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

## Tuesday, June 4th, 2019
## Biosciences Complex

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## Acknowledgments

A special thank you to Katherine Brennan-Rowcliffe for her invaluable assistance in organizing this meeting.

# The Twenty-Second Annual Scientific Meeting for Health Science Research Trainees

## Faculty of Health Sciences

## Queen's University

Tuesday, June 4th, 2019

Biosciences Complex, Atrium and School of Medicine

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Tuesday, June 4th, 2019

Poster Presentations taking place in Biosciences Complex, Atrium

Oral Presentations taking place in School of Medicine Building, Room 032A

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8:00 – 8:45 ***Registration and Poster Set-Up in Biosciences Complex, Atrium***

8:45 – 9:00 ***Introductory Remarks Rm 032A, School of Medicine Building***
Dr. Brian Bennett, Associate Dean, Graduate and Postdoctoral Education, Faculty of Health Sciences
Dr. Richard Reznick, Dean, Faculty of Health Sciences

9:00 – 9:30 ***Keynote Speaker***
Dr. Susan Bartels
Departments of Emergency Medicine and Public Health Sciences
"Turning Stories Into Data "

# *Oral Presentation - Session 1*

**Chair: Dr. Stephen Pang**

9:35 – 9:47 **CLASSIFYING NEUROENDOCRINE NEOPLASMS USING MICRORNA-375 AND MICRORNA EXPRESSION.** Jina Nanayakkara[1], Xiaojing Yang[1], Justin Wong[1], Kathrin Tyryshkin[1], Thomas Tuschl[2], Neil Renwick[1,2] [1]Laboratory of Translational RNA Biology, Department of Pathology and Molecular Medicine, Queen's University, Kingston, ON [2]Laboratory of RNA Molecular Biology, The Rockefeller University, New York, NY

9:47 – 9:59 **THE GUT MICROBIOTA AS A NOVEL RISK FACTOR FOR THE DEVELOPMENT OF ANTI-DRUG ANTIBODIES IN HEMOPHILIA A MICE.** Cormier M, Tarrant J, Nesbitt K, Dwyer C, Hough C, and David Lillicrap. Department of Pathology and Molecular Medicine, Queen's University Kingston, Ontario Canada.

9:59 – 10:11 **OUTDOOR LIGHT AT NIGHT AND RISK OF BREAST CANCER – IS THERE A LINK?** Jennifer Ritonja[1], Michael McIsaac, Eric Sanders, Christopher C.M. Kyba, Anne Grundy, John J. Spinelli, Kristan J. Aronson. [1]Department of Public Health Sciences

10:11 – 10:23 **INTERLEUKIN-33 MEDIATES MACROPHAGE RECRUITMENT AND ACTIVATION PROMOTING ENDOMETRIOSIS PATHOPHYSIOLOGY.** Ryan M. Marks, Jessica Miller, Lindsey Symons, & Chandra Tayade DVM, PhD**.** Department of Biomedical and Molecular Science, Queen's University, Kingston ON, Canada

10:25 – 10:45 ***Coffee Break (Biosciences Atrium)***

10:45 – 12:15 Poster Presentations (Odd Numbered Abstracts)

12:15 – 1:00 Lunch/Poster Set-Up

1:00 – 2:30 Poster Presentations (Even Numbered Abstracts)

# *Oral Presentation – Session 2*

**Chair: Dr. Mark Ormiston**

2:30 – 2:42 **NOVEL BIOLOGICAL NETWORKS ASSOCIATED WITH CHEMOTHERAPY RESPONSE IN HIGH-GRADE SEROUS OVARIAN CANCER.** D.G. Topouza[1], J. Choi[1], S. Nesdoly[2], A. Tarnouskaya[2], M. Koti[1], Q.L. Duan[1,2] [1]Department of Biomedical and Molecular Sciences, Queen's University, Kingston, Ontario, Canada; [2]Queen's School of Computing, Queen's University, Kingston, Ontario, Canada.

2:42 – 2:54 **DECREASED AND LESS SUSTAINED HEMOSTATIC STRESS RESPONSES MAY CONTRIBUTE TO ABNORMAL BLEEDING IN TYPE 1 VWD: PRELIMINARY RESULTS.** Matteo Zago-Schmitt BSc[1], Robbie Kloosterman[1], Julie Grabell[3], Lisa Thibeault[2], Lori Harpell[1], Mackenzie Bowman PhD[3], Wilma Hopman[2], Amer Johri MD[3], David Good MD[1], Paula James MD[3. 1] Department of Pathology and Molecular Medicine, Queen's University, Kingston, Ontario, Canada [2] Kingston Health Sciences Center, Kingston, Ontario, Canada[3] Department of Medicine, Queen's University, Kingston, Ontario, Canada.

2:54 – 3:06 **THE INTEGRATION OF VIRTUAL SIMULATION GAMING INTO UNDERGRADUATE NURSING RESUSCITATION EDUCATION: A PILOT STUDY**. Evan Keys, Marian Luctkar-Flude, Kim Sears, Kevin Woo, and Jane Tyerman

3:06 – 3:18 **FECAL SUPERNATANTS FROM INFLAMMATORY BOWEL DISEASE PATIENTS ACTIVATE LUMBER SPLANCHNIC AFFERENT NERVES.** Amal Abu Omar, David Reed and Alan E. Lomax. Centre for Neuroscience Studies, Queen's University, Kingston, Ontario, Canada K7L2V7.

3:18– 3:30 **DEVELOPING SYNTHETIC ACTIN TOXINS FOR TREATMENT OF METASTATIC CANCERS.** Kavan Shah[1], Xu Deng[1,2], Madhu Aeluri[2], Bhavin Pipaliya[2], P. Andrew Evans[2], John S. Allingham[1], and Andrew W. Craig[1] [1]Department of Biomedical and Molecular Sciences, Queen's University [2]Department of Chemistry, Queen's University

3:30 – 3:45 ***Coffee Break (Outside room 032A)***

# *Oral Presentation – Session 3*

**Chair: Dr. Madhuri Koti**

3:45 – 3:57 **EXPLORING THE GENOME-WIDE BINDING LANDSCAPE OF THE ONCOGENIC TRANSCRIPTION FACTOR TCF3-PBX1 IN ACUTE LYMPHOBLASTIC LEUKEMIA.** Alison M. Moore[1,2], Marina R. Lochhead[3], Kyster Nanan[1,2], Jesse H. Leblanc[1,2], Changnian Shi[1,2], Steven P. Smith[3] and David P. LeBrun[1,2] [1]Department of Pathology and Molecular Medicine, Queen's University, Kingston, Ontario, Canada[2]Division of Cancer Biology and Genetics, Queen's Cancer Research Institute, Kingston, Ontario, Canada [3]Department of Biomedical and Molecular Sciences, Queen's University, Kingston, Ontario, Canada

3:57 – 4:09 **IL-4 STIMULATION INDUCES AN ANTIVIRAL STATE IN M2 MACROPHAGES.** Andra Banete, Bruce Banfield, and Sam Basta**.** Department of Biomedical and Molecular Sciences, Queen's University, Kingston ON, Canada

4:09 – 4:21 **STRUCTURE-GUIDED INHIBITION OF POLYPHOSPHATE KINASE IN PSEUDOMONAS AERUGINOSA AS A NOVEL ANTIVIRULENCE STRATEGY.** Nolan Neville, Nathan Roberge, Zongchao Jia. Department of Biomedical and Molecular Sciences, Queen's University Kingston, Ontario Canada.

4:21 – 4:33 **TGFB AND INTERLEUKIN-15 SYNERGIZE THROUGH MAP KINASE PATHWAYS TO DRIVE THE CONVERSION OF NATURAL KILLER CELLS TO A TISSUE-RESIDENT PHENOTYPE.** Lindsey G. Hawke, Brandon Z. Mitchell, M.L. Ormiston. Queen's University Departments of Biomedical and Molecular Sciences, Medicine and Surgery, Kingston, Ontario, K7L 3N6, Canada

4:35 – 4:45 Concluding Remarks and Awards

5:00 – 7:00 Reception at The Grad Club, 2nd Floor
162 Barrie Street, Kingston, ON
Cash Bar

# *Oral Presentations*

## Cancer Research and Therapy

**EXPLORING THE GENOME-WIDE BINDING LANDSCAPE OF THE ONCOGENIC TRANSCRIPTION FACTOR TCF3-PBX1 IN ACUTE LYMPHOBLASTIC LEUKEMIA.** Alison M. Moore[1,2], Marina R. Lochhead[3], Kyster Nanan[1,2], Jesse H. Leblanc[1,2], Changnian Shi[1,2], Steven P. Smith[3] and David P. LeBrun[1,2] [1]Department of Pathology and Molecular Medicine, Queen's University, Kingston, Ontario, Canada [2]Division of Cancer Biology and Genetics, Queen's Cancer Research Institute, Kingston, Ontario, Canada [3]Department of Biomedical and Molecular Sciences, Queen's University, Kingston, Ontario, Canada

Current treatments for acute lymphoblastic leukemia (ALL) are effective but associated with severe long- and short-term toxicities. There remains a need to elucidate the biology of the disease in order to inform the development of less toxic, more effective therapies. The oncogenic transcription factor TCF3-PBX1 is expressed in 5% of ALL cases consequent to a somatic chromosomal translocation. Although it is broadly accepted that TCF3-PBX1 binds to DNA across the genome and affects gene transcription resulting in dysregulated proliferation, survival and/or differentiation of hematopoietic cells, the particular binding sites and genes affected have not been identified. We therefore aimed to identify where TCF3-PBX1 binds across the genome using chromatin immunoprecipitation followed by massively parallel DNA sequencing (ChIP-seq). We identified over 3500 TCF3-PBX1 binding sites, many of which localize near transcriptional start sites along with other well-known transcription factors. Many of these binding sites have attributes characteristic of *cis*-regulatory elements, including binding of the transcriptional co-activator p300, which appears to be recruited to novel sites by TCF3-PBX1. A total of 163 genes are 1) near a TCF3-PBX1 binding site; and 2) differentially expressed upon shRNA-mediated knockdown of TCF3-PBX1, suggesting that they are directly transcriptionally regulated by the fusion protein. These target genes and their associated cellular pathways will inform future studies to elucidate the manner in which lymphoid development is disturbed by TCF3-PBX1 in ALL. (Supported by the Canadian Institutes of Health Research).

