Hamid Ghaedi, Dr. David Berman. Department of Pathology and Molecular Medicine, Division of Cancer Biology and Genetics, Queen's University Cancer Research Institute, Queen's University, Kingston, Ontario, Canada.

Background

Transposable elements (TEs) are mobile genetic elements that can insert themselves within the genome, comprising a significant portion of the human genome. Dysregulated TE expression and transposition have been implicated in various diseases. Notably, bladder cancer exhibits one of the highest levels of TE expression among all cancers, making it an intriguing field for investigating the TE expression landscape.

Methods

This study aimed to investigate the potential interplay between transcriptional regulators and TE expression in bladder cancer. The methodology employed involved quantification of TE and gene expression in bladder cancer samples from The Cancer Genome Atlas, clustering samples into groups based on TE expression profiles, and identifying genes that are differentially expressed between the two clusters.

Results

Clustering analysis identified two distinct groups of samples based on their TE expression profiles, separating the samples into basal and luminal subtypes. 899 genes were found to be differentially expressed among the identified clusters.

Conclusion

The clustering analysis revealed a strong correlation between TE expression profiles and the biologic and clinical subtypes of bladder cancer. The identified differentially expressed genes serve as potential key players in the intricate transcriptional regulation of TEs. Future steps include identification and characterization of other potential regulators among the differentially expressed genes and exploration of additional layers of regulation.

Afternoon Session

1. Dure Khan (Field: Brain Cancer, Focused Ultrasound) **FOCUSED ULTRASOUND STIMULATED MICROBUBBLES MEDIATE BLOOD-BRAIN BARRIER OPENING AND BRAIN TUMOUR MICROENVIRONMENT CHANGES.**

Dure S. Khan[1], Christopher J. B. Nicol[2] & Ryan Alkins[1]. [1]Center for Neuroscience Studies, [2]Dept. of Pathology & Molecular Medicine, Queen's University, Kingston, Ontario, Canada.

Brain tumours are hard to treat due to Blood-Brain Barrier (BBB) limiting the passage of drugs into the parenchyma. Focused Ultrasound (FUS) stimulated Microbubbles (MBs) are a non-invasive, image-guided technology allowing for safe, transient disruption of the BBB. While preclinical studies have showed alterations in cytokines and chemokines of healthy brain post-sonication with MBs, elucidating the effects of FUS & MBs on tumour microenvironment (TME) is needed. This study aims to characterize the cytokine and chemokine profiles of breast-cancer metastases in the brain post-sonication with MBs. It is hypothesized that pro-inflammatory cytokines will be detected in higher concentrations in tumour versus healthy brain tissue post-FUS&MB treatment. 10,000 human metastatic MDA-MB-231 breast cancer cells were directly injected into the right frontal lobe of immunocompromised mice via a burr-hole.

Tumour growth was monitored by MRI, and mice were treated with FUS&MBs when tumours reached a diameter of 3-5mm. Pre-and post-treatment MRI scans were obtained to visualize the BBB-opening, validated with intravenous injections of 2% Evans Blue. Mice were euthanized 1-hour post-treatment and fresh and frozen samples were collected for analyses. Preliminary results from female mice (n=4) suggest Eotaxin, G-CSF, IL-9 and IL-12p70 levels were significantly increased ($p<0.05$) in tumour vs healthy samples. This study will improve understanding of tumour and TME alterations following FUS&MB treatment and guide future therapies for brain tumour patients.

2. Maria Korovina (Field: Medicine) **DIRECT AND INDIRECT CHALLENGE TESTS TO ASSESS ASTHMA, COUGH VARIANT ASTHMA AND CHRONIC COUGH: A LITERATURE REVIEW**

Maria Korovina[1, 2], Nicolle Domnik[1] and M. Diane Lougheed[1, 2]

[1]Queen's University, Kingston, ON Canada; [2]Kingston General Hospital Research Institute, Kingston, ON Canada

Background:

Asthma and cough variant asthma (CVA) are characterized by non-specific airway hyperresponsiveness. Recently, we have described individuals with chronic cough (lasting ≥8 weeks) who are suspected clinically to have CVA but have methacholine-induced cough with normal airway sensitivity (COUGH). We hypothesize that asthma, CVA and COUGH represent clinically relevant airway disease phenotypes, distinguishable by their responses to direct and indirect challenges and deep inspirations (DIs).

Objective:

To review the literature regarding responses to direct inhalation challenge tests, and bronchodilating and bronchoprotective effects of DIs, in asthma, CVA and COUGH.

Methods:

The OVID Medline database was used to search key terms in primary articles, systemic reviews, and published guidelines relating to cough variant asthma, chronic cough, and indirect inhalation challenges.

Results:

Despite developing small airway obstruction, dynamic hyperinflation, and gas trapping, individuals with COUGH experience significantly less bronchoconstriction than those with asthma. These symptoms partially resolve following a DI and a cough, but the clinical relevance is unknown. Hypertonic saline tests show enhanced cough sensitivity in individuals with chronic cough and asthma, while mannitol leads to more cough in individuals with asthma than healthy. Both groups have absence of significant bronchoconstriction during these tests.

Conclusion:

A DI and a cough may be a significant observational characteristic. Indirect challenges may highlight pathophysiological differences between CVA and COUGH, suggesting distinct phenotypical differences from classic asthma.

3. Chinmay Potdar (Field: Microbiology and Immunology) **COMBINING CYTOKINE MULTI-OMICS AND MACHINE LEARNING FOR INFLAMMATORY BOWEL DISEASE EVALUATION: IDENTIFYING NEW MECHANISTIC DISEASE CLASSIFICATIONS**

Chinmay Potdar, BSc[1,2], Olimpia Sienkiewicz, MSc[1,2], Carly van Wylick[1], Daniel J. Mulder, MD, PhD[1,2,3]

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: Personalized treatment of inflammatory bowel disease (IBD) is a difficult clinical challenge. Large variation in clinical presentation between IBD patients is a major reason for this problem. Cytokine concentrations in peripheral blood are known to be a driving force behind the complex inflammatory process in IBD and warrants understanding as they may be responsible for the large variation in presentation.

Hypothesis: Cytokine multiplex analysis of IBD patient peripheral blood samples provide mechanistic insight into IBD disease classification, that is not evident using current methods of standard clinical evaluation.

Methods: Both IBD (n=63) and control patients (n=118) were enrolled in this study (HSREB 6033229). Cytokine profiles were investigated using a 17-plex multi-fluorescent bead-based immunoassay on serum from control patients (n=8) and IBD patients (n=5 with active disease and n=10 in remission). Clinical data was collected by chart review. Machine learning was performed on the resulting data using custom R scripts utilizing the {tidymodels} and {XGBoost} packages.

Results: Uniform Manifold Approximation and Projection dimension reduction based on cytokine concentrations showed clustering differences between the groups. Extreme Gradient Boosting (XGBoost) is a machine learning model implementing decision trees to identify prediction factors for an outcome. An XGBoost model identified lower concentrations of MIP-1a as highly associated with IBD samples over controls. A Reciever Operator Characteristic (ROC) curve generated for MIP-1a as a negative predictor of IBD had an Area Under the Curve (AUC) value of 0.788.

Conclusions: Multiplex cytokine analysis with machine learning can identify novel molecular patterns in IBD patients.

4. Nasry Zane Bouzeineddine and Alecco Philippi (Field: Microbes, Immunity and Inflammation) **INVESTIGATING THE RESPONSE OF GM-CSF STIMULATED BONE-MARROW DERIVED CELLS TO LCMV INFECTION**

Nasry Zane Bouzeineddine, Alecco Philippi, Katrina Gee and Sam Basta

Department of Biomedical and Molecular Sciences, Queen's University, Kingston, ON, Canada, K7L 3N6

Macrophages are crucial immune cells that provide innate immunity against pathogens. Upon activation with specific mediators in the environment, known as cytokines, macrophages develop into classically activated macrophages (M1). Such cells are known to be pro-inflammatory and are associated with antiviral immune responses. Granulocyte Macrophage Colony Stimulating Factor (GM-CSF) is a cytokine that can affect macrophage development and differentiation towards an M1-like phenotype and can affect the development of other myeloid cells, such as dendritic cells. Few studies have investigated the direct response of GM-CSF activated macrophages during viral infections. To gain a greater understanding of the antiviral response of these cells upon infection, an *in vitro* model was used to elucidate the effects of GM-CSF on macrophages during lymphocytic choriomeningitis virus (LCMV) infection. LCMV is a well-established model for investigating immune responses to viral infection in the murine model. Our data suggests that GM-CSF does limit LCMV infection in cells cultured with GM-CSF and it induces the upregulation of antiviral genes. This work will elucidate the mechanisms by how this cytokine can affect macrophage immune functions during infections and highlights the potential use of GM-CSF for antiviral therapeutics.

5. Amelia Potts (Field: Cancer Research and Therapy) **DEVELOPING A CO-CULTURE MODEL OF OVARIAN CANCER TO TEST IMMUNOTHERAPIES TARGETING TGF-ß**

Amelia Potts, Jacob Kment, Anna Nicolela, Andrew Craig. Departments of Biomedical and Molecular Sciences, Queen's University, Kingston, Ontario, Canada.

Epithelial ovarian cancer (EOC) is the most fatal gynecological cancer, partly due to frequent late-stage diagnosis and high recurrence rates following primary treatment. Immune checkpoint inhibitors (ICIs) are not yet approved for treating EOC, as immunosuppressive resistance mechanisms dampen their therapeutic efficacy. Transforming growth factor beta (TGF-ß) is an immunosuppressive cytokine linked to poor prognosis in EOC. Among other functions, TGF-ß suppresses CD8+ T cell cytolytic effectors expression, including granzyme B (GzmB). We established a simplified, *in vitro* co-culture EOC model to

evaluate the role of TGF-ß in immunotherapy resistance. Murine BR5-Luc ovarian cancer cells expressing either a chicken ovalbumin (OVA) epitope, or a scrambled (SCR) epitope control, plus a GzmB-cleavable Förster resonance energy transfer (FRET) reporter were prepared. When co-cultured with purified OVA-specific OT-1 mouse splenic T cells, a decrease in FRET signal was observed compared to baseline levels. BR5 cell killing by OT-1 T cells was assessed with a propidium iodide uptake assay, in which increased killing was detected in co-cultures treated with Galunisertib (LY), a TGFßR1 inhibitor, compared to vehicle control. Further, LY treatment significantly increased the expression of GzmB in OT-1 T cells. Future co-culture studies will analyze TGF-ß inhibitors in combination with ICIs to characterize changes in T cell activation or exhaustion phenotypes. Overall, we aim to improve immunotherapy options for EOC patients by simplifying screening methods for ICI efficacy. (Supported by the Canadian Institute for Health Research.)

6. Heidi Scott (Field: Microbes, Immunity and Inflammation) **CHARACTERIZING THE ROLE OF TOLL-LIKE RECEPTOR 4 POLYMORPHISMS IN VIRAL PATHOGENESIS**.

Heidi M. Scott and Che C. Colpitts. Department of Biomedical and Molecular Sciences. Queen's University, Kingston, Ontario, Canada.

The recent SARS-CoV-2 pandemic, which killed millions, highlights our vulnerability to emergent RNA viruses. The pathogenesis of these viruses is closely tied to the expression of glycosylated viral proteins called glycoproteins. Several viral glycoproteins interact with innate immune receptors, such as Toll-like receptor 4 (TLR4). TLR4 activation by its well-characterized bacterial ligand, lipopolysaccharide (LPS), can initiate antiviral and/or proinflammatory responses via activation of IRF-3 or NF-κB transcription factors, respectively. Differences in TLR4 signaling are thought to influence the severity of disease outcomes. A hallmark of severe infection is an excessive and detrimental inflammatory response, termed cytokine storm. How viral glycoproteins activate TLR4 and its downstream signaling pathways is still unclear. Furthermore, genotypic variation in TLR4 exists within the population, and common polymorphisms (e.g., D299G, T399I) may impact disease outcomes, although the underlying mechanisms remain poorly understood. Through quantifying activation of IRF-3 and NF-κB using reporter assays, we are comparing the immunological consequences of activation by viral glycoproteins between TLR4 polymorphisms. We have observed slight differences in TLR4-mediated activation by D299G TLR4 in response to LPS, and are currently exploring how TLR4 polymorphisms impact responses to viral glycoproteins. This work will improve our understanding of why some individuals develop more severe disease than others as a result of viral infection. It may also inform the development of novel treatment options for viral disease.

7. Sanjana Kapuria (Field: Cardiovascular Disease) **THE ROLE OF EXTRARENAL TISSUE IN VITAMIN D METABOLISM IN CHRONIC KIDNEY DISEASE**

Sanjana Kapuria[1], Sono Khan[1], Tyler Rowsell[1], Michael A. Adams[1]
[1]Department of Biomedical and Molecular Sciences, Queen’s University, Kingston, ON

Vitamin D (VD) deficiency is characteristic in chronic kidney disease (CKD). Although circulating levels of $25(OH)D_3$ and $1,25(OH)_2D_3$ are common indicators of VD sufficiency, the extrarenal VD metabolome may more meaningfully inform VD metabolomics in CKD. Circulating levels of VD alone may not accurately predict VD biological action. Relying solely on circulating VD to optimize VD supplementation in CKD has led to off-target effects (e.g., vascular calcification), indicating the necessity to establish how the extrarenal VD metabolome contributes to VD status. Thus, it is important to quantify VD metabolites in extrarenal tissues, assessing its role in VD production. The presented study establishes the potential for extrarenal tissue to produce VD. Aiming to assess the capacity of calcifediol ($25(OH)D_3$) to supress parathyroid hormone (PTH) in CKD rats with secondary hyperparathyroidism, it was found that VD supplementation induced PTH suppression despite only marginally increasing circulating levels of active VD ($1,25(OH)_2D_3$). This suggested that extrarenal tissue-based production of active VD likely contributes to its biological action. Additionally, calcifediol treatment induced inconsistent changes in precursor and product VD metabolites, implying their extrarenal production. The results also implicated $1,24,25(OH)_3D_3$ as a novel bioactive metabolite. Overall, these findings provide support for analyzing the extrarenal VD metabolome in humans. By addressing the extrarenal contribution to VD metabolism, clinicians may better inform patient VD status, thereby optimizing VD-based treatment strategies for CKD patients.