**CLASSIFYING NEUROENDOCRINE NEOPLASMS USING MICRORNA-375 AND MICRORNA EXPRESSION.** Jina Nanayakkara[1], Xiaojing Yang[1], Justin Wong[1], Kathrin Tyryshkin[1], Thomas Tuschl[2], Neil Renwick[1,2] [1]Laboratory of Translational RNA Biology, Department of Pathology and Molecular Medicine, Queen's University, Kingston, ON [2]Laboratory of RNA Molecular Biology, The Rockefeller University, New York, NY

**Introduction:** Neuroendocrine neoplasms (NENs) are clinically diverse tumors that lack highly sensitive and specific biomarkers. microRNAs (miRNAs) are regulatory RNA molecules that demonstrate cell-type and disease-stage specificity. To date, there are no comprehensive biomarker studies of miRNAs in multiple NEN pathological types. We hypothesize that 1) there is a universal miRNA marker for NENs, and 2) machine learning models can identify NEN types using miRNAs. **Methods:** We performed barcoded small RNA sequencing on 224 archived NEN samples of 14 different pathological types. NENs were compared to neoplastic and non-neoplastic control tissue from a custom database of miRNA expression. We ranked highly expressed miRNAs and assessed their ability to discriminate NEN types. Selected miRNAs were incorporated into a hierarchical machine-learning model to classify NENs. **Results:** miR-375 was highly expressed in all NENs: contributing >10% of miRNA expression. In contrast, miR-375 was barely expressed in non-NEN cancers (n=933) and normal tissues (n=1081). Seventeen miRNAs strongly discriminated NEN pathological types. These miRNAs built a multi-layer machine-learning model that separated NENs on developmental origin (neuroectoderm vs. epithelium), then anatomic origin, with >95% accuracy at each layer. **Conclusions:** In summary, we identified miR-375 as a candidate universal marker of NENs. We demonstrated that machine-learning models can accurately classify NENs using only miRNAs. Further validation of these findings could establish miRNA-guided diagnostics for NENs and inform future NEN classification frameworks.

**DEVELOPING SYNTHETIC ACTIN TOXINS FOR TREATMENT OF METASTATIC CANCERS.** Kavan Shah[1], Xu Deng[1,2], Madhu Aeluri[2], Bhavin Pipaliya[2], P. Andrew Evans[2], John S. Allingham[1], and Andrew W. Craig[1] [1]Department of Biomedical and Molecular Sciences, Queen's University [2]Department of Chemistry, Queen's University

HER2 is a driver and clinical target in breast and ovarian cancers with high rates of metastasis. Despite advances in HER2 targeted therapies, not all metastatic cancers respond effectively. Here, we focus on "actin addiction" of metastatic cancers that require dynamic actin polymerization into filamentous-actin (F-actin)-based protrusions. We predict that F-actin-disrupting drugs may complement existing cancer therapies. A series of 20 simplified synthetic analogs of Mycalolide B (Myc B), a marine macrolide toxin that severs F-actin, were generated and profiled for activity in SKOV3 ovarian cancer cells. One analog, BVP-02-013, was exceptionally potent in HER2+ cancer cells, exhibiting dose dependent suppression of growth, viability and directional migration. Live cell imaging of SKOV3 cells expressing a LifeAct-GFP reporter showed that this analog severs cellular F-actin, leading to formation of aggresomes within minutes of treatment at doses in the low µM range. These results imply that simplified MycB analogs have potential as novel anti-cancer drugs and can form the warhead of new antibody-drug conjugates to target metastatic HER2+ cancers.

**NOVEL BIOLOGICAL NETWORKS ASSOCIATED WITH CHEMOTHERAPY RESPONSE IN HIGH-GRADE SEROUS OVARIAN CANCER.** D.G. Topouza[1], J. Choi[1], S. Nesdoly[2], A. Tarnouskaya[2], M. Koti[1], Q.L. Duan[1,2] [1]Department of Biomedical and Molecular Sciences, Queen's University, Kingston, Ontario, Canada; [2]Queen's School of Computing, Queen's University, Kingston, Ontario, Canada

Ovarian cancer has the lowest survival rate of all common gynaecological cancers. The standard of care is platinum-based chemotherapy; however, more than 30% of patients are resistant to this treatment. Previous studies have identified genes correlated with chemotherapy response in ovarian cancer, but the underlying mechanisms of resistance remain poorly understood. In this study, we analyzed RNA-seq data from 112 sensitive and 78 resistant high-grade serous ovarian cancer (HGSOC) patients (TCGA) to detect biological networks associated with platinum-based chemotherapy response. We applied machine-learning to determine connections among expressed transcripts from both mRNA and miRNA. Using these results, we constructed novel integrated mRNA-miRNA networks. Moreover, we integrated mRNA expression data with genome-wide polymorphism data, to identify variants potentially regulating gene expression, known as expression quantitative trait loci (eQTL). Our study identified 92 differentially expressed mRNAs that show a repression of adaptive immunity in resistant patients, and one mRNA network enriched for protein ubiquitination. We also identified 21 differentially expressed miRNAs that regulate the epithelial-mesenchymal transition (EMT) and the *MET/ERK2* oncogenic pathway, and three miRNA networks enriched for lipoprotein transport and angiogenesis. Finally, we located 75 eQTL associated with the expression of significant mRNAs. We characterised novel genes and networks that potentially regulate chemotherapy response in HGSOC patients. Our findings help elucidate the underlying mechanisms of resistance, ultimately leading to patient genetic screening and novel therapeutic targets.

## Cardiac, Circulatory, and Respiratory Sciences

**DECREASED AND LESS SUSTAINED HEMOSTATIC STRESS RESPONSES MAY CONTRIBUTE TO ABNORMAL BLEEDING IN TYPE 1 VWD: PRELIMINARY RESULTS.** Matteo Zago-Schmitt BSc[1], Robbie Kloosterman[1], Julie Grabell[3], Lisa Thibeault[2], Lori Harpell[1], Mackenzie Bowman PhD[3], Wilma Hopman[2], Amer Johri MD[3], David Good MD[1], Paula James MD[3. 1] Department of Pathology and Molecular Medicine, Queen's University, Kingston, Ontario, Canada [2] Kingston Health Sciences Center, Kingston, Ontario, Canada [3] Department of Medicine, Queen's University, Kingston, Ontario, Canada

Von Willebrand factor (VWF) levels are used to diagnose Type 1 Von Willebrand Disease (VWD), however there is significant heterogeneity in bleeding scores (BS) among those with Type 1 VWD and the correlation between VWF levels and BS is absent. The impact of the hemostatic stress response on bleeding has not been evaluated in Type 1 VWD. Desmopressin, used therapeutically, can also be used as a hemostatic stress surrogate. By running consecutive desmopressin trials, the response to repeat stress can be evaluated. The hemostatic response can also be characterized by studying subject-derived blood outgrowth endothelial cells (BOEC). Five adult Type 1s and eight controls were enrolled. Subjects participated in 2 desmopressin trials 24 hours apart. Desmopressin was administered sc and blood taken pre-administration and 1, 2, and 4 hours after and VWF levels measured. 24 hours later the same procedure was repeated. Controls had a significantly greater rise over baseline in VWF antigen up to 4 hours post desmopressin on day 1 compared to Type 1s. Controls also had a significantly greater rise over baseline in VWF activity (VWF:GPIbM) levels at peak. The greater rise in VWF:GPIbM at peak and at 4 hours negatively correlates with BS. These results may suggest that Type 1s are less able to respond and maintain a response to hemostatic stress and this may contribute to bleeding. (Supported by the Canadian Haemophilia Society)

## Health Policy, Population Health, and Epidemiology

**OUTDOOR LIGHT AT NIGHT AND RISK OF BREAST CANCER – IS THERE A LINK?** Jennifer Ritonja[1], Michael McIsaac, Eric Sanders, Christopher C.M. Kyba, Anne Grundy, John J. Spinelli, Kristan J. Aronson**.** [1]Department of Public Health Sciences

**Introduction:** Exposure to light at night (LAN) disrupts circadian rhythms, and this disruption is hypothesized to increase breast cancer risk, supported by findings from epidemiologic studies of female shift workers. We investigated whether residential outdoor LAN is associated with breast cancer risk. **Methods:** A population-based case-control study was conducted in Vancouver BC and Kingston ON. Participants were 778 incident breast cancer cases and 830 cancer-free controls who completed a questionnaire collecting many characteristics as well as lifetime residential histories. For each home address 10 to 20 years prior to study entry, outdoor LAN exposure was estimated from two sources of satellite imagery and these measurements were used to derive two time-weighted averages for each participant. Logistic regression estimated the relationship between outdoor LAN and breast cancer risk. **Results:** Higher outdoor LAN exposure is associated with a higher risk of breast cancer among pre-menopausal women. There was no association between outdoor LAN and breast cancer among post-menopausal women. Women who also work at night and who are exposed to higher levels of outdoor LAN may experience a higher risk of breast cancer. **Conclusion:** This study provides evidence that pre-menopausal women, those who work at night, and those who are exposed to higher LAN around their homes may be at an increased risk for breast cancer (Supported by CIHR, Breast Cancer Action Kingston, DAAD German Faculty Research Exchange).

## Inflammation, Infection and Immunity

**IL-4 STIMULATION INDUCES AN ANTIVIRAL STATE IN M2 MACROPHAGES.** Andra Banete, Bruce Banfield, and Sam Basta**.** Department of Biomedical and Molecular Sciences, Queen's University, Kingston ON, Canada

Macrophages (Mϕ) are innate immune tissue sentinels with a variety of functional phenotypes depending on their cytokine microenvironment. Mϕs exhibit distinct activation patterns across a continuum from pro-inflammatory (M1), induced by IFNγ, to anti-inflammatory (M2a), induced by IL-4. Given the critical role of Mϕ in regulating innate and adaptive immunity, it is not surprising that Mϕ activation status directly influences the outcome of viral infections. Viruses can exploit inflammation to bypass host barriers and support replication. Viruses such as lymphocytic choriomeningitis virus (LCMV) and Lassa virus directly suppress Mϕ activation leading to viral chronicity and severe disease. While the role of M1 Mϕ in viral infections has been defined, the mechanism behind altered responses of M2 Mϕ to virus infections is unknown. We investigated the response of activated spleen-derived Mϕ (SpM) to LCMV infection to understand how polarization influences the initiation of an adequate immune response. Following infection, polarized Mϕs show an altered resistance to LCMV infection, which was not due to modifications in viral entry. Protein expression and localization of innate recognition molecules and transcription factors involved in the RLR pathway was evaluated, including RIG-I, MAVS, STING, and IRFs, which play a crucial role in controlling infections. This research has implications for immunotherapy as broad-acting antivirals, as RLR agonists may be used in combination with other therapies to target the innate arm of immunity against emerging infections.
*Research funded by NSERC and CIHR.*

**THE GUT MICROBIOTA AS A NOVEL RISK FACTOR FOR THE DEVELOPMENT OF ANTI-DRUG ANTIBODIES IN HEMOPHILIA A MICE.** Cormier M, Tarrant J, Nesbitt K, Dwyer C, Hough C, and David Lillicrap. Department of Pathology and Molecular Medicine, Queen's University Kingston, Ontario Canada.

The human gut microbiota is a complex community of commensal microorganisms that line our gastrointestinal tract and a disruption of its composition, termed dysbiosis, is associated with the development of many immune-related pathologies. In part, immune regulation occurs through the immunosuppressive actions of various bacterially-produced metabolites that are critical for immune homeostasis. The development of anti-drug antibodies in up to one third of hemophilia A patients results from a dysregulated immune response that targets the therapeutic factor (F) VIII used to treat these patients. Here we investigate if gut dysbiosis is a contributing factor to the development of this adverse immune response. Ampicillin was administered to disrupt the gut microbiota of hemophilia A mice and then were treated with FVIII infusions. Relative to control mice, alterations in the microbiota of ampicillin-treated mice were observed and this dysbiosis was accompanied by a significant increase in levels of inhibitory anti-FVIII IgG ($P<0.0001$). In addition, nuclear magnetic resonance of caecal contents revealed significant depletion of immunosuppressive metabolites butyrate ($P=0.0035$), acetate ($P=0.0010$) and propionate ($P< 0.0001$). Further analysis of the gut microbial composition of these mice through next generation sequencing eloquently demonstrated a depletion of the relative abundances of bacteria that produce these metabolites. Taken together, these data illustrate that resident gut bacteria produce metabolites that likely modulate anti-drug antibody formation in hemophilia A patients. (Supported by the Canadian Institutes of Health Research and the National Institutes of Health)

**TGFB AND INTERLEUKIN-15 SYNERGIZE THROUGH MAP KINASE PATHWAYS TO DRIVE THE CONVERSION OF NATURAL KILLER CELLS TO A TISSUE-RESIDENT PHENOTYPE.** Lindsey G. Hawke, Brandon Z. Mitchell, M.L. Ormiston. Queen's University Departments of Biomedical and Molecular Sciences, Medicine and Surgery, Kingston, Ontario, K7L 3N6, Canada

Circulating Natural Killer (NK) cells are known to convert to a tissue-resident, or type 1 innate lymphoid cell (ILC1)-like phenotype in response to chronic transforming growth factor-β (TGFβ) exposure. However, the precise cellular changes defining this process, as well as the downstream signaling pathways guiding it, remain poorly defined, particularly in humans. We used CyTOF mass cytometry to model this phenotypic shift *in vitro* and identify a synergistic activity of TGFβ and interleukin-15 (IL-15) this cellular conversion. CyTOF profiling identified substantial heterogeneity in the propensity of NK cells to adopt an ILC1-like phenotype, dictated by the acquisition of various markers, including integrins CD9, CD103, CD49a, and CD69 in response to culture in the presence of both cytokines. Assessment of the TGFβ signaling pathways identified TAK1 mediated activation of p38 MAPK as the critical pathway driving conversion. IL-15 enhanced TGFβ, mediated conversion through Ras:RAC1-based P38 signaling as well as via the activation of the MEK/ERK pathway. Interestingly, the adoption of an ILC1-like phenotype was independent of the actions of IL-15 or TGFβ on mTOR, as culturing NK cells in the presence of mTORC inhibitors had no effect on conversion efficiency. In conclusion, we have used *in vitro* human culture systems and mass cytometry to define the conversion of circulating NK cells to an ILC1-like phenotype and clarify the pathways responsible for this process. (Supported by CIHR)

## Neuroscience Research

**FECAL SUPERNATANTS FROM INFLAMMATORY BOWEL DISEASE PATIENTS ACTIVATE LUMBER SPLANCHNIC AFFERENT NERVES.** Amal Abu Omar, David Reed and Alan E. Lomax. Centre for Neuroscience Studies, Queen's University, Kingston, Ontario, Canada K7L2V7.

Increased excitability of extrinsic afferent neurons has emerged as an important cause of visceral pain during inflammatory bowel disease (IBD). However, little is understood regarding what role, if any, the intestinal microbiota plays in pain development during IBD. Therefore, we aimed to determine the effects of fecal supernatants (FS) from patients with IBD on lumbar splanchnic nerves (LSN). Fecal samples from 9 patients with active IBD were suspended in normal saline solution at 0.5gm/4 ml. In vitro extracellular recordings technique to record from LSN of the distal from healthy mice. LSN afferents were classified based on their responses to colonic distention into LT (low threshold), HT (high threshold) and MIS (mechanically insensitive) units. FS was applied intraluminally for 20min and mechanical sensitivity to distention was re-assessed after 5 minutes. FS from IBD patients increased the basal firing frequency of afferent nerves compared to vehicle control. Furthermore, these FS augmented the mechanosensitivity of HT and MIS units. These changes would lead to an increased afferent input to the CNS, which could exacerbate pain. Further characterization of the active mediators responsible for the sensitization of afferent nerves has the potential to lead to new targets for pain management in IBD. Funding Agency: Crohn's and Colitis Canada

## Patient Care and Nursing

**THE INTEGRATION OF VIRTUAL SIMULATION GAMING INTO UNDERGRADUATE NURSING RESUSCITATION EDUCATION: A PILOT STUDY**. Evan Keys, Marian Luctkar-Flude, Kim Sears, Kevin Woo, and Jane Tyerman

**Background**: Virtual Simulation Games (VSG) offer a novel teaching modality that has shown promise globally. The VSG created for the study follows the Heart and Stroke Foundation of Canada's (HSF) guidelines for both Basic Life Support (BLS), and Advanced Cardiac Life Support (ACLS), and provides students with an engaging, innovative, way to learn life-saving skills. **Purpose**: The research team examined whether learners with the VGS pre-simulation preparation translated their experience into practical application gains better than those with traditional pre-simulation preparation. **Design**: A pilot study was designed, modelled after a randomised controlled trial, to observe the effects of VGS on nursing students' practical application skills. Control participants were provided the HSF BLS and ACLS guidelines and intervention participants were provided HSF BLS and ACLS Guidelines as well as the VGS. All participants then underwent an ACLS-style simulation while caring for a patient in ventricular fibrillation. **Results**: Due to the non-parametricity of the collected data, a Mann-Whitney U test found a statistically significant difference between the intervention and control group ($p = 0.003$); with the intervention group ($M = 11.5/12$) outperforming the control group ($M = 8.1/12$). **Implications**: These findings support the claim that virtual simulation gaming effectively supplements traditional training in the field of resuscitation science; helping students meet and exceed their educational goals.

## Protein Structure and Function

**STRUCTURE-GUIDED INHIBITION OF POLYPHOSPHATE KINASE IN PSEUDOMONAS AERUGINOSA AS A NOVEL ANTIVIRULENCE STRATEGY.** Nolan Neville, Nathan Roberge, Zongchao Jia. Department of Biomedical and Molecular Sciences, Queen's University Kingston, Ontario Canada.

*Pseudomonas aeruginosa* is one of the most dreaded hospital-acquired pathogens and the leading cause of morbidity and mortality in cystic fibrosis patients. Multidrug-resistant *P. aeruginosa* is emerging at an alarming rate, and the World Health Organization has deemed this bacterium a Priority 1 pathogen that is in urgent need of new antibiotics. An attractive target for new drugs is the enzyme polyphosphate kinase (PPK), which converts ATP to long chains of inorganic polyphosphate (polyP). Remarkably, polyP accumulation is essential for bacterial survival and persistence in the host, and PPK is required for *P. aeruginosa* virulence in animal models of infection. We have recently identified a family of small molecules that inhibit purified PPK with low-micromolar affinity *in vitro*. Moreover, these compounds significantly inhibit PPK-dependent virulence phenotypes in cultured *P. aeruginosa*, including biofilm formation, motility, and toxin secretion. Importantly, our inhibitors display little cytotoxicity in cultured mammalian cells. We aim to elucidate the molecular mechanisms by which our molecules inhibit PPK. Using a combination of X-ray crystallography, enzyme kinetic studies, and iterative rounds of analogue screening, a structure-activity relationship will be developed to identify moieties that are critical for inhibition. This information will guide subsequent chemical modifications to optimize potency and specificity for PPK. This work forms the basis of a potential new class of antivirulence therapeutics to treat multidrug-resistant *P. aeruginosa* infections. (Supported by Cystic Fibrosis Canada and NSERC)

## Reproductive and Sexual Function

**INTERLEUKIN-33 MEDIATES MACROPHAGE RECRUITMENT AND ACTIVATION PROMOTING ENDOMETRIOSIS PATHOPHYSIOLOGY.** Ryan M. Marks, Jessica Miller, Lindsey Symons, & Chandra Tayade DVM, PhD**.** Department of Biomedical and Molecular Science, Queen's University, Kingston ON, Canada