8. Farzaneh Afzali (Field: Cancer Research and Therapy) **PKR MODULATES OVARIAN CANCER CELLS ADAPTIVE RESPONSES THROUGH MECHANISMS INVOLVING RHO-GTPASES**

Farzaneh Afzali, Tyler Cooper, Bianca Hill, Lynne-Marie Postovit*

Ovarian cancer (OV) is the primary cause of mortality among female reproductive malignancies. The clinical outcome is closely linked to peritoneal dissemination, influenced by multiple factors. To metastasize, cells must survive as spheres in ascites fluid, but also must dissociate, survive, and proliferate in secondary tissues. Using proteomics and Western blotting, we determined that the high grade serous (HGSC) subtype of ovarian cancer has an abundant expression of Protein Kinase RNA-activated (PKR), a stress-activated kinase that governs translation initiation, NF-kB activation, cell to cell adhesion, and the transcriptional control of cell death and survival factors. We showed that PKR kinase activity (indicated by phosphorylation) is not induced upon 3D growth but that knocking down PKR increases the ability of OV cells to form spheres, concomitant with tighter aggregation patterns. Herein, I hypothesize that PKR increases OV cell adhesion and survival in a non-canonical (kinase independent) manner. Using single cell spheroid formation assays, PKR-KD (OVCAR8) cells generated a higher number of spheres. Substrate independent culturing of Tyk-nu and OVCAR8 PKR-KD cells showed a tighter pattern of aggregation in these cells. Cell trace violet flow cytometry proliferation assays and Trypan blue cell exclusion assays revealed a higher survival and proliferation rate in PKR-KD cells in 3D culture. 2D Matrigel Boyden chamber assay revealed a notable decrease in PKR-KD cells invasion. To investigate the underlying mechanisms, DATA-independent acquisition (DIA) mass spectrometry proteomics and RNA-sequencing were performed on Tyk-nu and OVCAR8 (shPKR vs shGFP), 2D and 3D cultured cells. Gene Set enrichment analysis was performed on the significant differentially expressed factors. Regulatory network analysis revealed significant associations between RHO-GTPases, cytoskeleton regulators, and adhesion markers, supporting our hypothesis. Our results indicate that PKR KD may improve adaptation to 3D culture via mechanisms

involving RHO GTPases. Future studies will be conducted to determine the roles of sub-regulatory networks in the process of metastasis in mice models.

*Funded by: CIHR

9. Cierra Perron (Field: Biomedical and Molecular Sciences) **SFRP1 PROMOTES MELANOMA METASTATIC PHENOTYPES.**

Cierra Perron, Douglas Quilty, Krista Vincent, Lynne-Marie Postovit*
Department of Biomedical and Molecular Science, Queen's University, Kingston, Ontario, Canada.

Melanoma accounts for only 2% of diagnosed skin cancers, however results in the majority of deaths associated with skin cancer. This is widely attributed to the high metastatic potential of melanomas and the subsequent acquisition of therapeutic resistance. Using RNA sequencing analysis, we identified Secreted Frizzled Related Protein 1 (sFRP1) as a top differentially expressed gene in invasive melanoma. sFRP1 is a member of a family of secreted, matricellular glycoproteins which function to modulate Wnt signalling pathways. Multiple branches of Wnt signaling exist, including the canonical, beta-catenin dependent pathway and non-canonical WNT/Ca2+ pathway. Both arms of Wnt signalling have been proposed to play a role in the phenotype switching between proliferative and invasive phenotypes. While the role of sFRP1 in melanoma is not well established, previous data from our lab has associated sFRP1 expression with poor clinical outcomes, tumour growth and invasive phenotypes in melanoma. As such, we hypothesize that sFRP1 promotes metastasis *in vivo,* through altering Wnt signaling pathways. Canonical (beta-catenin dependent) and non-canonical (beta-catenin independent) Wnt signaling pathways were assessed via western blot in 2D, 3D and Matrigel cultures. It was found that sFRP1 expression increases activation of canonical Wnt signalling, while activation of the non-canonical pathway remains unchanged in human melanoma cell lines. Future directions will assess the Wnt profiles in melanoma and to assess mechanisms by which sFRP1 promotes melanoma metastasis.

10. Jill Greenlaw (Field: Translational Medicine) **EVALUATING THE SAFETY OF A NOVEL PROTEASE FOR THE TREATMENT OF *CLOSTRIDIOIDES DIFFICILE* INFECTION IN A MURINE MODEL**

Jill Greenlaw[1,2], Katya Douchant[2,3], Shu Mei He[2], Calvin Sjaarda[4], Prameet M. Sheth[1,2,3,4]

1.Department of Translational Medicine, 2. Gastrointestinal Diseases Research Unit, 3. Department of Biomedical and Molecular Sciences, 4. Kingston Health Sciences Centre – Division of Microbiology

Introduction*: Clostridioides difficile* (CD) is the number one cause of antibiotic-associated diarrhea and infectious colitis. The pathogenesis of CD disease is mediated via the release of two toxins (TcdA and TcdB). Our laboratory recently isolated a protease from gut from CD disease *in vivo*. Although HTRA-1 appears to be well-tolerated in mice, the safety of HTRA-1 needs to be assessed both on the host and the gastrointestinal microbiota.

Methods: Based on a well-defined mouse model of CD infection, mice were given antibiotic drinking water for 3 days followed by treatment with either a vehicle control or 200, 400, or 800 ug of HTRA-1 administered daily by oral gavage. Mice were weighed daily and colons were harvested post-

euthanization. Stool specimens were collected pre-antibiotics, post-antibiotics, pre-HTRA-1, and 24-, 48-, and 72-hours post-HTRA-1, and processed for 16S Next Generation Sequencing (NGS).

Results: Stool microbiome analysis of mice treated with HRTA-1 found no changes in the relative abundances of *Akkermansia*, *Bacteriodes*, *Lactobacillus, Clostridium, Ruminococcus,* and *Turicibacter (p>0.1* for all) compared to mice exposed to vehicle control. Mice treated with HTRA-1 experienced no significant change in body weight (p=0.1156) or colon lengths (p=0.8857), both a sign of gastrointestinal inflammation in mice.

Conclusions: These findings suggest that HTRA-1 does not impact the murine gastrointestinal microbiota, and does not impact murine body weight, or colonic length.

This research was supported by an NIH R33 grant.

11. Karine Roversi (Field: Cancer Research and Therapy) **OPTOGENETIC ACTIVATION OF TUMOR-INNERVATING NEURONS STOPS HOST IMMUNITY**

Karine Roversi, Sebastien Talbot

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

The immune system detects and destroys abnormal cells, including cancer cells. However, the sustained activation of specific classes of membrane-bound receptors, leads to the functional exhaustion of immunocytes, greatly limiting their cytotoxic capabilities. Sensory nerves play a central role in cancer pathogenesis, driving tumor progression and dissemination, possibly by altering anti-tumor immunity. The optogenetic activation of skin nociceptors mediates anticipatory immunity against microbes, and can potentiate skin immunity by triggering the antidromic release of neuropeptides. Here, we evaluate whether the optogenetic stimulation of light-sensitive tumor-innervating neurons ($Na_V1.8^{cre}$::$ChR2^{fl/wt}$) modulates host immune responses against the cancer cells.

B16F10-mCherry-OVA ($5x10^5$ cells; i.d.) malignant cells were inoculated in the hind limb of 8-week-old female $Na_V1.8^{Cre}$::$ChR2^{fl/wt}$ mice. Once-daily, melanoma-innervating neurons were stimulated and tumor growth assessed.

When initiated when the tumor was visible or reaching $200mm^3$ volume, optogenetic activation of tumor-innervating neurons increased B16F10 tumor growth. Furthermore, intra-tumoral cytotoxic $CD8^+$ T-cells showed decreased expression of $IL\text{-}2^+$ compared to the control not blue-light stimulated. When blue-light stimulation started immediately after tumor inoculation, we observed a transient increase in tumor growth (days 5 and 6).

Tumor-innervating neurons gain-of-function increased tumor progression, in part, by modulating the function of intra-tumoral cytotoxic CD8 T-cells. Blocking the function of these neurons may help stop melanoma progression.

Supporting agency: Mitacs, CIHR, NSERC

12. Declan Gainer (Field: Cardiopulmonary Immunology) **SELECTIVE DELETION OF THE NATURAL KILLER CELL TYPE II TGFB RECEPTOR IMPAIRS MURINE LUNG DEVELOPMENT AND FUNCTION.**

D.J. Gainer[1], K.M. Coyle[1], K.L. Laverty[2], N.J. Domnik[2], M.L. Ormiston[1,2]

[1]Department of Medicine, Queen's University, Kingston, ON

[2]Department of Biomedical Sciences, Queen's University, Kingston, ON

Background: Bronchopulmonary dysplasia (BPD) is a lung disease of alveolar simplification and impaired pulmonary vascular development that is strongly linked to premature birth and inflammatory lung injury. We have shown that elevated transforming growth factor-β (TGFβ) signaling in a subset of innate lymphoid cells, termed natural killer (NK) cells, is associated with pathological vascular remodeling in adults with pulmonary arterial hypertension. However, the contribution of NK TGF-β signaling to neonatal pulmonary vascular development and BPD is unknown.

Methods and Results: Lung development was assessed from postnatal (P) day 1-7 in mice bearing a deletion of the type-II TGF-β receptor in their NK cells (*Tgfbr2*$^{NK-/-}$) and *Tgfbr2*$^{NK+/+}$ littermate controls. *Tgfbr2*$^{NK-/-}$ neonates exhibited reduced pulmonary vascular density by P3, which coincided with a surge in lung-localized NK cells relative to controls. Vascular defects preceded alveolar simplification, which was observed in *Tgfbr2*$^{NK-/-}$ mice by P5. This BPD-like phenotype manifested functional lung defects in adult (8-12 week) mice, including reduced pulmonary arteriolar density and mild pulmonary hypertension, as well as reduced inflation compliance (C_{Inf}) in male, but not female *Tgfbr2*$^{NK-/-}$ mice.

Conclusion & significance: We have identified NK cell TGF-β signaling as a key regulator of pulmonary vascular and airway development. Upcoming single cell RNA sequencing will define this phenotype at the transcriptional level, with the goal of developing novel immune-targeted treatments for a leading cause of neonatal mortality.

(Work supported by the CIHR)

13. Bianca Dauber (Field: Breast Cancer) **HYPOXIA-INDUCED TRANSCRIPTIONAL AND EPIGENOMIC ALTERATIONS REGULATE MRNA TRANSLATION BY TRANSCRIPTIONAL START SITE SWITCHING.**

Kathleen Watt[1], Laura Lee[2], Predrag Jovanovic[3], Bianca Dauber[4], Krzysztof Szkop[1],

Ranveer Palia[3], Ola Larsson[1], Ivan Topisirovic[3] & Lynne-Marie Postovit [4]

1 Department of Onkology-Pathology, Science for Life Laboratories, Karolinska Institutet, Stockholm, Sweden

2 Department of Oncology, University of Alberta, Edmonton, AB, Canada

3 Lady Davis Institute, Departments of Oncology and Biochemistry, Division of Experimental Medicine, McGill University, Montreal, QC, Canada

We previously observed that the selective translation of stem cell-associated factors (NODAL, SNAIL, NANOG) is enabled by the presence of multiple 5'UTR sequences. In this study, NODAL, SNAIL and NANOG protein levels accumulated despite marked reductions in global protein synthesis due to the presence of multiple 5'UTR sequences; at least one of which could be efficiently translated in hypoxia.

Using NanoCage sequencing from total and polysome associate mRNA fractions, we have revealed that transcriptional start site (TSS) switching, leading to alterations in 5'UTR sequences but not affecting the

proteoform, is a major regulator of mRNA translation in hypoxia. This switching allows for the emergence of known elements, such as uORFs, as well as hitherto unappreciated regulatory sequences that collaborate with the translational machinery to coordinate adaptive translation in response to hypoxia. In particular, TSS switching appears to regulate the production of proteins, including PDK1, involved in the requisite switch to glycolytic metabolism. Mechanistically, we determine that only a subset of translation-regulating TSS events are associated with HIF1a binding. Rather, a large proportion appear to be due to reductions in KDM5 activity, concomitant with an expansion of H3K4Me3.

14. Rachel Bishop (Cancer Research and Therapy) **TESTING EFFECTS OF TGFB INHIBITORS ON CHEMORESISTANCE IN OVARIAN CANCER**

Rachel Bishop, Stephanie Young, and Andrew Craig

Department of Biomedical and Molecular Sciences, Cancer Biology & Genetics/Queen's Cancer Research Institute, Queen's University, Kingston, Ontario, Canada

High grade serous ovarian carcinoma (HGSC) is the most fatal gynecological cancer, that often develops resistance to chemotherapies like Doxorubicin (Dox). High Transforming Growth Factor Beta (TGFβ) is linked to poor prognosis in HGSC, and acts as a driver of chemoresistance and metastasis by promoting epithelial to mesenchymal transition (EMT). TGFβ also suppresses Granzyme B (GzmB) expression in CD8+ T cells and suppresses immune responses during chemotherapy. We hypothesize that blocking TGFβ signaling with the TGFβR1 inhibitor Galunisertib (LY), will enhance chemotherapy response in mouse HGSC models. Treatment of ID8-Trp53$^{-/-}$; Nf1$^{-/-}$ cells with TGFβ increased EMT transcription factor levels and increased the Dox IC_{50}, which was reversed by co-treatments with LY. We extended these studies to syngeneic mouse HGSC models, treated with a vehicle, Dox, LY, or the combination. Mice were imaged weekly with IVIS, and all treatments significantly decreased the tumour burden with the combination having the greatest reduction. Furthermore, LY treatment both with and without Dox resulted in decreased SNAIL expression and increased GzmB expression in ascites cells. These results provide rationale for testing TGFβ inhibitors in combination with chemotherapy to prevent ovarian cancer recurrence and progression.