**Background:** Endometriosis is an inflammatory gynaecological disease characterized by the presence of proliferating ectopic endometrial-like lesions. Macrophage activation is critical for sustaining lesion neovascularization/development and facilitating associated peritoneal inflammation, however causal factors regulating pathologic macrophage activation in endometriosis remain elusive. Elevated interleukin (IL)-33 was identified in endometriotic lesions and is implicated in similar inflammatory pathologies. We hypothesize that IL-33 promotes peritoneal inflammation and lesion development via macrophage activation in a mouse model of endometriosis. **Methods:** Endometriosis was surgically induced in 8-10-week-old female C57BL/6 mice, treated twice weekly with recombinant IL-33 (1µg) or saline. Additionally, IL-33-macrophage depletion cohorts were generated with concurrent intraperitoneal clodronate-liposome treatment. Pathology hallmarks were evaluated as follows: 1) Peritoneal inflammation characterized by measuring peritoneal lavage cytokines via multiplex. 2) Peritoneal macrophage ($CD11b^{+}F4/80^{+}$) frequency was determined by flow cytometry of recovered peritoneal exudate cells. 3) Endometriotic lesions were evaluated by immunohistochemistry for vascularization (CD31), cellular proliferation (Ki-67), and macrophage infiltration (F4/80). **Results:** IL-33 treated mice developed significant peritoneal inflammation (TNFα, MCP-1, MIP-1β, TGF-β) compared to saline. Lesions from IL-33 treated mice had significantly greater vascular density (CD31) and cellular proliferation (Ki-67) and were accompanied with significant macrophage infiltration (F4/80). Importantly, macrophage depletion significantly reduced IL-33-induced inflammation, lesion vascularization and subsequent proliferation. **Conclusion:** IL-33 may regulate the endometriotic lesion microenvironment by mediating macrophage recruitment and activation, ultimately supporting lesion development and sustaining peritoneal inflammation.

# *Poster Presentations*

## Biomedical Engineering

1. **THE USE OF MULTIVARIATE REGRESSION TO QUANTIFY AGE-RELATED DEVELOPMENTAL CHANGES IN COORDINATION IN CHILDREN AND YOUTH**. Stephan C.D. Dobri[1], Stephen H. Scott[2], T. Claire Davies[1]1: Mechanical and Materials Engineering, 2: Biomedical and Molecular Sciences

## Cancer Research and Therapy

2. **CADHERIN-11 IS REQUIRED FOR ACTIVATION OF STAT3 BY V-SRC.** Hanad Adan, Stephanie Guy and Leda Raptis**.** Department of Pathology and Cancer Center, Queen's University, Kingston, Ontario, Canada.

3. **NEW COMBINATION THERAPIES FOR INFLAMMATORY BREAST CANCER USING INHIBITORS OF PI3K AND AURORA A KINASE**. Nadia Al Ali and Andrew W. Craig. Department of Biomedical and Molecular Sciences, Queen's University. Division of Cancer Biology and Genetics, Queen's Cancer Research Institute

4. **IDENTIFICATION OF NOVEL RECEPTORS TYROSINE KINASES (RTKS) REGULATING THE HIPPO SIGNALING PATHWAY IN TUMORIGENESIS**. Taha Azad, Kazem Nouri, Helena J Janse van Rensburg, Xiaolong Yang* Department of Pathology and Molecular Medicine, Queen's University, Kingston, K7L 3N6, Canada

5. **INVESTIGATING THE STING PATHWAY TO EXPLAIN MECHANISMS FOR BCG FAILURES IN NON-MUSCLE INVASIVE BLADDER CANCER.** Stephen Chenard[1, 3, 4], Sarah Nersesian[5], Thiago Vidotto[6], Alvaro Morales[4], D. Robert Siemens[1, 3, 4], Madhuri Koti*[1, 2, 3,4] *Department of Biomedical and Molecular Sciences, Queen's University, Kingston, Canada, Department of Obstetrics and Gynecology, Kingston Health Sciences Center, Queen's University, Kingston, Canada*, *Cancer Biology and Genetics, Queen's Cancer Research Institute, Queen's University, Kingston, Canada*, *Department of Urology, Kingston Health Sciences Center, Queen's University, Kingston, Canada*, *School of Medicine, Dalhousie University, Canada*, *Department de Genética, University of São Paulo, Brazil*

6. **EXAMINING THE EFFECTS OF BMP9 ON ANGIOGENESIS IN MURINE METASTATIC LUNG TUMOURS**. Devon V. Cole, Yan Gao, Matthew T. Rätsep, Patricia D. A. Lima, Melissa Mitchell, Peter A. Greer, and Mark L. Ormiston. Departments of Biomedical and Molecular Sciences and Medicine, Queen's University, Kingston, Ontario, Canada.

7. **CLINICAL IMPACT OF NEXT-GENERATION SEQUENCING ON MYELOID CANCER DIAGNOSIS, RISK AND TREATMENT ASSESSMENT**. Christina Ferrone[1], Henry Wong[2], Laura Semenuk[2], Brooke Snetsinger[1], Xiao Zhang[1], Patricia Farmer[1], Annette E. Hay[3], David Good[1], Graeme Quest[1], Harriet E. Feilotter[1,2], Michael J. Rauh[1] *[1]Department of Pathology and Molecular Medicine, Richardson Laboratory, Queen's University, [2]Molecular Genetics Laboratory, Kingston Health Sciences Centre (KHSC), [3]Department of Medicine, Queen's University, Kingston, ON, Canada*

8. **POLY(I:C)-MEDIATED DEATH OF HUMAN PROSTATE CANCER CELL LINES IS INDUCED BY IL-27 TREATMENT.** Olena Kourko[1], Robin Smyth[1], Daniela Cino[1], Kyle Seaver[1], Carlene Petes[1], Sally Eo[1], Sam Basta[1], and Katrina Gee[1] [1]Department of Biomedical and Molecular Sciences, Queen's University, Kingston ON.

9. **INVESTIGATING THE STAT1 ASSOCIATED IMMUNE CHECKPOINT GENE EXPRESSION AND SPATIAL PROFILES OF ADAPTIVE IMMUNE CELLS IN HIGH-GRADE SEROUS OVARIAN CANCER.** Deyang Li[1,4], Thiago Vidotto[1], Noor Shakfa[1,4], Afrakoma-Afriyie Asante[1,4], Nichole Peterson[2], Manon de Ladurantaye[3], Anne-Marie Mes-Masson[3], Julie-Ann Francis[2], Madhuri Koti[1,2, 4] *[1]Department of Biomedical and Molecular Sciences, Queen's University [2]Department of Obstetrics and Gynecology, Queen's University*, *Centre de recherche du Centre hospitalier de l'Université de Montréal (CRCHUM)/Institut du cancer de Montréal and Université de Montréal, Montreal, QC, Canada. [4]Division of Cancer Biology and Genetics, Cancer Research Institute, Queen's University*

10. **POST-TRANSLATIONAL MODIFICATION OF PROGRAMMED DEATH LIGAND (PD-L1) IN TRIPLE NEGATIVE BREAST CANCER.** Min Ling, Dr. Xiaolong Yang, Department of Pathology and Molecular Medicine, Queen's University Kingston, Ontario Canada

11. **ROLE OF THE PROGRAMMED DEATH LIGAND 1 (PD-L1) IMMUNE CHECKPOINT IN THE ACQUISITION OF DRUG RESISTANCE IN TUMOUR CELLS.** Minassian L[1,2], Sanwalka D[1,2], Macdonald-Goodfellow S[1,2], Ghaffari A[1,2], Craig A, Siemens[1,2,3] DR, Graham CH[1,2,3] *1) Biomedical and Molecular Sciences, Queen's University, Kingston, Ontario, Canada. 2) Cancer Research Institute, Queen's University, Kingston, Ontario, Canada. 3) Department of Urology, Kingston General Hospital, Kingston, Ontario, Canada.*

12. **EFFECTS OF MEN2 MUTATIONS ON RET RECEPTOR LOCALIZATION AND FUNCTION**. Eduardo Reyes-Alvarez, Brandy Hyndman, Eric Lian, and Lois M. Mulligan. Department of Biomedical and Molecular Sciences, Queen's University, Ontario Canada. Department of Pathology and Molecular Medicine, Cancer Research Institute Division of Cancer Biology and Genetics, Queen's University, Ontario Canada.

13. **CAN THE GENOTYPE OF HIGH-GRADE SEROUS OVARIAN CANCER CELLS AFFECT THEIR RESPONSE TO CHEMO-IMMUNOTHERAPY?** Noor Shakfa[1,2], Elizabeth Lightbody[1,2], Afrakoma Afriyie-Asante[1,2], Vinicius Kannen[1,2], Madhuri Koti[1,2,3] *Department of Biomedical and Molecular Sciences, Queen's University, Kingston, ON, Canada*, *Division of Cancer Biology and Genetics, Queen's Cancer Research Institute, Kingston, ON, Canada*, *Department of Obstetrics & Gynaecology, Queen's University, Kingston, ON, Canada.*

14. **ELUCIDATING ISOFORM SPECIFIC ROLES FOR CALPAIN-1 AND CALPAIN-2 IN BREAST CANCER.** Ivan Shapovalov, James MacLeod, Yan Gao, and Peter Greer. Department of Pathology and Molecular Medicine, Queen's University, Division of Cancer Biology and Genetics, Queen's Cancer Research Institute, Kingston, Ontario, Canada.

15. **ROLE OF INNATE IMMUNE MEMORY IN RESPONSE TO BCG IMMUNOTHERAPY OF BLADDER CANCER.** William Tran, Jean-François Paré, Charles Graham

16. **TARGETING THE HIPPO PATHWAY FOR TRIPLE-NEGATIVE BREAST CANCER THERAPY.** Liqing Wu; Xiaolong Yang. Department of Pathology & Molecular Medicine, Queen's University Kingston, Ontario Canada.

## Cardiac, Circulatory, and Respiratory Sciences

17. **THE ROLE OF VON WILLEBRAND FACTOR IN THE PATHOGENESIS OF DEEP VEIN THROMBOSIS.** Choi SJ, Swystun L, Michels A, Dwyer C, Nesbitt K, and Lillicrap D. Department of Pathology and Molecular Medicine, Queen's University, Kingston ON, Canada.

18. **TYROSINE-PROTEIN KINASE SRC REGULATES Kv1.5 CHANNEL ACTIVITY AND MEMBRANE EXPRESSION THROUGH INTERACTION WITH THE N-TERMINUS OF THE CHANNEL**. Taylore Dodd, Tingzhong Wang and Shetuan Zhang. Department of Biomedical and Molecular Sciences, Queen's University Kingston, ON, Canada.

19. **EXAMINING THE IMPACT OF ENDOTHELIAL BONE MORPHOGENETIC PROTEIN RECEPTOR 2 LOSS ON INTERLEUKIN-15 SIGNALING AND THE PATHOGENESIS OF PULMONARY ARTERIAL HYPERTENSION.** L. Rhiannon Hilton[1,2], Matthew Rätsep[1,3], Patricia Lima[1], Melissa Mitchell[1] and Mark. Ormiston[1,2,3,4] [1] Queen's Cardiopulmonary Unit, Queen's University, Kingston, Canada. [2] Department of Biomedical and Molecular Sciences, Queen's University, Kingston, Canada. [3] Department of Medicine, Queen's University, Kingston, Canada [4] Department of Surgery, Queen's University, Kingston, Canada

20. **ZONE 0/1 FROZEN ELEPHANT TRUNK WITH WHOLE BODY PERFUSION IN AORTIC DISSECTION.** Stephanie Jiang, Syed Hassan, Darrin Payne M.D., Andrew Hamilton M.D., Dimitri Petsikas M.D., Gianluigi Bisleri M.D. Department of Surgery, Kingston Health Sciences Centre, Ontario Canada.

*21.* **INTRAOPERATIVE HIGH-DENSITY MAPPING DURING SIMULTANEOUS HYBRID ABLATION FOR THE TREATMENT OF ATRIAL FIBRILLATION**. Camila Mayorga Palacios, Syed M. Ali Hassan, Benedict Glover, Damien Redfearn, Andreas Enriques, Gianluigi Bisleri. Division of Cardiac Surgery, Queen's University, Kingston, ON, Canada.

22. ***BMPR2* LOSS CAUSES A PROLIFERATIVE SHIFT IN THE ENDOTHELIAL RESPONSE TO BMP9.** Anne L. Theilmann[1], Lindsey G. Hawke[1], L. Rhiannon Hilton[1], Mara K.M. Whitford[1], Jodi L. Mackeil[1], Kimberly J. Dunham-Snary[2], Paula D. James[2], Donald H. Maurice[1], Stephen L. Archer[2], Mark L. Ormiston[1,2,3] [1] Department of Biomedical and Molecular Sciences, Queen's University, Kingston, Canada[2] Department of Medicine, Queen's University, Kingston, Canada .[3] Department of Surgery, Queen's University, Kingston, Canada.

23. **PAPILLARY MUSCLE RELOCATION WITH A MULTI-LOOP TECHNIQUE AS AN ADJUNCT FOR TREATMENT OF ISCHEMIC MITRAL REGURGITATION.** Maria Theresa Dela Cruz Servito BHSc,[1] Lawrence Torkan BSc,[1,2] Gianluigi Bisleri MD, FRCSC.[1] [1] Department of Surgery, Queen's University School of Medicine, Kingston ON, Canada**.** [2] Department of Mechanical and Materials Engineering, Queen's University, Kingston ON, Canada

24. **TOWARDS INTRAOPERATIVE USE OF AMBIENT MASS SPECTROMETRY IMAGING FOR CARDIAC TISSUE.** Randy E. Ellis[1,2,3], Alice M.L Santilli[2], Diane E. Tomalty[1], John F. Rudan[3,4], Martin Kaufmann[1,4], Gianluigi Bisleri[4]. [1]Department of Biomedical and Molecular Sciences, Queen's University; [2]School of Computing, Queen's University; [3]Human Mobility Research Centre, Queen's University, [4]Department of Surgery, Queen's University

## Health Policy, Population Health, and Epidemiology

25. **THE ASSOCIATION BETWEEN HIGH DIAGNOSTIC TESTING AND THE SPECIALIST DIAGNOSTIC INTERVAL IN SYMPTOMATIC BREAST CANCER PATIENTS.** Lucas Cohen, Colleen Webber, Andrea Eisen, and Patti Groome**.** Department of Public Health Sciences, Division of Cancer Care and Epidemiology, Queen's Cancer Research Institute

26. **SLEEP, MELATONIN, AND CIRCADIAN GENE METHYLATION AS POTENTIAL PATHWAYS BETWEEN SHIFT WORK AND BREAST CANCER RISK.** Felske LR, Tranmer J, Ritonja J, and Aronson KJ. Department of Public Heath Sciences, Queen's University Kingston, Ontario, Canada.

27. **MODELLING THE PATHWAYS FROM SHIFT WORK TO CARDIOVASCULAR DISEASE RISK AMONG FEMALE HOSPITAL WORKERS.** Haley Golding[1], Kristan Aronson[1], Jennifer Ritonja[1], and Joan Tranmer[1,2]. [1]Department of Public Health Sciences and [2]School of Nursing, Queen's University.

28. **CONFOUNDING BY INDICATION, SURVIVAL AND SELECTION BIAS IN INFECTIVE ENDOCARDITIS RESEARCH.** Olivia Moir, MSc Epidemiology Student, Department of Public Health Sciences, Queen's University**.** Dr. Yingwei Peng, Department of Public Health Sciences, Faculty of Health Sciences, Queen's University. Dr. Susan B Brogly, Department of Surgery, Faculty of Health Sciences, Queen's University and ICES Queen's

## Inflammation, Infection and Immunity

29. **EXPLORING THE DIVERGENT EFFECTS OF HYPOXIA AND TGFB ON THE PHENOTYPIC CONVERSION AND ANGIOREGULATORY CAPACITY OF CIRCULATING PRIMARY NK CELLS.** Lindsey G. Hawke, Mara K.M. Whitford, Mark L. Ormiston. Queen's University Departments of Biomedical and Molecular Sciences, Medicine and Surgery, Kingston, Ontario, K7L 3N6, Canada.

30. **TARGETING GENE NETWORKS OF SPINAL CORD INJURY PAIN.** Courtney A. Bannerman, Jihoon Choi, Julia P. Segal, Mitra Knezic, Scott Duggan, Qingling Duan, Nader Ghasemlou

31. **INTERLEUKIN-27 ALTERS THE POLARIZATION AND RESPONSIVENESS OF HUMAN PMA-THP-1 MACROPHAGES.** Katelyn Gray, Olena Kourko, Natalya Odoardi, Katrina Gee**.** Department of Biomedical and Molecular Sciences

32. **NOVEL TYROSINE KINASE INHIBITORS REGULATE INTESTINAL SMOOTH MUSCLE CELL GROWTH *IN VITRO.*** Jay Kataria, Sandra Lourenssen, and Michael Blennerhassett. Department of Medicine.

33. **CONTROL OF SOMATOSENSATION BY NEUTROPHILS AND ENDOGENOUS OPIOIDS**. Mitra Knezic and Nader Ghasemlou. Department of Biomedical and Molecular Sciences, Queen's University Kingston, Ontario Canada.

34. **IL-27 DIFFERENTIALLY MODULATES TLR7 AND TLR8 RESPONSIVENESS IN HUMAN MONOCYTES AND MACROPHAGES INDEPENDENTLY OF TYPE I IFN.** Natalya Odoardi[1], Olena Kourko[1], Carlene Petes[1], Katrina Gee[1]. [1]Department of Biomedical & Molecular Sciences, Queen's University, Kingston, Ontario, Canada, K7L 3N6.

35. **ABERRANT INFLAMMATION IN RAT PREGNANCY CONTRIBUTES TO RISK FACTORS ASSOCIATED WITH CARDIOVASCULAR DISEASE AND PREGNANCY COMPLICATIONS IN SUBSEQUENT GENERATIONS**. Nicole Protopapas, Takafumi Ushida, Tiziana Cotechini, Shannyn K. Macdonald-Goodfellow, Charles H. Graham. Department of Biomedical and Molecular Sciences. Queen's University, Kington, Ontario Canada.

36. **THE CONTRIBUTION OF DENDRITIC CELLS TO MECHANISMS OF INFLAMMATORY PAIN.** Madeline Robinson[1], Pascale Patenaude[1], Jaqueline Raymondi Silva[1], Nader Ghasemlou[1,2]**.** [1]Department of Biomedical and Molecular Sciences, [2]Department of Anesthesiology and Perioperative Medicine.

37. **EVALUATING PROPHYLACTIC VACCINATION MODELS TO ASSESS TUMOURIMMUNE CELL INTERACTIONS FOLLOWING TUMOUR ENGRAFTMENT.** Kyle Seaver[2], Peter Greer[1] and Sam Basta[2]. [1]Division of Cancer Biology and Genetics, Cancer Research Institute, Department of Pathology and Molecular Medicine, Queen's University, Kingston, Ontario, Canada. [2]Department of Biomedical & Molecular Science Queen's University, Kingston, Ontario, Canada.

38. **NEUROIMMUNE MECHANISMS UNDERLYING CIRCADIAN CONTROL OF PAIN IN A MOUSE MODEL OF MULTIPLE SCLEROSIS.** Julia Segal, Courtney Bannerman, Ian Gilron, Nader Ghasemlou, Department of Biomedical and Molecular Science.

39. **LCMV-ARMSTRONG INFECTION DIFFERENTIALLY AFFECTS GM-CSF AND M-CSF DERIVED MACROPHAGE EARLY ACTIVATION SIGNALING PATHWAYS.** Evan Trus[1], Torki Alothaimeen[1], Katrina Gee[1], Sam Basta[1] [1]Department of Biomedical and Molecular Sciences, Queen's University, Kingston, ON, Canada, K7L 3N6.