15. Alison McCallion (Field: Reproductive and Developmental Sciences) **DEFINING THE TH9-MAST CELL RELATIONSHIP IN ENDOMETRIOSIS PATHOPHYSIOLOGY**

Alison McCallion (PhD Candidate), Danielle Sisnett (PhD Candidate), Katherine Zutautas (PhD Candidate), Chandrakant Tayade. Department of Biomedical and Molecular Sciences (Reproductive and Developmental Sciences), Queen's University.

Introduction: The interface of endocrine and immune dysregulation affecting endometriosis (EM) lesion growth is an evolving concept. Interleukin-9 (IL-9) is a cytokine with roles in inflammation and fibrosis. IL-9 is a vital growth factor for mast cells, which have been implicated in EM pathophysiology. T helper 9 (Th9) cells are IL-9-producing T cells involved in inflammatory diseases like cystic fibrosis and endometrial carcinoma. Within endometrial carcinoma, IL-9 production by Th9 has been found to be

regulated by estrogen. The relationships between Th9, mast cells and and E2 have not been defined in EM pathophysiology.

Aims: Understand involvement of IL-9 and Th9 cells in EM pathophysiology, both independently and with respect to mast cell activity. Define the role of Th9 cells within a mouse model of EM and discern effects of E2 on IL-9-producing immune cell populations.

Methods: Using our EM mouse model, groups of C57Bl/6 mice were treated with or without E2 and received adoptive transfer of Th9-like lymphocytes or saline control. Peritoneal fluid (PF), blood plasma and EM lesions were collected; flow cytometry was used to classify immune cell populations in PF and spleen. Using primary human peripheral blood mononuclear cells (PBMC), CD4+ T cells were driven in culture towards Th9 phenotype. Then, *in vitro* experiments were conducted with hormonal treatments of estrogen and progesterone to capture cytokinic signalling responses within human Th9 cell populations. Human mast cell line HMC-1 cells were "co-cultured" with conditioned media from Th9-PBMC culture supernatants to capture cytokinic signalling relationships.

Results: E2-treated mice showed a significant reduction in splenic populations of IL-9-producing immune cells (Th, mast cells) and IL-9R-expressing cells (neutrophils, CD8a+ dendritic cells). E2 increased PF concentration of IL-9-modulating cytokines (IL-6, CXCL-10). Plasma concentrations of IL-9 significantly decreased in E2-treated mice but not untreated mice. *In vitro*, E2 treatments significantly increased IL-9 production from human Th9-driven PBMC, along with several other chemotactic and inflammatory cytokines. Co-culture experiments with HMC-1 cells revealed rich cell crosstalk influenced by hormonal conditions, where cytokinic secretory responses were significantly magnified downstream upon mast cell exposure to Th9-driven PBMC media.

Significance: These results demonstrate an impact of E2 on IL-9-producing T cells and Th9/mast cell signalling within EM. Continuing to decode the complex immunopathophysiology of EM will lead to new opportunities in therapeutic development.

Funding source: CIHR, Queen's University.

16. Hailey Gowdy (Field: Neuroimmunology) **CIRCAHEALTH: EXAMINING THE CIRCADIAN CONTROL OF BIOPSYCHOSOCIAL FACTORS IN CHRONIC PAIN ON A NATIONAL LEVEL.**

Hailey Gowdy[1], Doriana Taccardi[1], Amanda Zacharias[1], Mitra Knezic[1], Lesley Norris Singer[2], Jennifer Daly-Cyr[2], Etienne J. Bisson[3-5], Scott Duggan[3], Manon Choinière[6], Zihang Lu[7], Qingling Duan[1,8], M. Gabrielle Pagé[6], and Nader Ghasemlou[1,3,4]. [1]Department of Biomedical and Molecular Science, Queen's University, Canada. [2]Chronic Pain Network, McMaster University, Canada. [3]Department of Anesthesiology and Perioperative Medicine, [4]Centre for Neuroscience Studies, [5]School of Rehabilitation Therapy, Queen's University, Canada. [6]Department of Anesthesiology and Pain Medicine, Université de Montréal, Canada. [7]Department of Public Health Sciences, [8]School of Computing, Queen's University, Canada.

20% of the Canadian population lives with chronic pain, for which current treatments are insufficient. In the pursuit of individualised treatment strategies, it is crucial to know *why* and *when* someone has pain. As such, pain fluctuations represent an interesting and understudied research target. It is hypothesized that circadian rhythms play a role in the pathogenesis of chronic pain, given their importance in regulating neuroimmune function. Our study CircaHealth used an online survey to examine the circadian control of chronic pain in the Canadian population.

Following an initial questionnaire, participants completed a series of electronic symptom-tracking diaries (ecological momentary assessments), in which they rated their pain intensity, negative affect, and fatigue on a 0-10 scale at 3 timepoints (08:00, 14:00, 20:00) daily for one week.

Distinct patterns of pain rhythmicity were identified (e.g., constant, rhythmic, mixed), with different pain condition cohorts displaying diversity in the 'phenotypic patterns' observed in their participants. Further analysis determined associations between pain rhythmicity patterns and other variables, such as type of chronic pain, anxious and depressive symptoms, exercise, and sleep habits.

This work will deepen our understanding of 24-hour pain fluctuations by uncovering distinct pain rhythmicity patterns and potential predictors for their occurrence, which may help to develop new management and preventive strategies for chronic pain. (Supported by CIHR-SPOR Chronic Pain Network.)

17. Marty VandenBroek (Field: Endothelial Cell Biology) **CIRCULAR RNA PROFILING IDENTIFIES A CAPRIN-1-DEPENDENT ROLE FOR *BMPR2*-DERIVED TRANSCRIPTS IN PULMONARY ENDOTHELIAL TRANSLATION AND PROLIFERATION**

M. Martin VandenBroek, BSc[1], Mackenzie C. Sharp, BSc[2], Patrick Thompson[3], Emmanuel O. Fagbola[1], Douglas Quilty[3], Jeffrey D. Mewburn[1], Anne L. Theilmann, MSc[3], Stephen L. Archer, MD[1], Neil Renwick, MD, PhD[4], Mark L. Ormiston, PhD[1,3]

1. Queen's University Department of Medicine, Kingston, Canada
2. Queen's University School of Computing, Kingston, Canada
3. Queen's University Departments of Biomedical and Molecular Sciences and Surgery, Kingston, Canada
4. Queen's University Department of Pathology and Molecular Medicine, Kingston, Canada

Background – Mutations in *BMPR2*, the gene encoding the type-II BMP receptor, are strongly associated with pulmonary arterial hypertension (PAH) and the pathological proliferation of pulmonary artery endothelial cells (PAECs). While studies of *BMPR2* mutation in disease primarily focus on loss of the protein receptor, the functional contribution of *BMPR2* transcripts, including circular RNAs (circRNAs), is unknown.

Methods and Results – Ultra-deep RNA sequencing identified *circ5078*, a novel *BMPR2* derived circRNA. Silencing *circ5078* doubled PAEC proliferation via increased Cyclin D1 translation, an effect not observed with silencing of linear *BMPR2*, or linear and circular transcripts in combination. This effect was mediated by Caprin-1, a known translational regulator and component of stress granules (SGs). Polyribosome profiling identified an increase in ribosomal subunit assembly with *BMPR2* transcript loss, suggesting a role for these RNAs in the regulation of translational initiation. Moreover, silencing linear and circular *BMPR2* transcripts had opposing effects on arsenite-induced SG formation, with linear *BMPR2* loss suppressing SGs and *circ5078* depletion dramatically increasing SG assembly. Reduced SG formation was

also identified in the endothelium of mutation-bearing PAH patients, who lack linear but not circular *BMPR2* transcripts.

Conclusions – We have identified contrasting roles for circular and linear *BMPR2* transcripts as functional regulators of endothelial translation, proliferation and stress responses via interaction with Caprin-1. This work is essential to understanding the wholistic contribution of *BMPR2* to health and disease.

18. Dominique Hancock (Field: Neuroscience) **INVESTIGATING COLD SPREADING DEPOLARIZATION (COLD SD) IN RODENT BRAIN SLICES.**

Dominique A. Hancock, Edwin R. N. Kiarie, Peter J. Gagolewicz, and R. David Andrew. Centre for Neuroscience Studies, Queen's University, Kingston, Ontario, Canada.

Neuronal damage induced by mild hypothermia (<15°C) was first demonstrated in non-hibernating mammals where cold temperatures caused cerebral edema, with an associated accumulation of Na^+ intracellularly and K^+ extracellularly (Thauer, R., & Brendel, W., 1962). As well, a so-called 'chill coma' has been documented in insects (locusts, fruit flies) when they are exposed to near-freezing (n/f) temperatures, evoking spreading depolarization (SD) (Robertson, R. M., et al., 2020). We propose that, surprisingly, exposure to n/f temperature causes an SD-like event in mammalian higher brain, which we term `cold SD`. SD is a mass wave of propagating cellular depolarization caused by failure of the Na^+/K^+ pump particularly in neurons. SD during ischemia causes cell swelling, neuronal injury, and death as during stroke, traumatic brain injury, and sudden cardiac arrest. We hypothesize that cold-SD can be caused by compromised Na^+/K^+ pump function due to reduced ATP production at n/f temperatures. This study builds on research that shows neuronal swelling and dendritic beading in rodent slices upon cooling to ~6°C (Kirov, S., et al., 2004) and on our previous lab findings that n/f temperature (3-6°C) evokes cold SD in rodent slices, observable by imaging changes in light transmittance (LT). As bath temperature dropped from 10°C to 3-6°C over 200 to 300 seconds, LT decreased in neocortex coinciding with a slow positive drift in extracellular voltage of 2-3 mV recorded with a KCl pipette. Then, when SD initiation was imaged, a negative DC shift of 2 to 4 mV coincided with the front passing the pipette, so this is a classic SD event with a slower propagation and reduced peak cell swelling as compared to OGD-SD. As well, slices that underwent cold SD when slowly warmed could generate OGD-SD, demonstrating clear recoverability from cold-SD. The TRPM8 receptor is involved with cold reception, but its specific antagonist PBMC (25nM) did not affect cold SD parameters. We conclude that 1) the standard technique of preparing rodent slices at n/f temperatures often induces cold SD from which slices recover upon warming; 2) Surprisingly, energy to drive cold-SD is maintained even in n/f mammalian gray matter because the Gibbs-Donnan equilibrium remains intact by Na^+/K^+ ATPase transport. The pump maintains the chemical and electrical energy differential between the intra- and extracellular compartments. So, while heat can be pulled from the tissue by lowering temperature, SD can still be generated so long as the pump functions. 3) Although non-hibernating mammals cannot survive n/f temperatures due to systemic failure, certain bat species likely undergo cold SD and survive in the real world, much like insects.

References

Kirov, S. A., Petrak, L., Fiala, J., & Harris, K. (2004). Dendritic spines disappear with chilling but proliferate excessively upon rewarming of mature hippocampus. Neuroscience, 127(1), 69-80. doi: 10.1016/j.neuroscience.2004.04.053

Robertson, R. M., Dawson-Scully, K. D., & Andrew, R. D. (2020). Neural shutdown under stress: an evolutionary perspective on spreading depolarization. *Journal of Neurophysiology*, *123*(3), 885–895. https://doi.org/10.1152/jn.00724.2019

Thauer, R., & Brendel, W. (1962). Hypothermie. *Progress In Surgery*, 73-271. doi: 10.1159/000386265

19. Madeleine Carew (Field: Pathology and Molecular Medicine) **INVESTIGATING THE ROLE OF PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR Γ (PPARΓ) IN INVASIVE DUCTAL CARCINOMA (IDC)**

Madeleine Carew[1], Natasha Iaboni[1], Rachel R. Rubino[2] and Christopher JB Nicol[1,2].

[1]Dept. of Pathology & Molecular Medicine; [2]Division of Cancer Biology & Genetics, Cancer Research Institute. Queen's University, Kingston, ON, Canada

Invasive ductal carcinoma (IDC) is a form of breast cancer originating from the lining of the milk ducts that has invaded into surrounding breast tissue. Due to its invasive nature, IDC can further metastasize throughout the body, affecting lymph nodes and other organs. Current research is interested in investigating and validating prognostic biomarkers in an attempt to better understand IDC and improve patient care. Our lab focuses on the peroxisome proliferator-activated receptor gamma (PPARγ), a ligand-activated transcription factor involved in normal sugar and lipid metabolism. PPARγ is also expressed in a wide variety of cells in the breast and our lab provided the first direct evidence that PPARγ suppresses breast tumor progression, although its role in IDC remains unknown. Here, we hypothesized that the activation of PPARγ signaling with a gold standard antidiabetic activator drug, Rosiglitazone (ROSI), would decrease the invasive potential of IDC cells. To test this, we used a human IDC (BT-549) cell line and initially characterized the expression of PPARγ protein. Western blotting revealed a lack of significant endogenous PPARγ expression in BT-549 cells. We then generated stable, inducible $PPAR\gamma^{WT}$ expressing BT-549 cells to define the effects of PPARγ expression and activation (+/- ROSI) on *in vitro* cancer cell phenotypic changes including invasion (Boyden Chamber Assay) and migration (Scratch Wound Assay). (Supported by Britton Smith Chair, KHSC)

20. Natasha Iaboni (Field: Pathology and Molecular Medicine) **DISTINGUISHING IN SITU VERSUS INVASIVE DUCTAL CARCINOMA USING DESI**

Natasha Iaboni[1], Teaghan Kooster[1,2], Sonal Varma[1,2], Martin Kaufmann[3], Rachel R. Rubino[4], Amoon Jamzad[5], Kevin Yi Mi Ren[1,2], John F. Rudan[2,3], Parvin Mousavi[5], Gabor Fichinger[5] and Christopher JB Nicol[1,4].

Affiliations (Kingston, ON, Canada)

1. Dept of Pathology & Molecular Medicine, Queen's University
2. Kingston Health Sciences Centre (KHSC)
3. Dept of Surgery, KHSC
4. Division of Cancer Biology & Genetics, Queen's Cancer Research Institute
5. School of Computing, Queen's University

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 small molecules and 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=16) breast tumour samples. Sections of 10μm FFPE samples were analyzed by DESI in negative ionization scanning mode, then H&E stained and annotated for tumour and non-tumour regions by a pathologist. Multivariate analyses (*m/z* 50-1200 range) were performed on n≅100 randomized regions of interest per pathological region. Significant ions of interest were identified using reference libraries. We observed significantly increased apoptosis-related glycerophosphoserine (*m/z* 812.5444;GPS) in DCIS, while phosphatidylinositol phosphate (*m/z* 1053.4772;PIP) is elevated in IDC. Our novel data suggests DESI detectable profiles may distinguish DCIS and IDC, and eventually enhance rapid prognostic DCIS characterization to improve patient outcomes. Correlations with clinical outcomes (survival, recurrence and metastasis) are underway to help define prognostic profiles. (Supported by Dean's Doctoral, QHS)

21. Reginald Smyth (Experimental Medicine) **Systemic Determinants of Exercise Intolerance in Patients with Fibrosing Interstitial Lung Disease and a Severely Impaired DL_{CO}**

Reginald M. Smyth[1,2], Matthew D. James[1], Sandra G. Vincent[1], Kathryn M. Milne[1,3], Mathieu Marillier[4], Nicolle J. Domnik[1,2], Christopher M. Parker[1], Juan P. de-Torres[1,5], Onofre Moran-Mendoza[1], Devin B. Philips[1,6], Denis E. O'Donnell[1], J. Alberto Neder[1]

[1] Respiratory Investigation Unit, Division of Respirology, Department of Medicine, Queen's University and Kingston Health Sciences Centre, Kingston General Hospital, Kingston, ON, Canada, [2] Department of Biomedical and Molecular Sciences and Department of Medicine, Queen's University, Kingston, ON, Canada,[3] Centre for Heart Lung Innovation, Providence Health Care Research Institute, University of British Columbia, St. Paul's Hospital, Vancouver, BC, Canada,[4] HP2 Laboratory, INSERM U1300, Grenoble Alpes University, Grenoble, France; [5] Pulmonary Department, Clínica Universidad de Navarra and Instituto de Investigación Sanitaria de Navarra (IdiSNA), Navarra, Spain. [6] School of Kinesiology and Health Science, Faculty of Health, York University, Toronto, ON, Canada.

Background: The mechanisms driving poor exercise tolerance in patients with fibrotic interstitial lung diseases (*f*-ILD) showing a severe impairment in single-breath lung diffusing capacity (DL_{CO}<40% predicted) are not fully understood. A severely impaired DL_{CO} may signal multiple deranged physiologic adjustments to exercise which jointly increase the burden of exertional symptoms in *f*-ILD.

Methods: 67 patients (24 showing DL_{CO}<40%) and 22 controls underwent pulmonary function tests and an incremental cardiopulmonary exercise test with serial measurements of operating lung volumes and 0-10 Borg dyspnea and leg discomfort scores.

Results: The DL_{CO}<40% group showed lower spirometric values, more severe restriction, and lower transfer coefficient (K_{CO}) compared to controls and patients with DL_{CO}≥40% (p<0.05). Peak work rate was ~45% (vs. controls) and ~20% (vs. DL_{CO}≥40%) lower in the former group, being associated with lower O_2 pulse, an earlier anaerobic threshold, heightened submaximal ventilation, and lower O_2 saturation. Moreover,

critically high inspiratory constrains were reached at lower exercise intensities in the DL_{CO}<40% group and they reported the highest dyspnea and legs discomfort scores (p<0.05).

Conclusion: A DL_{CO}<40% in *f*-ILD signals multiple interconnected derangements (cardiovascular impairment, early anaerobic metabolism, excess ventilation, inspiratory constraints, and hypoxemia) which ultimately lead to exercise limiting dyspnea and leg discomfort. DL_{CO}<40%, therefore, might help in clinical decision making to indicate *f*-ILD patients who might derive particular benefit from interventions aimed at lessening these systemic abnormalities.

22. Isabella Delano (Field: Biomedical and Molecular Sciences) **CHARACTERIZING THE ROLE OF GUANYLATE BINDING PROTEINS IN THE ANTIVIRAL ACTIVITY OF IFNγ AGAINST CORONAVIRUS REPLICATION**

Isabella Pellizzari-Delano, Nicole Coman, Che C. Colpitts

Department of Biomedical and Molecular Sciences, Queen's University, Kingston, Ontario, Canada.

Novel antiviral approaches are needed to protect against future emerging coronaviruses (CoVs). IFNγ inhibits the replication of positive-sense single-stranded RNA viruses, including SARS-CoV-2, although its direct antiviral mechanisms remain poorly understood. Interestingly, IFNγ has been shown to inhibit other viral infections via the induction of antiviral IFNγ-inducible proteins known as guanylate binding proteins (GBPs). We hypothesized that IFNγ inhibits CoV replication by inducing the expression of GBPs. Using the endemic human coronavirus HCoV-229E as a model, we showed that IFNγ potently inhibits HCoV-229E infection in A549 lung epithelial cells, while concomitantly inducing expression of GBP1-5 in A549 cells. To determine if IFNγ requires GBP1-5 expression to inhibit HCoV-229E infection, we silenced expression of GBP1-5 in A549 cells using siRNA. Notably, the antiviral effect of IFNγ against HCoV-229E infection was impaired when GBP1 or GBP5 expression was silenced. Since GBP2 has been implicated in the inhibition of other RNA viruses by IFNγ, we used CRISPR/Cas9 to generate GBP2 knockout A549 cells to more robustly evaluate the role of GBP2. The antiviral effect of IFNγ against HCoV-229E was significantly reduced in the absence of GBP2. These data suggest that GBP1, 2 and 5 may at least partially mediate the inhibitory effect of IFNγ against HCoV-229E infection. While mechanistic studies are ongoing, these findings are enhancing our understanding of the direct antiviral mechanisms of IFNγ against CoV infection.

23. Alexa Toews (Field: Reproductive and Developmental Sciences) **THE EFFECT OF INFLAMMATION IN PREGNANCY ON SUBSEQUENT MATERNAL CARDIOVASCULAR FUNCTION**

Alexa Toews (M.Sc. Candidate), Gabrielle Fava (M.Sc. Candidate), Nakeisha Lodge-Tulloch (Ph.D. Candidate) and Dr. Charles Graham. Department of Biomedical and Molecular Sciences, Queen's University, Kingston, Ontario, Canada.

Background: Inflammatory pregnancy has been associated with an increased risk of future maternal cardiovascular disease (CVD). We propose that a second inflammatory stimulus, such as one induced by a high fat diet, may be necessary to produce overt cardiovascular dysfunction.

Methods: C57BL/6 mice were injected with lipopolysaccharide (LPS, 50 µg/kg; to induce pregnancy loss) or with phosphate-buffered saline (PBS; controls) on gestational day 10.5. Fourteen days after injection,

dams were transitioned to a high fat diet (60% kCal from fat) for 10 weeks to produce chronic low-grade inflammation. Groups consisted of pregnant mice on a high fat diet (PHF) or regular fat diet (PRF), and non-pregnant mice on a high fat diet (NPHF) or regular fat diet (NPRF). Echocardiography was used to assess systolic and diastolic function.

Results: Results revealed reduced mitral valve no-flow time in PRF mice treated with LPS (P=0.0382) and PBS (P=0.0269) compared to LPS-treated NPRF mice. The mitral valve A peaks in PBS-treated PHF mice were significantly lower than those of PBS-treated PRF mice (P=0.0138), LPS-treated PHF mice (P=0.0370), and LPS-treated PRF mice (P=0.0437). At low heart rates (350 bpm), PBS-treated PHF dams had significantly increased E/A ratios compared to PHF dams treated with LPS (P=0.0176).

Significance: These results may help to inform care and nutrition guidelines for women following complicated pregnancies to mitigate their risk of future heart disease.

24. Gabryella Pinheiro (Field: Cancer Research and Therapy) **ROLE OF PROSTATE CANCER INNERVATING NEURONS**

Gabryella Pinheiro [1], Alexander Birbrair [2], Sebastien Talbot [1]
[1] Department of Biomedical and Molecular Sciences, Queen's University, Kingston, Canada
[2] Department of Dermatology, University of Wisconsin–Madison, Wisconsin, USA

The tumor microenvironment is highly complex, composed of heterogeneous cancer cells and normal cell types at the tumor's site of origin. In this environment, cancer cells secrete molecular signals to guide their infiltration into peripheral nerves, a process known as perineural invasion. Furthermore, these cancer cells can induce peripheral nervous system (PNS) remodeling, dysfunction, and axonogenesis through a neurotrophic surge. Conversely, nerves stimulate tumor growth and metastasis through the production of neurotransmitters and growth factors. In this study, we examined the spontaneous crosstalk between cancer cells and peripheral sympathetic and sensory neurons. By depleting the peripheral nerves of transgenic mice with prostate cancer, our goal was to understand the changes in prostate cancer development, perineural invasion, and the interaction between nerves and tumors. Our results demonstrated sensory and sympathetic nerve infiltration into the prostate microenvironment during tumor development. Notably, both sympathetic nerve infiltration and vasculature increased in sensory neuron-depleted mice. This study identifies the cellular changes that underpin a pro-tumorigenic niche and provides mechanistic insights into the process of perineural invasion and cell communication within the perineural microenvironment of prostate cancer. The findings have significant implications for therapeutic treatments promoting an anti-tumorigenic niche.

25. Sofia Skebo (Field: Biochemistry and Cell Biology) **DESIGNING A FLUORESCENT REPORTER FOR THE LIVE-CELL ASSESSMENT OF ENDOTHELIAL BMP9 SIGNALING KINETICS**

S.I. Skebo[1], M.M.VandenBroek[1], M.L. Ormiston[1,2]

[1]Department of Medicine, Queen's University, Kingston ON

[2]Department of Biomedical and Molecular Sciences, Queen's University, Kingston ON

Rationale: Bone morphogenetic protein-9 (BMP9) regulates angiogenesis through 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. We aim to design a reporter to monitor Smad1 nuclear translocation in live cells as a measure of BMP9 signaling kinetics.

Methods and Results: HiFi assembly was used to create constructs encoding N-terminally GFP-tagged Smad1 under the control of either an HSV (low expression) or CMV (high expression) promoter which were transfected into human embryonic kidney (HEK-293) cells alongside a Histone 2B-mCherry plasmid to visualize the nucleus in live cells. 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 both endogenous and GFP-Smad1 by immunoblotting. While BMP9 stimulation induced a marked accumulation of phosphorylated endogenous Smad1 in the nucleus, a similar effect was not seen for the GFP-tagged construct.

Conclusions: The GFP-Smad1 reporter construct produces a stable protein product in HEK cells. However, the N-terminal tag interferes with Smad1 phosphorylation and nuclear localization. Ongoing work will optimize the linker length and flexibility, with the goal of creating a tool for screening novel drugs targeting BMP9-linked angiogenesis.

26. Kristin MacLeod (Field: Experimental Medicine) **CPAP THERAPY: IMPACT ON DAILY PHYSICAL ACTIVITY AND EXERCISE CAPACITY IN CHRONIC OBSTRUCTIVE PULMONARY DISEASE—OBSTRUCTIVE SLEEP APNEA OVERLAP SYNDROME.**

Kristin E MacLeod, Helen S Driver, Izzy Silot, Christina Liak, Amirali Mahpour, Sophie J Crinion, J Alberto Neder, Nicolle J Domnik.
Department of Biomedical and Molecular Sciences, Queen's University, Kingston, Ontario, Canada.

Many patients with chronic obstructive pulmonary disease (COPD) also develop obstructive sleep apnea (OSA), termed Overlap syndrome. COPD and OSA are each associated with fatigue and impaired exercise capacity. Continuous positive airway pressure (CPAP) is used to treat OSA, but the impact of CPAP therapy on fatigue, physical activity and exercise capacity in Overlap is largely unknown. We hypothesize that treating OSA in Overlap with CPAP will decrease fatigue and improve daily physical activity and exercise capacity. The impact of CPAP therapy on daily physical activity (1 -week actigraphy) and functional capacity (6-minute walk test [6MWT]) will be assessed in 30m:30f with Overlap before/three months after CPAP initiation. The impact of CPAP on exercise capacity in Overlap will be assessed with cardiopulmonary exercise testing in 10m:10f with Overlap before/three months after CPAP initiation. Outcomes for the physical activity study include daily physical activity (e.g., step count, sit time), 6MWT (distance), and fatigue questionnaire scores. Outcomes for the exercise capacity study include exercise time, peak workload, perceived exertion scores and fatigue questionnaire scores. If treating OSA in Overlap reduces fatigue and promotes physical activity and exercise, our findings would highlight the need to proactively screen for and treat OSA in patients with COPD. (Supported by Spear/Start Health Sciences Internal Award and DBMS Research Initiation Grant, Queen's University).

27. Innocent Ojobile (Field: Breast Cancer Research) **ELUCIDATING THE ROLE RIPK2 OF IN INFLAMMATORY BREAST CANCER.**

Innocent Ojobile, Alaa Zare, Einav Renert and Lynne-Marie Postovit. Department of Biomedical and molecular sciences, Queen's university, Kingston, Ontario, Canada.

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 threonine/serine kinase protein, which is 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 a 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 wound healing and bead assay indicate that RIPK2 promotes proliferation and angiogenesis respectively. Our study has so far demonstrated the role of RIPK2 in progression of IBC therefore can be imperative therapeutic target in the treatment of IBC. Therefore, Using IBC cell lines and humanised mouse models, we will seek to identify and determine the potency of RIPK2 inhibitors in the treatment IBC.