## Neuroscience Research

40. **NOVEL STRAIN-BASED ANALYSIS OF TISSUE MECHANICS INFORMS ABOUT CHANGES IN STRUCTURAL INTEGRITY WITHIN THE CORPUS CALLOSUM FOLLOWING EXPOSURE TO SUB-CONCUSSIVE IMPACTS.**
**Allen A. Champagne,[1] BSc, BA,** Emile Peponoulas,[1] BSc, Itamar Terem,[2] BSc, Andrew Ross, MSc, Maryam Tayebi,[3] MSc, Yining Chen,[1] MSc, Nicole S. Coverdale,[1] PhD, Poul M. F. Nielsen,[3,6] PhD, Alan Wang,[3] PhD, Vickie Shim,[3] PhD, Samantha J. Holdsworth,[4] PhD, Douglas J. Cook,[1,5] MD, PhD. [1]Centre for Neuroscience Studies, Queen's University, Kingston, ON, Canada. [2]Department of Radiology, Stanford University, Stanford, CA, United-States of America. [3]Auckland Bioengineering Institute, University of Auckland, Auckland, New Zealand . [4]Departement of Anatomy and Medical Imaging & Centre for Brain Research, Faculty of Medical and Health Sciences, University of Auckland, Auckland, New Zealand. [5]Department of Surgery, Queen's University, Kingston, ON, Canada. [6]Department of Engineering Science, Faculty of Engineering, University of Auckland, Auckland, New Zealand.

41. **IMPACT OF DOSE RESPONSE FOR THE GENE THERAPY TREATMENT OF AB-VARIANT GM2 GANGLIOSIDES IN A MOUSE MODEL USING SELF-COMPLIMENTARY ADENO-ASSOCIATED VIRUS SEROTYPE 9**
Natalie M. Deschenes[1], K. Osmon[1], S. Kot[2], Z. Chen[3], A. Ryckman[1], B. Quinville[1], A. Jadav[1], M. Mitchell[3], S. J. Gray[4] and J. 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/Departments of Pediatrics, Queen's University, Kingston, Ontario, Canada, K7L 2V7; [4]Department. of Pediatrics, UT Southwestern Medical Center; Dallas, TX, USA.

42. **GM2 GANGLIOSIDES: BIOMARKERS OF DISEASE PROGRESSION IN A SANDHOFF MOUSE MODEL.**
Deirdre Hindmarch[1] & Jagdeep Walia [1,2]. [1] Centre for Neuroscience Studies, Queen's University, Kingston, Ontario, Canada [2] KGH Research Institute.

43. **MAPPING PSILOCYBIN-ASSISTED THERAPY: A SCOPING REVIEW.**
Ioudovski, Paul; McKeown, Sandra; Goldie, Craig; Dumont, Eric; Ron, Shore. Department of Biomedical and Molecular Science, Queen's University, Kingston, Ontario, Canada.

44. **USING EYE TRACKING TO IDENTIFY BIOMARKERS OF EATING DISORDER IN ADOLESCENTS.**
Ryan H. Kirkpatrick, Linda Booij, Sarosh Khalid-Khan, and Douglas Munoz. Centre for Neuroscience Studies, Queen's University, Kingston, Ontario, Canada.

45. **TARGETING NECROPTOSIS VS APOPTOSIS IN ISCHEMIA-INDUCED MYENTERIC NEURON DEATH.**
Savio Cyril Kocherry, Sandra Lourenssen, & Michael Blennerhassett. Department of Medicine, Queen's University

46. **ISOLATING ENDOGENOUS MOLECULAR TRIGGER OF SPREADING DEPOLARIZATION RELEASED BY NEURAL TISSUE UNDER ABRUPT HYPERTHERMIA.**
Kelly Lee, Nikita Ollen-Bittle, R.D. Andrew, A.Y. Jin
Center for Neuroscience Studies, Queen's University, Kingston ON, Canada.

47. **EXPRESSION OF NA+/K+-ATPASE ISOFORMS IN HIGHER AND LOWER BRAIN REGIONS FOLLOWING FOCAL ISCHEMIA IN MICE: A PRELIMINARY ANALYSIS.**
Chloe Lowry, Brian Bennett, R. David Andrew
Centre for Neuroscience Studies, Queen's University, Kingston, ON, Canada.

48. **MORPHOMETRIC AND SPINE DENSITY ANALYSIS OF PYRAMIDAL NEURONS IN A MOUSE MODEL OF SPORADIC ALZHEIMER'S DISEASE.**
R. H. Mehder, M. Yoon, B. M. Bennett, R.D. Andrew. Department of Biomedical and Molecular Sciences, Queen`s University, Kingston, ON, Canada K7L3N6.

49. **INVESTIGATING A POTENTIAL ACTIVATOR OF SPREADING DEPOLARIZATION RELEASED BY ISCHEMICALLY STRESSED GRAY MATTER.**
N.K. Ollen-Bittle, K.H. Lee, M.S. Fisher, R.D. Oleschuk, A.Y. Jin, R.D. Andrew. Centre for Neuroscience Studies, Queen's University Kingston, Ontario Canada.

50. **INVESTIGATION INTO THE CORRECTION OF GM2 GANGLIOSIDOSIS - IMPACT OF THE AGE OF ADMINISTRATION OF AAV9 MURINE AND HUMAN BICISTRONIC HEXOSAMINIDASE VECTORS IN SANDHOFF MICE.** Karlaina J. L. Osmon[1], Natalie M. Deschenes[1], Eminet Bogale[2], Shalini Kot[2], Zhilin Chen[3], Melissa Mitchell[2], Clifford Heindel[4], John G. Keimel[4], William F Kaemmerer[4], Steven J. Gray[5], 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/Departments of Pediatrics, Queen's University, Kingston, Ontario, Canada, K7L 2V7; 4 New Hope Research Foundation, North Oaks, Minnesota, USA 5 Department of Pediatrics, University of Texas Southwestern Medical Center, Texas, United States.

51. **THE EFFECTS OF NEURONAL NITRIC OXIDE SYNTHASE AND APOPTOSIS ON NEURAL STEM CELL. PROLIFERATION WITHIN THE ADULT ENTERIC NERVOUS SYSTEM**. Catherine Parisien & Alan E. Lomax. Centre for Neuroscience Studies, Queen's University Kingston, Ontario, Canada.

52. **QUANTIFYING RAPID CORRECTIVE RESPONSES IN THE STROKE POPULATION USING THE UPPER LIMB.** Kayne Park and Stephen H. Scott. Centre for Neuroscience Studies, Queen's University, Kingston, Ontario, Canada.

53. **PROGRESS TOWARDS THE INDUCTION OF IMMUNE TOLERANCE TO THE HYBRID HEXM ENZYME FOLLOWING INTRAVENOUS GENE THERAPY IN A MOUSE MODEL OF SANDHOFF DISEASE.** Brianna M. Quinville[1], Shalini Kot[2], Zhilin Chen[3], Melissa Mitchell[2], Karlaina J.L. Osmon[1], Natalie M. Deschenes[1], Deirdre Hindmarch[1], John G. Keimel[4], William F. Kaemmerer[4], Steven J. Gray[5] 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 [5]Department of Pediatrics, University of Texas Southwestern Medical Center, Texas, USA.

54. **ASSESSMENT OF COGNITIVE PERFORMANCE IN DP(16)1/YEY/+ MOUSE MODEL OF DOWN SYNDROME.** Negin Rezaie, Dr. Brian Bennett.

55. **USING EYE TRACKING TO IDENTIFY SACCADE BIOMARKERS OF NEURODEGENERATIVE DISEASE.** Heidi C. Riek, Brian C. Coe, Don Brien, Sandra Black, Michael Borrie, Dar Dowlatshahi, Elizabeth Finger, Morris Freedman, David Grimes, Donna Kwan, Anthony Lang, Connie Marras, Mario Masellis, Gustavo Saposnik, Rick Swartz, Carmela Tartaglia, Lorne Zinman, the ONDRI Investigators and Douglas P. Munoz. Centre for Neuroscience Studies, Queen's University Kingston, Ontario, Canada.

56. **ALZHEIMER'S DISEASE BIOMARKERS IN CEREBROSPINAL FLUID OF NONHUMAN PRIMATES.** Emma Robertson[1], Susan Boehnke[1,2], Brittney Armitage-Brown[1], Robert Wither[1], Natalia Lyra e Silva[1], Andrew Winterborn[3], DJ Cook[1,4], Ron Levy[1,4], Fernanda De Felice[1,5], Douglas Munoz[1,2]**,** [1]Centre for Neuroscience Studies, [2]Department of Biomedical and Molecular Sciences, [3]Animal Care Services, [4]Department of Surgery, [5]Department of Psychiatry, Queen's University.

57. **NOVEL CHARACTERIZATION OF IMPACT BIOMECHANICS REVEALS DIFFERENCES ACROSS POSITIONAL GROUPS IN CANADIAN HIGH SCHOOL FOOTBALL PLAYERS.** Kaden Shearer,1 BSc, Allen A. Champagne,1 BSc, BA, Emile Peponoulas,1 BSc, Douglas J. Cook,1,2 MD, PhD**.** 1Centre for Neuroscience Studies, Queen's University, Kingston, ON, Canada**.** 2Department of Surgery, Queen's University, Kingston, ON, Canada.

58. **CEREBROVASCULAR PROTEINS INVOLVED IN AMYLOID B DISPOSITION IN A MOUSE MODEL OF SPORADIC ALZHEIMER'S DISEASE: A PRELIMINARY ASSESSMENT.** Kaitlyn A. Tresidder, Brian M. Bennett.

59. **EVIDENCE FOR EFFECTS OF PHOENIXIN ON NEURONS OF THE PARAVENTRICULAR NUCLEUS.** Emma Walton and Alastair V. Ferguson. Centre for Neuroscience Studies, Canadian Institutes of Health Research.