28. Temeara Barrett (Field: Reproduction and Developmental Sciences) **ASSESSING PLACENTAL PHENOTYPIC RESCUE VIA DHA/EPA DIETARY SUPPLEMENTATION IN Δ9-THC-EXPOSED RAT PREGNANCIES.**

Temeara Barrett[1], Bryony Natale[2], Sofia Allen[1], Kendrick Lee[3], Daniel Hardy[3,4], David Natale[1,2].

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

[2]Dept. of Obstetrics and Gynaecology, Queen's University, Kingston, Ontario, Canada.

[3]Dept. of Physiology and Pharmacology, Schulich School of Medicine and Dentistry, Western University, London, Ontario, Canada.

[4]Dept. of Obstetrics and Gynaecology, Schulich School of Medicine and Dentistry, Western University, London, Ontario, Canada.

Cannabis Δ9-tetrahydrocannabinol (Δ9-THC) concentration and maternal *cannabis* usage in Canada have both increased significantly following its recreational legalization in 2018. Maternal cannabis use is the third highest risk factor for low-birth-weight (LBW) babies. Maternal Δ9-THC exposure causes symmetrical fetal growth restriction (FGR) and placental deficits in the labyrinth zone, the site of maternal/fetal nutrient and gas exchange. LBW and FGR are associated with adverse long-term offspring cardiovascular, metabolic, and neurodevelopmental outcomes. Dietary supplementation of Δ9-THC-exposed rat dams with omega-3 poly-unsaturated fatty acids (n-3 PUFAs) can recover fetal weight, although the mechanism by which this occurs is unknown. This project aims to validate the placenta's

role in fetal phenotypic rescue by characterizing labyrinth-zone components deficient in Δ9-THC-exposed rat pregnancies. Rat dams were given daily vehicle or Δ9-THC IP injections from GD6.5-19.5 and either control or DHA/EPA (n-3 PUFAs) supplemented diets. Pups were collected and weighed at GD19.5 (VEH (n=22) mean±SEM = 1.618±0.031g; Δ9-THC (n=24) mean±SEM = 1.505±0.021g; Δ9-THC+n-3PUFAs (n=36) mean±SEM = 1.584±0.032g. One-way ANOVA: VEH vs. Δ9-THC $p<0.05$*, VEH vs. Δ9-THC+n-3PUFAs $p=0.64$). Placentas were formalin-fixed, paraffin-embedded, and serially sectioned. Labyrinth-zone cell populations, ECM components, maternal/fetal blood spaces, and glucose transporters will be assessed via immunohistochemistry, imaged by Brightfield microscopy, and analyzed using Celleste Image Analysis software (Thermofisher). This work may elucidate mechanisms underlying the potentially therapeutic role of n-3 PUFAs in Δ9-THC-exposed pregnancies. (Supported by CIHR)

29. Kelly Lee (Field: Neurophysiology**) IONOTROPIC ACETYLCHOLINE RECEPETOR DESENSITIZATION IN NEUROENDOCRINE CELLS INVOVLES MEMBRANE TRAFFICKING**

Kelly H Lee and Dr. Neil S. Magoski

Department of Biomedical and Molecular Sciences, Queen's University, Ontario, Canada.

Cholinergic signalling is often required to initiate long-term changes in neural activity, such as, executive attention, learning, and neuroendocrine control. The sea snail, *Aplysia*, reproduces when a brief cholinergic input to neuroendocrine bag cell neurons triggers a lengthy afterdischarge and egg-laying hormone secretion. Once exposed to acetylcholine, these neurons are less responsive to successive applications, and only recover after ~24 hrs, similar to the ~18-hr refractory period following the afterdischarge *in vivo*. To understand this prolonged desensitization, cultured bag cell neurons were whole-cell voltage-clamped. Consecutive pressure-ejections of acetylcholine at 10-, 30-, 60-, 90-, or 360-min intervals onto bag cell neurons demonstrated a uniform ~40% decrease in the 2nd current. These effects appear to be mediated by the ionotropic receptor itself, as the metabotropic acetylcholine receptor blocker, phenyl-trimethyl ammonium, failed to affect the desensitization. That stated, opening of the channel was not necessary for desensitization given that concurrent exposure to acetylcholine with the pore-blocker, hexamethonium, did not preserve subsequent currents to any greater degree than control. Moreover, pre-treatment for ≥12 hrs with the proteasome antagonist, lactacystin, lessened the desensitization to ~20%. Hence, the retrieval of ionotropic acetylcholine receptors from the membrane may underlie the extended desensitization and, thus, contribute to both the refractory period and reproductive timing.

(Supported by the Canadian Institute of Health Research)

30. Regan Bucciol (Field: Women's and Children's Health Research) **Tissue Factor-Microparticles as a Biomarker for Increased Risk of Breast Cancer-Associated Thrombosis**

Regan Bucciol [1] (BSc 4th year), Maha Othman (MD PhD)[1,2]
Department of Biomedical and Molecular Sciences, Queen's University, Kingston, Ontario, Canada
School of Baccalaureate Nursing, St Lawrence College, Kingston, Ontario, Canada

Introduction: Cancer-associated thrombosis (CAT), such as venous thromboembolism (VTE), is a complication in cancer patients, resulting in poor prognosis. Breast cancer is not highly thrombogenic but is highly prevalent, resulting in increased VTE cases. Many cancers express tissue factor (TF); a glycoprotein that triggers coagulation and were shown to release substantial amounts of TF-positive microparticles (MPTF), associated with a prothrombotic state. This narrative review evaluated the current use of the procoagulant MPTF as a biomarker for thrombosis risk in breast cancer.

Method: Omni database, an academic search tool standardized between 18 Ontario University libraries, was searched. Keywords included", and "Breast Cancer", and "Microparticle", and "Tissue Factor", or "EVTF", or "Extracellular Vesicle", and "VTE".

Results: One hundred and seven articles resulted from this search. Tumors of epithelial origin with elevated TF expression have been associated with increased VTE incidence. Thus, studies have affirmed the use of MPTF biomarkers for VTE risk in many cancers. Patients with metastatic breast cancer and CAT were found to exhibit procoagulant microparticles in vitro, due to TF expression. The silencing of TF was associated with decreased microparticle release in breast carcinoma cell lines, associated with decreased coagulation.

Conclusion: Based on this literature review, it is proposed that MPTF may be an effective biomarker for thrombosis risk in breast cancer patients and needs to be systematically examined.

31. Emmanuel Zangio (Field: Kinesiology) **CAN A SINGLE BOUT OF "SPRINT" INTERVAL EXERCISE INCREASE RAPID ONSET VASODILATION RESPONSE TO MUSCLE CONTRACTION?**

Emmanuel Zangio, Taylor Liu, Zac Tofflemire, Michael E. Tschakovsky,

Institution: Queen's University, Kingston, ON

INTRODUCTION: The blood flow increase at the onset of exercise is due to rapid onset vasodilation (ROV) and can impact exercise tolerance. Whether a single bout of sprint interval training (SIT) increases the ROV response at exercise onset remains unknown. We tested the hypothesis that a single SIT session would increase ROV.

METHODS: Twelve participants (6 males, 6 females) performed a single 2 second forearm contraction at 20%, 40%, and 60% of maximal voluntary contraction (MVC) force prior to and 10 minutes following SIT and matched duration rest. The SIT session consisted of 8x20-second maximal sprints with 10-second rest intervals. Forearm blood flow (FBF; Doppler ultrasound), muscle excitation (EMG; electromyography), and arterial blood pressure (ABP; finger photoplethysmography) were measured. Forearm vascular conductance (FVC), which quantifies vasodilation was calculated as FBF/MAP.

RESULTS: Peak FVC increase following a single contraction was greater post vs. pre for SIT (Peak (ml·min−1·100 mmHg−1) ; 20% MVC 181.9$\pm$58.938 vs. 118.2 $\pm$ 54.62 , 40% MVC 264.6$\pm$83.6 vs.192.7$\pm$ 39.5 ,60% MVC 323.7$\pm$99.4 vs. 230.1 ± 61.8. $all\ p < 0.05$) .

Muscle excitation (EMG) during single contraction was greater post vs. pre for SIT (20%, 18.03 vs 24.20, 40% 48.67% vs 38.69% , 60% MVC 69.01 % vs 56.00% all p <0.05).

CONCLUSION: A single SIT session increases ROV. A greater EMG response may suggest increase muscle fiber release of K+ (a vasodilator) that may explain increase ROV.

32. Gabrielle Fava (Field: Reproduction and Development) **THE EFFECT OF ABERRANT INFLAMMTION DURING PREGNANCY ON SUBSEQUNT RISK OF MATERNAL METABOLIC DISEASE.**

Gabrielle Fava, Alexa Toews, Nakeisha Lodge-Tulloch, Charles Graham. Department of Biomedical and Molecular Sciences. Queen's University, Kingston, Ontario, Canada.

Background: Pregnancy complications are often associated with aberrant inflammation and an increased risk of future metabolic disease. Additionally, murine studies demonstrated development of risk factors for metabolic disease after inflammatory pregnancy, but not overt disease. We hypothesised that a second source of inflammation is necessary for disease development.

Methods/Materials: C57BL/6 mice were injected with lipopolysaccharide (LPS) to induce fetal loss on gestational day 10.5. Fourteen days after injection, mothers were transitioned to a high-fat diet for 10 weeks to induce chronic low-grade inflammation. Alterations in glucose metabolism were assessed using an intraperitoneal glucose tolerance test (IPGTT). Blood glucose levels were measured at 0-, 15-, 60-, and 120-minutes post-glucose bolus.

Results: Results showed a significant increase of blood glucose concentration at the 60-minute time point in LPS-treated pregnant mice compared with LPS-treated non-pregnant mice, both groups on the regular fat diet (P = 0.0138), indicating a deviation from typical glucose metabolism. There was also a trend showing an increase in blood glucose concentration at the 60-minute time point (P = 0.0958) in high-fat-fed PBS pregnant mice compared to high-fat-fed non-pregnant PBS mice, indicating a potential pregnancy specific effect on the development of insulin resistance following a high fat diet.

Conclusion: These results indicate that, independent of inflammation, pregnancy may exacerbate the risk of metabolic disease when combined with a high fat diet.

(Supported by CIHR)

33. Sam Prosserman (Field: Neurophysiology) **PHARMACOLOGICAL CHARACTERIZATION OF NEONICOTINOID-SENSITIVE CHOLINERGIC RECEPTORS IN IDENTIFIED MOLLUSCAN NEURONS.**

Samuel D. Prosserman and Neil S. Magoski. Department of Biomedical and Molecular Sciences, Queen's University, Kingston, Ontario, Canada.

Neonicotinoids activate nicotinic-type acetylcholine receptors in insects, although they may impact nontarget species. For example, exposing the pond snail, *Lymnaea stagnalis*, a food-web intermediary abundant in North America and Europe, to field concentrations of the neonicotinoid, imidacloprid, proves lethal. To study the neurophysiological basis of this mortality, we examined the effect of imidacloprid on Visceral Dorsal 1 (VD1) and Right Parietal Dorsal 2 (RPD2), a pair of electrically coupled central neurons that are essential for cardio-respiratory function. Prior work showed that applying

acetylcholine to VD1 and/or RPD2, under sharp-electrode current clamp in the isolated brain, caused depolarization. We now find that imidacloprid did not mimic this effect, but instead served as an antagonist of the acetylcholine response. Depolarization was elicited by the nicotinic agonist, tetramethylammonium, whereas the muscarinic agonist, arecoline, produced only hyperpolarization. Moreover, the muscarinic antagonist, phenyltrimethylammonium, not only failed to eliminate the depolarization but extended the duration of the acetylcholine response. Finally, the muscarinic antagonist, a-conotoxin ImI, blocked acetylcholine-induced excitation. Overall, acetylcholine likely acts through a depolarizing nicotinic-type receptor, which is tempered by a hyperpolarizing muscarinic-type receptor. If imidacloprid interferes with this cholinergic input to VD1/RPD2, it may compromise cardio-respiratory control and survival.

34. Zhi Fang (Experimental Medicine) **NETWORKS OF CO-OCCURRING HUMAN MILK MICROBIOTA ARE ASSOCIATED WITH HOST GENOMICS AND THE DEVELOPMENT OF CHILDHOOD ATOPY AND ASTHMA IN THE CHILD COHORT STUDY**

Zhi Yi Fang[1], Sara A. Stickley[1], Amirthagowri Ambalavanan[1], Yang Zhang[2], Kelsey Fehr[3,4], Shirin Moossavi[3,4], Charisse Petersen[5,6], Stuart E. Turvey[5,6], Piushkumar J. Mandhane[7], Elinor Simons[3], Theo J. Moraes[8], Malcolm R. Sears[9], Padmaja Subbarao[8], Meghan B. Azad[3,4], Qingling Duan[1,2]

[1]Department of Biomedical and Molecular Sciences, Queen's University, Kingston, Ontario, Canada; [2]School of Computing, Queen's University, Kingston, Ontario, Canada; [3]Section of Allergy and Immunology, Department of Pediatrics and Child Health, University of Manitoba, Winnipeg, Manitoba, Canada; [4]Manitoba Interdisciplinary Lactation Centre (MILC), Children's Hospital Research Institute of Manitoba, Winnipeg, Manitoba, Canada; [5]Division of Allergy and Immunology, Department of Pediatrics, University of British Columbia, Vancouver, British Columbia, Canada; [6]Department of Pediatrics, Child and Family Research Institute and British Columbia Children's Hospital, Vancouver, British Columbia, Canada; [7]Department of Pediatrics, University of Alberta, Edmonton, Alberta, Canada; [8]Department of Pediatrics, Hospital for Sick Children and University of Toronto, Toronto, Ontario, Canada; [9]Department of Medicine, McMaster University, Hamilton, Ontario, Canada

The microbial community in human milk has been proposed to influence children's health such as atopy and asthma. Earlier studies have identified non-genetic determinants of the human milk microbiota (HMM). However, the impact of host genomics on HMM remain poorly understood. This is the first genome-wide association study of HMM and the first study to employ a network analysis approach to investigate clusters of co-occurring microbes, which may influence risk of childhood atopy among 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, $P=1.3\times10^{-11}$) and 10 (e.g., rs11009644, $P=1.6\times10^{-8}$) 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.

35. Hannah Doherty-Plummer (Field: Biomedical and Molecular Sciences**) SMARCA4 LOSS IN ENDOMETRIAL CANCER CELLS INDUCES THE SENESCENCE ASSOCIATED SECRETORY PHENOTYPE AND DEDIFFERENTIATION CONCOMITANT WITH ALTERATIONS TO THE TRANSLATIONAL MACHINERY**

Hannah Doherty-Plummer, Bianca Dauber, Mackenzie Coatham, Einav Renert, Guihua Zhang, Jiahui Liu, Tyler Cooper, Cheng Han-Lee, and Lynne Postovit

The prevalence of endometrial cancer is rising, with ~8100 diagnoses in Canada in 2022. While the prognosis for localized endometrial cancer is excellent, certain patients develop dedifferentiated endometrial cancer, wherein the five-year survival rate is less than 25%. We have discovered that the loss of *SMARCA4*, the catalytic subunit in the SWItch/Sucrose Non-Fermentable (SWI/SNF) complex, causes well differentiated endometrial cancer cells to dedifferentiate. SWI/SNF is a critical epigenetic modifier, which remodels chromatin by sliding and evicting nucleosomes. Evidence from our lab suggests that SMARCA4 loss enables dedifferentiation by causing epigenomic chaos in endometrial cells. In an apparent paradox, this dedifferentiation occurs only upon passaging in a mouse, following an initial period wherein the cells manifest a senescent-like phenotype, indicated by reduced growth, expression of b-galactosidase and manifestation of the Senescence Associated Secretory Phenotype (SASP). This suggests that features of the growing tumour, such as hypoxia, may enable dedifferentiation and that the SASP may characterize a transitional cell state. The mammalian target of rapamycin (mTOR) pathway, which regulates metabolism, survival, and mRNA translation, has been shown to promote the SASP and is normally inactivated in response to cellular stresses, such as hypoxia. We hypothesize that SMARCA4 loss promotes the senescent-like phenotype by altering mTOR signalling, and that it allows cells to dedifferentiate in response to cellular stresses by enabling adaptations in mRNA translation. Preliminary data shows that relative phosphorylation of key mTOR mediators (S6, and 4EBP1) remains unchanged in SMARCA4 KO versus WT cells but that the total levels 4EBP1 are markedly increase. Notably, in contrast to other cell types, these cells did not respond to hypoxia with a reduction in mTOR activity (indicated by 4EBP1 phosphorylation). Phosphorylation of eIF4E (a cap binding protein involved in translation initiation) is significantly decreased in *SMARCA4* KO, under hypoxia and in normoxia. This may suggest an alteration in the targeting of mRNA transcripts for translation, rather than an impact on global translation. Future directions will be to perform polysome analysis to determine if translational efficiency and targeting of specific mRNA transcripts is altered with *SMARCA4* KO.

36. Michael Vermeulen (Field: Cancer Research) **IDENTIFYING NOVEL CANCER THERAPY OPPORTUNITIES THROUGH SYNTHETIC LETHAL GENE COMBINATIONS**

Michael Vermeulen, Doris Coto Villa, Tomas Babak and Andrew Craig

Cancer cell lines have numerous characteristics that make them favorable pre-clinical research models, yet they are notoriously poor at predicting drug response in the clinic. Here we investigate the utility of synthetic lethal (SL) interactions discovered from large-scale in vitro CRISPR functional screens (i.e. the

BROAD and Sanger Cancer Dependency Maps or "DepMap") as predictors of targets that validate in patients. Synthetic lethality is a phenomenon where the combined inactivation of two specific genes or pathways leads to cell death, providing a potential therapeutic strategy for cancer treatment. Using a random forest classifier, we created a genome-wide SL interaction network for each well represented cancer type, highlighting lineage-specific targeting opportunities. When overlapping our SL interactions with TCGA, we found enriched mutually exclusivity in patient tumors when they included a driver mutation (tumor-suppressor/oncogene). These SL interactions represent targeting opportunities with the advantage of clear patient selection criteria based on their driver mutation status. In an effort to identify drugs that target these proteins as potential repurposing opportunities, we found that pharmacogenomic inhibition rarely invokes the same target dependencies as a genetic deletion of the drug target. Nonetheless, we identified several "clean" drugs with potential for repositioning and are currently being evaluated using isogenic cell lines. Although tumours are more heterogeneous than cancer cell lines, we show that cell line viability readouts linked to single-gene/drug perturbations can yield biologically and clinically meaningful predictions when tied to tumor-driver biology.

Queen's University
Dept. Biology
Work carried out in QCRI – Craig Lab
Co-supervisors: Dr. Tomas Babak and Dr. Andrew Craig
Supporting agency: CIHR, OGS

37. Caitlin Piccone (Field: Rehabilitation Science) **NATURAL SUPPORT FOR CANADIANS WITH DISABILITIES: A SCOPING REVIEW.**

Heather Plyley, Julia Jansen-Van Vuuren, Caitlin Piccone, Navjit Gaurav, Rebecca Pauls Monique Nelson, Donna Thomson, Linda Perry, & Heather Aldersey. Department of Rehabilitation Science, Queen's University, Kingston, Ontario, Canada.

Natural supports play a crucial role in the lives of people with disabilities and their families by providing emotional, informational, and instrumental support. Providing and receiving natural supports (typically unpaid) is often seen as essential for realization of a good life. These supports help maximize benefits received from more formalized rehabilitation services. Despite their critical role in our society, Canadian policies often fail to create environments in which natural supports flourish.

Our scoping review assess how published literature defines natural supports in the Canadian context. We found that there is a dearth of evidence examining how natural supports for adults with disabilities and their families are defined in Canada. Oftentimes, the definitions and examples identified did not fully capture the complexity of natural support. Moreover, what evidence does exist largely lacks an exploration of how natural supports are used in the lives of families who are further marginalized, including those who are Indigenous, live in rural communities, identify as LGBTQIA+, and those who are racialized. We highlight the need for future research that more comprehensively captures the essence of natural support experiences in Canada. Additionally, we advocate for increased research and policy to support natural care to complement, not supplant, formal paid care. We recommend further examination of the voices represented in existing natural support literature and improved recognition and integration of equity-deserving community knowledges.

38. Gabriella Stefan (Field: Inflammation, Infection and Immunity) **INVESTIGATING THE IMPACT OF ASTHMA ON HEMATOPOIETIC STEM AND PROGENITOR CELLS**

Gabriella Stefan[1], Mckenna Perlin[1], Conrad Pietrzak[1], and Eva Kaufmann[1]

[1]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 through 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 pulmonary allergen sensitization is driven by epigenetic reprogramming of HSPCs that leads to the generation of innate immune cells with an elevated allergic reaction potential.

To investigate this hypothesis, C57BL/6J mice are sensitized with house dust mite extract and lung 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 leads to significant depletion of HSPCs in the BM and decreased cytokine responses of deriving monocytes to infectious stimulation.

Together, these results suggest that immunophenotypes in asthma are driven by both quantitative and qualitative changes in BM HSPCs.

39. Sydney Shephard (Field: Cancer Research) **A DNA METHYLATION-BASED LIQUID BIOPSY ASSAY FOR THE DETECTION AND MONITORING OF OVARIAN CANCER**

Sydney Shepherd, Keira Frosst, Dr. Christopher Mueller

Ovarian cancer is the 5th deadliest cancer for women in Canada, with an average 5-year survival rate for all stages of only 43%. The current tools for detection include symptom assessment, transvaginal ultrasounds, and the CA-125 biomarker; however, these are non-specific, have a high frequency of false positives and have low sensitivity in early-stage disease. The ovarian cancer Methylation DETEction of Circulating Tumour DNA (mDETECT) assay is a DNA methylation-based ctDNA liquid biopsy designed for the optimal detection and monitoring of ovarian cancer. We sought to integrate RNase H-dependent PCR technology into the previously designed ovarian cancer assay in order to develop a more sensitive and easier to perform test. The primers were validated the using synthetically methylated peripheral blood mononuclear cells (mePBMC) in a variety of singleplex, multi-singleplex, and multiplex PCRs. 30 of the 39 probes were successfully converted to RNase H-dependent primers and the resulting assay was sensitive to at least 0.1% mePBMC to PBMC, mimicking ctDNA to cfDNA ratios seen in the blood. The assay was

also able to quantify molecules of ctDNA using unique molecular identifier technology. The overall goal of this project is to improve upon the gold standard, the use of the CA-125 biomarker, by developing a one-tube liquid biopsy mDETECT assay for the detection and monitoring of ovarian cancer.

Sydney Shepherd, Incoming MSc Student, Department of Pathology and Molecular Medicine Queen's University, 18sjms2@queensu.ca

Keira Frosst, PhD Student, Department of Pathology and Molecular Medicine Queen's University, 16kmf4@queensu.ca

Dr. Christopher Mueller, Professor, Department of Biomedical and Molecular Sciences Queen's University, muellerc@queensu.ca

40. Bri Quinville (Field: Neuroscience) **CORRECTIVE GENE THERAPY IN A MOUSE MODEL OF SANDHOFF DISEASE**

Brianna M. Quinville[1], Alex E. Ryckman[1], Natalie M. Deschenes[1], Melissa Mitchell[2], John G. Keimel[4], William F. Kaemmerer[4], and Jagdeep S. Walia[1,2,3]
[1]Centre for Neuroscience Studies, Queen's University, Kingston, Ontario, Canada, K7L 3N6;
[2]Department of Biomedical and Molecular Sciences, Queen's University, Kingston, Ontario, Canada, K7L 3N6;
[3]Medical Genetics/ Department of Pediatrics, Queen's University, Kingston, Ontario, Canada, K7L 2V7;
[4]New Hope Research Foundation, North Oaks, Minnesota, USA

Sandhoff disease (SD) is a genetic, neurodegenerative disorder caused by the excessive accumulation of GM2 gangliosides in neuronal cells. Typically, GM2 are hydrolyzed by an enzyme, HexA, but mutations in the genes encoding HexA lead to improper functioning of the enzyme. A novel isoenzyme of HexA, HexM, has been developed and shown to hydrolyze GM2 *in vivo*, following gene transfer of the *HEXM* gene packaged in a self-complementary adeno-associated viral vector, serotype 9 (scAAV9), increasing SD mice (*Hexb*$^{(-/-)}$) lifespan.

This study examines the dose response of the *scAAV9-HEXM* treatment in ten cohorts of SD mice through concurrent dual delivery of treatment via intra-cisterna magna (3 possible doses) and intravenous (6 possible doses) routes. Repeated behavioural testing and blood collections were done. At termination, blood, gross organs, brain, and spinal cord were collected for analysis of GM2 accumulation, Hex enzyme activity, immune response, and histology.

Results show increased survival in all treatment groups compared to the vehicle-only control group ($p < 0.001$). The treatment cohort with the longest survival lived two years compared to four months for the vehicle cohort. This treatment cohort also showed a >12-fold decrease in accumulated GM2 compared to controls ($p < 0.0001$) and a >51-fold increase in serum Hex activity compared to heterozygous controls ($p < 0.05$).

41. Eve Racette (Field: Neuroscience) **SEXUALLY DIMORPHIC PHENOTYPES IN A MOUSE MODEL OF NEXMIF LOSS**

Authors: Eve Racette[1], Jagdeep Walia[1,2,3]

[1]Centre for Neuroscience Studies, Queen's University, Kingston, Ontario, Canada, K7L 3N6;

[2]Department of Biomedical and Molecular Sciences, Queen's University, Kingston, Ontario, Canada, K7L 3N6;

[3]Medical Genetics/ Department of Pediatrics, Queen's University, Kingston, Ontario, Canada, K7L 2V7;

Five to ten percent of intellectual disability (ID) cases in males are linked to mutations of various genes located on the X chromosome, one of which is the Neurite Extension and Migration Factor gene (*NEXMIF*). Interestingly, NEXMIF loss affects male and female patients in a sexually dimorphic fashion. While its role is poorly understood, studies have suggested its involvement in neurite outgrowth and orientation, in neuronal migration and in gene expression. Consistent with the clinical hallmarks, a recently generated mouse model of Nexmif loss displays cognitive impairments, as well as neuroanatomical and electrophysiological alterations. While these experiments were only carried out in males, they shed light on some of the symptoms experienced by patients. Our hypothesis is that the loss of Nexmif causes a variety of phenotypes due to the alteration of neurodevelopmental processes, such as motility, neurite growth, synaptic formation, gene expression, etc. To verify this hypothesis, we designed a battery of behavioural assays administered to mice of both sexes, while also monitoring body weight and seizure frequency. While preliminary, our results suggest alterated speed of locomotion in affected females, and alterations in body weight and both seizure prevalence and frequency in affected males. Together, these results suggest that Nexmif plays a fundamental role in both males and females.

42. Eric Fernandes (Field: Cardiac, Circulatory, and Respiratory Sciences) **CHARACTERIZING THE LABILITY OF CALCIPROTEIN PARTICLES: ROLE OF THE LYSOSOMAL ENVIRONMENT**

Eric B P Fernandes BHScH,[1] Tyler S Rowsell BScH,[1] Olivia M Novosel BScH,[1] Heshanth Rasalingam,[1] Trisha Singh,[1] Nelson Chen,[1] Rachel M Holden MD,[1,2] Michael A Adams PhD[1]

[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

Calciprotein particles (CPPs) are fetuin-A containing complexes which may chaperone circulating calcium and phosphate and are believed to be internalized in lysosomes after extravasation. While CPP maturation has been studied, the effect of particle maturity and lysosomal pH on CPP bound phosphate lability (i.e., stability) is unclear.

CPP lability was measured *in vitro* using a novel assay whereby *de novo* CPPs were isolated via differential centrifugation and reconstituted into new solutions with lower total mineral concentrations. Specifically, by subtracting the total solution and supernatant phosphate concentrations, the phosphate sequestered in CPPs was quantified and compared following reconstitution. To assess the effect of particle maturity, variable incubation periods were used to generate immature CPP-Is (10min) and mature CPP-IIs (72hrs). Further, the effect of lysosomal pH on CPP-II lability was determined by using reconstitution solutions of pH 4.5, 5.5, and 7.4.