## Patient Care and Nursing Research

**60. EXAMINING QUEEN'S UNIVERSITY NURSES' PERCEPTIONS OF HOSPITAL ORIENTATIONS AND THEIR TRANSITION TO PRACTICE.** Katherine Gregory, Marian Luctkar-Flude, and Kim Sears. Faculty of Health Sciences, School of Nursing, Queen's University Kingston, Ontario Canada.

## Protein Structure and Function

**61. UNDERSTANDING THE UNIQUE MECHANISM OF MICROTUBULE LENGTH CONTROL BY KINESIN-8.** Byron Hunter, Matthieu Benoit, Ana Asenjo, Caitlin Doubleday, Daria Trofimova, Hernando Sosa, and John Allingham. Department of Biomedical and Molecular Sciences, Queen's University, Kingston, Ontario, Canada Department of Physiology and Biophysics, Albert Einstein College of Medicine, Bronx, New York, USA.

**62. ENGINEERING A MULTI-FUNCTIONAL CAZYME COMPLEX WITH ENHANCED AGAROSE-DEGRADING PROPERTIES.** Keegan B. Turner-Wood, Julie Grondin, Benjamin Pluvinage, Alisdair B. Boraston, Holly L. Spencer, and Steve Smith**.** Department of Biomedical and Molecular Sciences, Queen's University Kingston, Ontario Canada. (Supported by NSERC)

## Reproductive and Sexual Function

**63. EFFECT OF CARBON MONOXIDE ON VASCULAR ADAPTATIONS DURING PREGNANCY.** Megan A Dickson[1], Nichole Peterson[1], Karalyn E McRae[1], Jessica Pudwell[2], Chandrakant Tayade[1], Graeme N Smith[1,2]. [1]Department of Biomedical and Molecular Sciences, Kingston, ON, Canada; [2]Department of Obstetrics and Gynaecology, Kingston Health Sciences Centre, Kingston, ON, Canada.

**64. INVESTIGATION OF THE NEGATIVE IMPACT ON FEMALE SEXUAL FUNCTION AS A RESULT OF MIS-URETHRAL SLING AND LOOP ELECTROSURGICAL EXCISION PROCEDURES: INNERVATION STUDIES.** O Giovannetti, M Monaghan, MA Adams.

**65. THE ORIGIN AND CHARACTERIZATION OF SURFACE-BORNE GLUTATHIONE-S-TRANSFERASE OMEGA 2 WITHIN MOUSE AND BOAR CAPACITATION.** Lauren E. Hamilton[1], Wei Xu[1], Michal Zigo[2], Jiude Mao[2], Peter Sutovsky[2,3], and Richard Oko[1]**.** [1]Department of Biomedical and Molecular Sciences, Queen's University, Kingston, Ontario, CA**.** [2]Division of Animal Sciences, College of Agriculture, Food and Natural Resources, University of Missouri, Colombia, Missouri, USA**.** [3]Department of Obstetrics, Gynecology and Women's Health, School of Medicine, University of Missouri, Colombia, Missouri, USA.

**66. ESTABLISHING AN EFFECTIVE PROTOCOL FOR THE SELECTIVE EXTRACTION OF NON-NUCLEAR CORE HISTONES FROM THE MOUSE PT.** Morgan Lion[1], Lauren Hamilton[1], Nicole Protopapas[1], Wei Xu[1], and Richard Oko[1]. [1]Department of Biomedical and Molecular Sciences, Queen's University, Kingston, ON, Canada.

**67. LOCALIZATION OF PLCZ1: A CANDIDATE SPERM-BORNE OOCYTE ACTIVATING FACTOR.** Ruben Warkentin, Nicole Protopapas, Lauren E. Hamilton, Wei Xu, Richard Oko. Department of Biomedical and Molecular Sciences, Queen's University, Kingston, Ontario, CA.

**68. HUMAN OVGP1 ENHANCES TYROSINE PHOSPHORYLATION OF PROTEINS IN THE FIBROUS SHEATH INVOLVING AKAP3 AND INCREASES SPERM-ZONA BINDING.** Yuewen Zhao and Frederick W. K. Kan. Department of Biomedical and Molecular Sciences, Queen's University, Kingston, Ontario, Canada.

## Therapeutics and Toxicology

69. **DNA DAMAGE AND PERTURBED TOPOISOMERASE IIA AS A TARGET OF 1,4-BENZOQUINONE TOXICITY IN MURINE FETAL LIVER CELLS.** Trent H. Holmes[1] and Louise M. Winn[1]. [1]Department of Biomedical and Molecular Sciences, Queen's University Kingston, Ontario Canada.

70. **CHARACTERIZATION OF THE CONTRIBUTION OF THE NF-KB-MEDIATED SIGNALING PATHWAY ON THE TERATOGENICITY OF VPA FOLLOWING IN VIVO EXPOSURE IN CD-1 MOUSE EMBRYOS.** Sidra Shafique1, Louise M. Winn1,2. Department of Biomedical and Molecular Sciences, Queen's University, Kingston1
School of Environmental Studies, Queen's University, Kingston2.

71. **EXAMINATION OF DNA DOUBLE-STRAND BREAKS AND OXIDATIVE DNA DAMAGE FOLLOWING TREATMENT OF MICE WITH THE NUTRACEUTICAL SULFORAPHANE**. Kristen Zamperoni and Thomas E. Massey. Department of Biomedical and Molecular Sciences, Queen's University, Kingston, Ontario Canada.

## Women's and Children's Health Research

72. **GENOME-WIDE ASSOCIATION STUDY OF HUMAN MILK OLIGOSACCHARIDES AMONG LACTATING MOTHERS IN CANADIAN LONGITUDINAL BIRTH COHORT.** Amirthagowri Ambalavanan[1], Le Chang[1,2], Jihoon Choi[1], Amel Lamri[3], Bianca Robertson[4], Chloe Yonemitsu[4], Stuart E. Turvey[5,6], Piushkumar J. Mandhane[7], Allan B. Becker[8,9], Theo J. Moraes[10], Sonia S. Anand[11], Guillaume Paré[12], Diana L. Lefebvre[11], Malcolm R. Sears[11], Padmaja Subbarao[10], Lars Bode[4], Meghan B. Azad[8,9], 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]Department of Clinical Epidemiology and Biostatistics, McMaster University, Hamilton, Ontario, Canada; [4]Department of Pediatrics and Larsson-Rosenquist Foundation Mother-Milk-Infant Center of Research Excellence, University of California San Diego, La Jolla, California, USA; [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]Manitoba Developmental Origins of Chronic Diseases in Children Network (DEVOTION), Children's Hospital Research Institute of Manitoba, Winnipeg, Manitoba, Canada; [9]Department of Pediatrics and Child Health, University of Manitoba, Winnipeg, Manitoba, Canada; [10]Department of Pediatrics, Hospital for Sick Children and University of Toronto, Toronto, Ontario, Canada; [11]Department of Medicine, McMaster University, Hamilton, Ontario, Canada; [12]Pathology and Molecular Medicine, McMaster University, Hamilton, Ontario, Canada.

73. **THE EFFECTS OF *IN UTERO* BENZENE EXPOSURE ON FETAL NF-κB CELL SIGNALLING IN CD-1 MICE.** Peter Chun Wan Lu, Louise M. Winn, Department of Biomedical & Molecular Sciences, Queen's University, Kingston, Ontario, Canada.

74. **EXPLORING CHANGES IN THE FATHERS' FAMILIAL ROLE AND ITS ASSOCIATION WITH CHILD OUTCOMES IN MONGOLIA.** Lesley A. Pablo and Colleen Davison. Department of Public Health Sciences, Queen's University Kingston, Ontario Canada.

75. **CHARACTERIZATION OF NEUTROPHIL INVOLVEMENT IN ENDOMETRIOSIS PATHOPHYSIOLOGY.** Lindsey K Symons[1], Jessica E Miller[1], Bruce A Lessey[2], Steven L Young[3] & Chandrakant Tayade[1] [1]Department of Biomedical and Molecular Sciences, Queen's University, Kingston, CAN [2]Department of Obstetrics and Gynecology, Greenville Health System, Greenville, USA [3]Department of Obstetrics and Gynecology, University of North Carolina, Chapel Hill, USA

**76. PLACENTAL MORPHOLOGY AND THE PREDICTION OF UNDERLYING CARDIOVASCULAR RISK FACTORS.** Aida Zaza[1], Jessica Pudwell[2], Shannon Bainbridge[3], Kristin Connor[4], Graeme N Smith[1, 2] [1]Department of Biomedical and Molecular Sciences, Queen's University, [2]Department of Obstetrics and Gynaecology, Queen's University, [3]Department of Cellular and Molecular Medicine, University of Ottawa, [4]Department of Health Sciences, Carleton University

# *Abstracts*

## Biomedical Engineering

1. **THE USE OF MULTIVARIATE REGRESSION TO QUANTIFY AGE-RELATED DEVELOPMENTAL CHANGES IN COORDINATION IN CHILDREN AND YOUTH.** Stephan C.D. Dobri1, Stephen H. Scott2, T. Claire Davies11: Mechanical and Materials Engineering, 2: Biomedical and Molecular Sciences

Background: Motor control processes coupled with child development are affected by neurological milestones. Quantifying age-specific progress of motor function and coordination in typically developing children can facilitate quantification of differences in pathological populations. The KINARM Exoskeleton has been used to evaluate motor impairments in adults and, to a lesser extent, in children. Objective: To develop normative models of age-related developments in motor function and coordination for children aged 5-18 years. Methods: Two-hundred and eighty-eight participants from 5-18 years old (mean: 13 ± 3 years, 190 male) performed the Object Hit and Avoid Task. Multivariate regression analysis (MVRA) was used to generate normative models of participant performance based on age, sex, and handedness. The algorithm accuracy and repeatability were assessed using simulated data generated with different sample populations and noise levels. After these analyses were run, the algorithm was used to create models of typical development for the KINARM task. Results: The algorithm accuracy and repeatability were found to increase with number of participants and decreasing noise. No significant differences in performance were observed between sexes, and significant improvement with age was observed for several task parameters. Conclusions: MVRA has been used to create normative models of motor function and coordination. These models can be used in future to compare to children with different pathologies (such as developmental coordination disorder and cerebral palsy). NSERC Discovery Grants 513272-17 and RGPIN-2016-04669

## Cancer Research

2. **CADHERIN-11 IS REQUIRED FOR ACTIVATION OF STAT3 BY V-SRC.** Hanad Adan, Stephanie Guy and Leda Raptis. Department of Pathology and Cancer Center, Queen's University, Kingston, Ontario, Canada.