Under physiological pH, CPP-Is and CPP-IIs were found to be approximately 53% and 14% labile, respectively (***p*<0.0001**). Furthermore, significant stepwise changes in CPP-II lability were observed after reconstituting in solutions possessing lysosomaly relevant acidities (***p*<0.0001**), with pH 4.5 and 5.5 yielding approximately 99% and 51% lability, respectively.

CPP lability was quantified through a simple *in vitro* assay where both particle maturity and solution pH substantially altered lability. These findings may support the development of innovative CPP characterization protocols and the identification of lysosomal CPP handling mechanisms.

43. Olimpia Sienkiewicz (Field: Inflammation, Infection and Immunity) **HISTOLOGIC SCORING OF GUT MUCOSAL SAMPLES FROM INFLAMMATORY BOWEL DISEASE PATIENTS IS LINKED TO CLINICAL DISEASE ACTIVITY**

Olimpia Sienkiewicz, MSc[1], Daniel J. Mulder, MD, PhD[1,2]

Affiliations:

1. Gastrointestinal Diseases Research Unit (GIDRU), Queen's University, Kingston, Ontario, Canada
2. Department of Pediatrics, Queen's University, Kingston, Ontario, Canada

Background: Crohn's disease (CD) and ulcerative colitis (UC) are related disorders characterized by gastrointestinal inflammation which are increasing in prevalence. The current diagnostic process for these diseases is challenging, requiring invasive procedures such as endoscopy and mucosal biopsies. Histological healing is the gold standard indicator of IBD prognosis. However, there is a lack of understanding of non-invasive signs of disease activity reflect histological healing. Linking non-invasive IBD characteristics with quantification of immune cells subsets in the gut mucosa, could provide valuable information about disease mechanisms and reduce the need for invasive testing.
Objective: To establish that histological inflammation aligns with reported disease activity in patients.
Methods: Mucosal biopsies from 6 IBD patients (2 active UC, 3 remission UC, 1 remission CD) were routinely stained. 4 non-overlapping images from each disease location were scored using the validated Naini & Cortina score.
Results: In this preliminary study, we have assigned a score of inflammation to 24 images.
Significance: These results could be used as a baseline to examine further non-invasive molecular markers of disease can be linked to mucosal changes, such as metabolomic information, cytokine profiles, and immune cell populations, establishing reliable connections between mucosal changes and non-invasive molecular markers of disease.

44. Sakura Koner (Field: Inflammation, Infection and Immunity)) **WNK1 CONTRIBUTES TO THE DEVELOPMENT OF SPINAL CORD INJURY PAIN**

Sakura Koner[1], Courtney Bannerman[1], Amanda Zacharias[1], Rosalin Dubois[1], Jihoon Choi[1], Margot Gunning[1], Qingling Duan[1,2], Nader Ghasemlou[1,3,4]

[1]Dept. of Biomedical & Molecular Sciences, [2]School of Computing, [3]Dept. of Anesthesiology & Perioperative Medicine, [4]Centre for Neuroscience Studies, Queen's University, Kingston, Ontario, Canada.

Almost 86,000 people suffer from traumatic spinal cord injuries (SCI) every year in Canada. Most of these injuries result in the development of persistent neuropathic pain. Neuroinflammation in the central nervous system, caused by the influx of peripheral immune cells and activation of resident glial cells, is a

major contributor to neuropathic pain. Previous work has shown that deletion of the HSN2 exon of neuronal WNK1 results in reduced mechanical allodynia and cold hyperalgesia in mice with nerve-injury induced neuropathic pain. It has also been shown that WNK1 and its downstream target, NKCC1, have increased expression and phosphorylation in the peak, acute and chronic phases of spinal cord injury. WNK1 and its downstream targets are a family of serine-threonine kinases capable of controlling chloride influx and efflux in macrophages, which may contribute to inflammation by altering activation states of immune cells recruited to the CNS. Analysis of a human dataset identified WNK1 as a potential contributor to intractable SCI pain. Transcriptomic and proteomic expression of WNK1 and its downstream targets (e.g., SPAK, OSR1, NKCC1 and KCC1) was completed at various timepoints after injury, showing increased expression early after SCI. Systemic treatment with the WNK1 inhibitor STOCK2S following SCI resulted in reduced pain outcomes. Further in vitro studies will shed light on the impact loss of WNK1 has on peripheral immune cell activation states. This work suggests that altering WNK1 expression could provide a new therapeutic avenue to reduce chronic pain in SCI by targeting the peripheral immune system.

Supported by Craig H. Neilson Foundation

45. Sara Sahlabadi (Field: Cancer Research and Therapy) **SENSORY NEURON ABLATION IMPACTS DENDRITIC CELL MIGRATION IN THE TUMOR DRAINING LYMPH NODES OF MICE BEARING MELANOMA**.

Sara Nikoofal Sahlabadi [1], Mohammad Balood [2], Maryam Ahmadi[2], Sebastien Talbot [1,3]

1. Department of biomedical and molecular sciences, Queen's University, Kingston, Ontario, Canada.

2. Département de Pharmacologie et Physiologie, Université de Montréal, Montréal, Quebec, Canada

3. Department of pharmacology and physiology, Karolinska Institutet, Stockholm, Sweden.

Introduction: This study sought to determine the effect of sensory neurons on the migration and function of dendritic cells (DCs) in the microenvironment of tumors.

Methods: B16F10-ova-mcherry melanoma cells were inoculated into $Na_v1.8$ and TRPV1 ablated mice, chemically ablated mice, and their littermates as controls. 14 days post-inoculation, tumor-draining lymph nodes (TDLNs) were collected, and resident and migratory DC subpopulations were evaluated using flow cytometry.

Results: The TDLNs of TRPV1-ablated mice contained significantly more $CD103^+$ (12562±1381) and $CD11b^+$ (13502±1461) migratory DCs than those of TRPV1-intact mice ($CD103^+$: 8458±1022, $CD11b^+$: 8818±1293; $P<0.03$). There were no differences between resident DC subpopulations. DC subsets exhibited no changes in $Na_v1.8$-ablated mice. In contrast, chemically ablated mice, either by QX or QX gel, exhibited a significant increase in $CD103^+$ and $CD11b^+$ migratory DCs compared to intact mice ($P\leq0.03$, $P<0.01$; respectively), whereas there were no differences in resident DCs.

Conclusion: Ablation of sensory neurons influences migration of DC subsets within TDLNs. Differential responses among ablation models suggest that sensory neuron subsets may uniquely influence DC migration, highlighting potential therapeutic targets for enhancing the immune response in melanoma. To elucidate the underlying mechanisms, additional research is needed.

46. Abhishek Shastry (Field: Cardiac, Circulatory and Respiratory Sciences**) CONFIDENCE: AN APP FOR CROSS-PLATFORM DIFFERENTIAL GENE EXPRESSION ANALYSIS AND ITS USE IN STUDIES OF METABOLISM AND OXIDATIVE STRESS**

Abhishek Shastry[1], Charles C. T. Hindmarch, MSc, PhD[1,2,3*], & Kimberly Dunham-Snary, MPS, PhD[1,3*]
[1]Department of Medicine, Queen's University, Kingston, ON
[2]Queen's CardioPulmonary Unit, Queen's University, Kingston, ON
[3]Department of Biomedical & Molecular Sciences, Queen's University, Kingston, ON

*These authors contributed equally to this work

An increasing emphasis has been placed on the role of abnormal and dysregulated gene expression as markers of diet-induced cardiometabolic diseases. Numerous platforms have been developed for differential gene expression analysis on data obtained from RNA-Sequencing. However, the use of these platforms and interpretation of results rely on substantial bioinformatics and programming knowledge. We present CONFIDENCE, a simple, user-friendly program that allows users to input gene count files and obtain a list of differentially expressed genes, functional pathways, and high-quality figures. The app utilizes a diverse set of differential expression platforms to provide a Confidence Score for outputted genes, and a Weighted Score which incorporates each gene's p-value and Log2(Fold Change) for functional analysis. To test the functionality of CONFIDENCE, a publicly available dataset representing the skeletal muscle transcriptomes of mice fed either a high fat diet or control diet were analyzed. Numerous genes were upregulated in high fat diet-fed animals vs control, including *Fasn*, *Ech1*, *Acly*, and *Acaca*. These significant genes were identified through their high confidence and weighted scores which indicate their consistency and magnitude of expression across various platforms. The gene targets obtained from this 'cross-platform' program can be modulated in future studies with greater assurance, which increases the efficiency of result translation. To promote the initiative of open science, the CONFIDENCE application will be made publicly available as an R package.

(Supported by my supervisors Canada Research Chair, Canada Foundation for Innovation, Garfield Kelly Cardiovascular Research & Development Fund, Banting Research Foundation, and Queen's University Department of Medicine.)

47. Isaac Emon (Field: Cardiac, Circulatory and Respiratory Sciences) **SOMATIC *DNMT3A* MUTATIONS IN HEMATOPOIETIC STEM CELLS PROMOTES ADVERSE PULMONARY VASCULAR REMODELLING, RIGHT VENTRICULAR DYSFUNCTION AND PULMONARY ARTERIAL HYPERTENSION**

Isaac Emon[1], Ruaa Al-Qazazi[1], Francois Potus[2], Ashley Martin[1], Patricia Lima[3], Kuang-Hueih Chen[1], Danchen Wu[1], Michael Rauh[4], Stephen Archer[1,3 *]

1- Department of Medicine, Queen's University, Kingston, Ontario, Canada
2- Pulmonary Hypertension Research Group, Institut Universitaire de Cardiologie et de Pneumologie de Québec Research Center, Laval University, Quebec City, Canada
3- Queen's Cardiopulmonary Unit, Queen's University, Kingston, Ontario, Canada
4- Department of Pathology, Queen's University, Kingston, Ontario, Canada

Background: Pulmonary Arterial Hypertension (PAH) is a fatal cardiopulmonary disorder that occurs due to remodeling and stiffening of small pulmonary arteries. Inflammation plays a major role in the pathogenesis of PAH. We study the role of clonal hematopoiesis of indeterminate potential (CHIP), which is known to promote a proinflammatory phenotype, driven by somatic mutations in *DNA Methyltransferase 3A* (*DNMT3A*) in the development of inflammation and PAH.

Methods: We produced a conditional, Vav-Cre driven hematopoietic *Dnmt3a* knockout model (*Dnmt3a*$^{-/-}$) and used *Dnmt3a*$^{f/f}$ as controls. 3-month-old male and female mice were exposed to a second hit (hypoxia, 3 weeks) to accelerate PAH development (N=3-4/group). Hemodynamics were assessed using echocardiography and right-heart catheterization. Pulmonary artery medial wall thickness (PAMWT) was assessed using light microscopy. Immune cell infiltration of the lung was measured via confocal microscopy and flow cytometry.

Results: *Dnmt3a*$^{-/-}$ mice had evidence of PAH including elevated right ventricular systolic pressure (*p*=0.0007), reduced pulmonary artery acceleration time (*P*=0.004) and reduced tricuspid annular plane systolic excursion (*P*=0.014) compared to hypoxic *Dnmt3a*$^{f/f}$ mice. There is an increase in PAMWT of *Dnmt3a*$^{-/-}$ mice compared to controls (57.83±3.9% *Dnmt3a*$^{-/-}$, 49.73±2.8% *Dnmt3a*$^{f/f}$; p=0.002). There was increased CD45^{+} leukocyte infiltration in the lungs, primarily macrophages and B cells.

Conclusion: Deletion of *DNMT3A* in the hemopoietic system is sufficient to provoke PAH in mice, identifying a new role of DNMT3A in PAH pathogenesis.

48. Ansha Nega (Field: Rehabilitation Science) **A CALL FOR INTEGRATION OF REHABILITATION IN WORKPLACES OF DEVELOPING COUNTRIES: LESSONS FROM THE LONG-TERM CONSEQUENCES OF TRAUMATIC INJURY ON WORKING ADULTS IN ETHIOPIA**

Ansha Nega Ahmed*[1,2], Rosemary Lysaght[2], Adamu Addissie[1], Ayalew Zewdie[3], and Marcia Finlayson[2]

[1] School of Public Health, Addis Ababa University, Ethiopia
[2] School of Rehabilitation Therapy, Queen's University, Canada
[3] Addis Ababa Burn, Emergency and Trauma Hospital, St. Paul's Hospital Millennium Medical College, Ethiopia

Injury survivors often experience ongoing health challenges and employment participation restrictions. In Ethiopia, individuals of working age have a high risk of injury; their return to work (RTW) has health and economic implications. However, there is paucity of evidence about the long-term consequences of injuries. This study aimed to characterise injury survivors by their preinjury status and post-injury outcomes one year after the injury. An institution-based cross-sectional study was employed in a large public hospital in Addis Ababa, Ethiopia. Files of all emergency patients (n=2042) who visited the hospital over a three-month period were reviewed after a year to identify eligible participants. Of these, 254 completed a questionnaire through telephone interview. Descriptive statistics were used. Road traffic accident and violence were the main mechanisms of injury. 61% had moderate some form of disability, only 16% received compensation for the injury, and 41% were not able to return to work one year after the injury. Further, 56% reported problems related to physical stressors when they attempt to resume work. Of the returned (n=150), 46% returned within 12 weeks, 78% returned to the same employer and informal supports were the main sources, while workplaces offered supports for a small number of

participants. It is imperative to focus on integration of rehabilitation into workplace health programs, and to embrace informal support systems to prevent long-term consequences of injuries.

This research project was supported by Mastercard Foundation Scholars Program (Queen's University _ University of Gondar partnership) for field related activities and the SGPS at Queen's University for travel expense.

49. Vina Li (Field: Neuroimmunology) **THE ROLE OF CIRCADIAN RHYTHM IN THE PROGRESSION AND PATHOGENESIS OF EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS**

Vina W. Li[1*], Julia P. Segal[1*], Hailey Gowdy[1], Mitra Knezic[1], Nader Ghasemlou[1,2,3]

[1] Department of Biomedical & Molecular Sciences, [2] Department of Anesthesiology, [3] Centre for Neuroscience Studies, Queen's University, Kingston, ON K7L 3N6, Canada

*equal contribution

Multiple sclerosis (MS) is a debilitating autoimmune disease characterized by inflammatory myelin lesions in the central nervous system. Although therapeutics exist, this disease remains incurable, and the underlying mechanisms are largely unknown. Evidence suggests that circadian disruption may be a risk factor for MS. For examples, people with abnormal circadian rhythms, including long-term shift workers and people living at higher latitudes, are at an increased risk for MS. We sought to investigate the role of circadian rhythm in MS using the experimental autoimmune encephalitis (EAE) mouse model. Mice were entrained under either a control 12:12 or an abnormal 10:10 light cycle using light-controlled cages for 4 weeks before disease induction. Symptom progression was monitored using the 5-point Clinical Score scale, demyelination was assessed using immunohistochemistry, and inflammatory response was determined by qPCR and flow cytometry. Mice under the 10:10 cycle showed exacerbated demyelination, proinflammatory cytokine expression, and immune cell infiltration into the spinal cord relative to the control group. Correspondingly, these mice experienced more severe paralysis as assessed by clinical score. Simultaneously, mice under the LD cycle experienced disruptions to their endogenous circadian rhythm at peak disease, as indicated by locomotor activities and spinal cord clock genes expressions. The results confirm circadian disruption as a potential risk factor for MS. Future therapeutics targeting the circadian system may be used to alleviate MS symptoms and disease progression.

Funding sources: Multiple Sclerosis Society of Canada; NSERC; Canadian Pain Society

50. Paola Nasute Fauerbach (Field: Medicine) **POOR SURVIVAL IN YOUNG WOMEN WITH BREAST CANCER IN SOUTHEAST ONTARIO (SEO): NATURE OR NURTURE?**

Paola V. Nasute Fauerbach, Janushan Hariharan, Sophia E. Bakyta, David M. Berman

Department of Pathology and Molecular Medicine and Queen's Cancer Research Institute, Queen's University, Kingston, Ontario, Canada (*PVNF and DMB*). Faculty of Health Sciences, Queen's University, Kingston, Ontario, Canada (*JH and SEB*)

Background: In SEO, the 5-year cancer-specific mortality rate of women aged 40-49 is almost double the rate province-wide. Inherited breast cancers (5%-10%) are aggressive and more common in young

women. Asymptomatic mutation carriers can join the High-Risk Ontario Breast Screening Program (HR-OBSP) and have better outcomes than symptomatic patients.

Purpose: To determine the percentage of patients with genetic mutations; clinicopathological characteristics were assessed and recurrence rates were compared in women who underwent genetic testing.

Materials and Methods: Clinicopathological, imaging, and genetic testing data were collected for consecutive primary breast cancer patients 40 to 49 between 2009 and 2013. Chi-square tests were used to compare patient outcomes with and without genetic mutations.

Results: Of 222 patients, only 11 (4.9%) were asymptomatic, and 59 (26.6%) underwent genetic testing. Mutations were found in 14/59 (6.3%) patients: *BRCA1/2* (nine), and *DICER1*, *PTEN*, *TP53*, *CHEK2* and *CDH-1* (one each). Overall, tumors were large (median=20mm), but inherited cancers were smaller than sporadic cancers (median=19mm vs. 21mm). Additionally, inherited and sporadic carcinomas had similar rates of high-risk pathological features, including triple negative (9.4% vs 11.3%) and HER2+ subtypes (1.9% vs 16.9%). In this age group, a high rate of metastasis was found in patients with sporadic and inherited cancers (68.4% and 31.6% respectively, p=0.33).

Conclusion: In SEO, young women have low rates of inherited breast cancers (6.3%). Poor outcomes may result from reduced screening or delayed treatment.

Funding: Paola V. Nasute Fauerbach received the Dean's Doctoral Award, Department of Pathology and Molecular Medicine, Queen's University, Kingston, Ontario, Canada

51. Nakeisha Lodge-Tulloch (Field: Reproductive Immunology) **CROSS-GENERATIONAL IMPACT OF MATERNAL INFLAMMATION ASSOCIATED WITH FETAL GROWTH RESTRICTION**.

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

Background: Pregnancy complications are often associated with abnormal maternal inflammation and an increased risk of disease in mothers and their children. We hypothesize that the increased risk of disease following inflammatory pregnancy is partly mediated by abnormal innate immune reprogramming. The latter may involve memory acquisition by monocytes, which occurs after exposure to an inflammatory stimulus, and is characterized by an enhanced or dampened response to a secondary inflammatory stimulus. Innate immune memory may persist across generations following an inflammatory pregnancy.

Methods: C57BL/6 mice (F0) were mated and on gestational day 10.5 they received 20 µg/kg of lipopolysaccharides (LPS); controls received saline. Four weeks after delivery a male and female F1 offspring were euthanized, and bone marrow was collected for monocyte isolation. Isolated monocytes were exposed to an inflammatory stimulus and cytokine release was analyzed. Remaining F1 offspring were crossbred, and weights of pups were recorded on postnatal day 1.

Results: Supernatants of ex vivo cultures of monocytes from F1 offspring revealed a significant decrease in TNFα concentrations after secondary challenge with an inflammatory stimulus. Additionally, F2

offspring born to LPS-lineage F1 offspring crossed with saline-lineage F1 offspring were significantly larger than offspring born to F1 saline-lineage crosses.

Conclusion: Maternal exposure to inflammation during pregnancy affects innate immune cell reprogramming of F1 offspring and subsequently affects fetal growth of the F2 generation.

(Supported by the Canadian Institutes of Health Research (CIHR).

52. Jayne Dent (Field: Biochemistry and Cell Biology) **FUNCTIONAL CHARACTERIZATION OF THE CHROMOSOME 19 MIRNA CLUSTER IN SKIN CUTANEOUS MELANOMA MODELS**

Jayne Dent, Mike Vermeulen, Nicolas Fera, Stephanie Young, Kathleen Watt and Andrew Craig

Department of Biomedical and Molecular Sciences, Cancer Biology & Genetics/Queen's Cancer Research Institute, Queen's University

Metastatic skin cutaneous melanoma (SKCM) accounts for the majority of skin cancer-related deaths, and improved therapies are needed. We recently discovered that 10% of SKCM tumors in a large cohort express the chromosome-19 miRNA cluster (C19MC), with significant links to risk of metastasis. C19MC encodes 46 miRNA genes, with normal expression restricted to placental and embryonic stem cells. While this was a novel finding in melanoma, C19MC expression has been reported as poor prognosis in several other cancers in adults and children. In melanoma, we predict that C19MC activation drives invasive and metastatic phenotypes and that identifying mRNA targets of the cluster will reveal actionable targets. Here, we shave created isogenic cell lines using CRISPR/dCas9-based Synergistic Activation Mediator (SAM) system to induce expression of the C19MC. While no significant differences in cell growth rates were observed in culture or as xenograft tumors, we have preliminary data showing increased melanoma cell invasion and lung metastasis with C19MC expression. We are expanding our cohorts and will use RNA sequencing to identify relevant target transcripts for further studies. Together, these studies will define pathways linked to metastasis in melanoma that can be targeted in future to improve outcomes.

Supporting Agency: Canadian Institute of Health Research (CIHR)

53. Kasthuri Ravishanker (Field: Endometriosis) **DETERMINING THE ROLE OF CANNABINOIDS IN MODULATING NEUROINFLAMMATORY PATHWAYS IN ENDOMETRIOSIS (EM)**

Kasthuri Ravishanker

Department of Biomedical and Molecular Sciences

Background: Dorsal root ganglia **(**DRGs) have a role in chronic pain. There may be a close association between pain symptoms and the lumbar DRGs in EM patients. The role of immune cells in modulating DRG functions is unknown. Though cannabinoids have been shown to produce an analgesic effect, CBD and THC remain under-researched as EM therapeutics.

Hypothesis: The EM lesion microenvironment modulates neuroinflammatory pathways through DRGs, and treatment with cannabinoids will alter the immune microenvironment and modulate pain pathways in EM.

Objective 1: Characterize the immune cell composition of DRGs in EM. Lumbar DRGs from a murine model of EM will be digested for analysis of immune cells using flow cytometry.

Objective 2: Establish the impact of cannabinoid treatments on the DRG immune microenvironment. Various cannabinoid treatments will be administered to lumbar DRG cell cultures. Cytokine analysis and flow cytometry will be used to perform an analysis of immune cell populations and gauge DRG cell secretory response after treatment.

Results: CXCL5, CCL2, CCL3, IL-12p70, IL-9 and IL-15 were detected in baseline DRGs through cytokine analysis. Preliminary flow cytometry experiments indicate the presence of CD45+, CD3+ and CD11b+ cells within DRGs.

Significance: Identifying novel mechanistic pathways in the treatment of EM-associated pain in mice will help define DRGs as a valuable target for cannabinoid-based pain management therapies and open new therapeutic avenues for pain relief.

This research was supported with funding from the CIHR

54. Kody Klupt and Matthew Fishman (Field: Protein Function) **ILLUMINATING BIOMOLECULES: UNFOLDING THE THERAPEUTIC POTENTIAL BEYOND PROTEIN STRUCTURE ANALYSIS USING CIRCULAR DICHROISM**

Kody Klupt*, Matthew Fishman*, and the Protein Function Discovery Facility

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

We present a unique perspective on the use of circular dichroism (CD) in the medical sciences, expanding its applications beyond traditional protein structural analysis. CD, a powerful tool sensitive to the chirality of molecules, offers valuable insights into molecular interactions and conformation. It is the preferred method for studying the high-order structures of biological macromolecules due to its broad utility, requiring minimal sample, being label-free, and having limited data analysis requirements. In showcasing CD as a tool to study the intrinsic dynamics of protein structure, we demonstrate how it can enhance our understanding of disease mechanisms, biomolecular responses to environmental changes, and contribute to the design and testing of therapeutics. Moreover, CD plays a vital role in drug discovery by providing information on binding affinity and the stabilities of drug-target interactions. By utilizing CD at the Protein Function Discovery Facility, researchers can simplify the complexities surrounding dynamic biomolecules and shed light on their functions.

(Supported by the Natural Sciences and Engineering Research Council of Canada and the Ontario Graduate Scholarship Program.)

55. Emils Matiss and Kaitlyn Hoesterey (Field: Computational Neuroscience) **A BIOPHYSICALLY-BASED NETWORK MODELLING APPROACH TO SENSORIMOTOR CONTROL WITH MAMMALIAN CORTICAL CYTOARCHITECTURE AND HIERARCHICAL STRUCTURE**

Kaitlyn J. Hoesterey, Emils M. Matiss, Matthew L. Laporte, Dominic I. Standage, and Gunnar Blohm. Centre for Neuroscience Studies, Queen's University, Kingston, Ontario, Canada.

Keywords: control of movement, spiking neural network, reservoir computing

Large strides have been made in understanding the anatomy of the brain, however, how this anatomy contributes to complex computations remains more elusive. One approach to unravelling this mystery is to build cortical models. However, most systems neuroscience models only consider one generic group of excitatory and inhibitory neurons with connectivity that weakly represents biology. This hinders the discovery of how different functional neuron types and their interconnectivity contribute to computation. Here we built a network with cytoarchitecture and hierarchical connectivity inspired by the thalamocortical pathway to perform motor control in a ballistic reaching task. Izhikevich models of pyramidal, large basket, and spiny stellate neurons were simulated in Brian2. Physiological data of mammalian microcircuitry was used to inform how populations of these neurons are interconnected within and between network areas. Subsequently, a linear decoder was trained to transform network activity into motor commands to move a point mass on a 2D plane. The reference trajectory was generated using a linear quadratic Gaussian regulator and simulated feedback was provided to the network in an open-loop paradigm. The model was successful in generating motor commands that closely match those of optimal trajectories in the ballistic reaching task. Beyond its ability to perform sensorimotor control, our biophysically-based network model also paves the way for future research investigating how individual anatomical components contribute to cortical computations.

(Supported by the Natural Sciences and Engineering Research Council of Canada.)

56. Miruna Jurj (Field: Neuroscience) **BRAIN-BODY CROSSTALK: CHARACTERIZING GUT MICROBIAL DYSBIOSIS IN AUTISM SPECTRUM DISORDER (ASD) AND ATTENTION-DEFICIT/HYPERACTIVITY DISORDER (ADHD)**

Miruna Jurj[1,2], Calvin Sjaarda[1,2,3]

Centre for Neuroscience Studies, Queen's University, Kingston, Ontario, Canada;
Queen's Genomics Lab at Ongwanada (QGLO), Ongwanada Resource Center, Kingston, Ontario, Canada; Department of Psychiatry, Queen's University, Kingston, Ontario, Canada,

Children with attention-deficit/hyperactivity disorder (ADHD) and autism spectrum disorder (ASD) often experience comorbid gastrointestinal (GI) issues and engage in selective eating behaviours which can affect gut microbial composition. The aim of this exploratory study is to determine whether changes in gut microbial composition are associated with GI symptoms, behavioural symptoms and diet composition. Participants consisted of children ages 6-17 inclusive (n=23), with a physician-confirmed

diagnosis of ASD and/or ADHD. Each participant received a study package containing diet questionnaires, behavioural questionnaires, and stool sample collection kits. Questionnaires and stool samples were collected daily across a continuous 14-day study period. The microbial community of the stool samples were determined by 16S rRNA sequencing. A preliminary principal component analysis (PCA) of four individuals demonstrated that three participants shared similar microbial profiles while the remaining participant displayed independent clustering. To our knowledge, this is the first study analyzing daily temporal changes in the gut microbiome of children with ADHD and ASD and attempts to relate those changes to diet and behaviour of the child. As we continue to sequence samples and analyze our data, findings will contribute to our understanding of the gut-brain connection and neurodevelopmental conditions.