The signal transducer and activator of transcription-3 (Stat3) plays an important role in cancer etiology. Stat3 is activated by phosphorylation by growth factor receptors and oncogenes at ptyr705, dimerizes and translocates to the nucleus where it activates genes involved in cell division and survival. We recently discovered a novel pathway of Stat3 activation, triggered by cadherins: Engagement of E- or N-cadherin or cadherin-11 is followed by a striking upregulation of the Rac GTPase, IL6 and Stat3-ptyr705, and this offers a potent survival signal. Oncogenes such as Src are known Stat3 activators. Src also negatively regulates the epithelial (E)-cadherin and cadherin-11 (Cad11), through a number of different mechanisms. Through stable expression of graded levels of activated Src in mouse Balb/c3T3 fibroblasts we now demonstrate that the levels of active Stat3-ptyr705 increased with increasing Src, as expected. Paradoxically however, at high Src levels (when Cad11 was undetectable), Stat3-ptyr705 dropped to baseline. Moreover, Src overexpression in Cad11-knockdown cells could not increase Stat3-ptyr705 levels, indicating that Src is unable to activate Stat3 in the face of Cad11 deficiency. These results suggest that, despite the fact that Src downregulates Cad11, Src also requires at least certain levels of Cad11 in order to activate Stat3. Most importantly, we report that Src-expressing cells deficient in Cad11 succumb to apoptosis, pointing to a significant role of the Cad11/Stat3 axis in tumour cell survival signalling.

3. **NEW COMBINATION THERAPIES FOR INFLAMMATORY BREAST CANCER USING INHIBITORS OF PI3K AND AURORA A KINASE**. Nadia Al Ali and Andrew W. Craig. Department of Biomedical and Molecular Sciences, Queen's University. Division of Cancer Biology and Genetics, Queen's Cancer Research Institute

   Inflammatory breast cancer (IBC) is an aggressive cancer that afflicts young women. Due to rapid progression IBC therapies are very aggressive, including radical mastectomy, taxane chemotherapy and radiation therapy. In triple negative IBC, lacking ER/PR/HER2 expression, there are no options for targeted therapy. Genome sequencing and functional screening have identified phosphatidyl inositol 3' kinase (PI3K) and Aurora A (AurA) kinases as candidates in the SUM-149 IBC cell line. Here, we test for synergistic effects between clinical grade inhibitors of PI3K (Buparlisib) and AurA (Alisertib) in SUM-149 cells. Using a cytotoxicity assay, we show that SUM-149 cells were resistant to Buparlisib or Alisertib alone, but the combination treatments resulted in synergistic killing. The non-IBC breast cancer MDA-MB-231 cells were sensitive to Buparlisib alone. Combined treatments with Buparlisib and Alisertib led to suppression of AKT pathway, expression of SSH1 phosphatase that regulates actin filament dynamics, and cell cycle arrest at G2/M phase in SUM-149 cells. In soft agar colony assays using SUM-149 cells, the combination of Buparlisib and Alisertib was more effective than either inhibitor alone in suppressing colony formation. This study provides rationale for our continued testing of PI3K and AurA inhibitors in preclinical IBC tumour models. If successful, our findings will lead to clinical trials of this novel combination targeted therapy for IBC patients.

4. **IDENTIFICATION OF NOVEL RECEPTORS TYROSINE KINASES (RTKS) REGULATING THE HIPPO SIGNALING PATHWAY IN TUMORIGENESIS.** Taha Azad, Kazem Nouri, Helena J Janse van Rensburg, Xiaolong Yang*
   Department of Pathology and Molecular Medicine, Queen's University, Kingston, K7L 3N6, Canada

   Several studies have shown that the Hippo pathway plays an important role in tissue growth, organ size, and cell death. Deregulation of the Hippo pathway contributes to loss of cell contact inhibition and continuing cell proliferation, which is observed during tumorigenesis. Although many studies have been done to clarify the role of the Hippo pathway in organ size control, cell proliferation and tumorigenesis, currently the connection between the Hippo pathway and its potential upstream kinase regulators is not very clear. Since the overexpression and deregulation of receptor protein kinases (RTKs) have a pivotal role in many cancers, we hypothesize that some receptor tyrosine kinases may be involved in tumorigenesis by inhibiting the Hippo pathway. To test the hypothesis, we developed a biosensor based on split-luciferase complementation assay1. To find new RTKs which regulate the Hippo pathway, gain of function and kinase-inhibitor screenings were performed. The screenings revealed several novel RTKs that regulate the Hippo pathway such as FGFR and VEGFR. Remarkably, we found three new tyrosine-phosphorylation sites on YAP, which regulate its function and stability. Our studies indicate that some RTKs also regulate the Hippo pathway through interaction or phosphorylation of LATS, YAP, and TEAD. In conclusion, these findings highlight the pivotal role of the Hippo pathway in mediating RTK-MAPK/PI3K signalling and provide a compelling rationale for targeting YAP/TAZ in RTK-driven cancer therapies. 1 Azad, T. et al. A LATS biosensor screen identifies VEGFR as a regulator of the Hippo pathway in angiogenesis. Nature communications 9, 1061 (2018).

5. **INVESTIGATING THE STING PATHWAY TO EXPLAIN MECHANISMS FOR BCG FAILURES IN NON-MUSCLE INVASIVE BLADDER CANCER.** Stephen Chenard1, 3, 4, Sarah Nersesian5, Thiago Vidotto6, Alvaro Morales4, D. Robert Siemens1, 3, 4, Madhuri Koti*1, 2, 3,4 Department of Biomedical and Molecular Sciences, Queen's University, Kingston, Canada, Department of Obstetrics and Gynecology, Kingston Health Sciences Center, Queen's University, Kingston, Canada, Cancer Biology and Genetics, Queen's Cancer Research Institute, Queen's University, Kingston, Canada, Department of Urology, Kingston Health Sciences Center, Queen's University, Kingston, Canada, School of Medicine, Dalhousie University, Canada, Department de Genética, University of São Paulo, Brazil

   Intravesical Bacillus Calmette Guerin (BCG) based immunotherapy has been the gold standard therapy to treat high-risk non-muscle invasive bladder cancer (NMIBC) for over 40 years. We hypothesize that the attenuation of Mycobacterium bovis for clinical use as BCG leading to loss of its ability to activate the "Stimulator of Interferon Genes" (STING) pathway, limits local anti-tumor immune activity and subsequent BCG responsiveness due to reduced induction of CXCL10. The TICE strain of BCG (oncoTICE) was used in combination with STING agonist to determine STING pathway activation and CXCL10 production in THP-1 monocytic cell line, THP-1 defNLRP3, THP-1 dual STING knock out cells, RT112 bladder cancer cells and primary bladder epithelial cells. NanoString platform-based gene expression profiling and multiplex cytokine analysis revealed that synergistic activation of STING pathway enhanced the effect of BCG induced inflammasome and STING pathway genes in monocytes and bladder cancer cells. Addition of a STING agonist to BCG led to significantly increased levels of chemokines CXCL10 in THP-1 cells and primary bladder epithelial cells. Findings from our study are the first evidence demonstrating the significant enhancement in interferon induced chemokine production via the synergistic action of BCG and STING agonist combination. STING pathway activators are promising new innate immune modulators with a potential to synergize with or replace BCG therapy in the treatment of NMIBC. Supporting Agency: SAEMO Innovation Fund Lab: Cancer Research Institute 313

6. **EXAMINING THE EFFECTS OF BMP9 ON ANGIOGENESIS IN MURINE METASTATIC LUNG TUMOURS.** Devon V. Cole, Yan Gao, Matthew T. Rätsep, Patricia D. A. Lima, Melissa Mitchell, Peter A. Greer, and Mark L. Ormiston. Departments of Biomedical and Molecular Sciences and Medicine, Queen's University, Kingston, Ontario, Canada.

   One protein that has not been explored but may play a role in cancer development and tumour angiogenesis is bone morphogenetic protein 9 (BMP9). Normally BMP9 is a vascular quiescence factor. It elicits these effects through signalling of Type I and II receptors, including Bone Morphogenetic Protein Receptor II (BMPRII). BMP9 can bind to a variety of Type I and II receptors, potentially leading to bidirectional effects on angiogenesis. Although previous research on the role of BMP9 in cancer has been conducted, the results are contradictory. Some groups have demonstrated that the addition of BMP9 in vivo resulted in increased tumour growth while others have shown that it inhibits tumour growth. The overarching question explored in our research is, "Does the loss of BMPR2 in the pulmonary endothelium influence the contribution of BMP9 to the vascularization of solid tumours?" The two main aims were to investigate whether the loss of Bmpr2 in the pulmonary endothelium of mice results in increased vascularization of lung metastases in response to endogenous BMP9 and to determine whether administration of recombinant BMP9 suppresses the vascularization of lung metastases in wildtype mice, while enhancing it in mice lacking pulmonary endothelial Bmpr2. This project will give insight into the effects of BMP9 on angiogenesis in cancer development and whether its effects are modified with the loss of endothelial BMPR2. (Supported by CIHR).

7. **CLINICAL IMPACT OF NEXT-GENERATION SEQUENCING ON MYELOID CANCER DIAGNOSIS, RISK AND TREATMENT ASSESSMENT.** Christina Ferrone1, Henry Wong2, Laura Semenuk2, Brooke Snetsinger1, Xiao Zhang1, Patricia Farmer1, Annette E. Hay3, David Good1, Graeme Quest1, Harriet E. Feilotter1,2, Michael J. Rauh1 1Department of Pathology and Molecular Medicine, Richardson Laboratory, Queen's University, 2Molecular Genetics Laboratory, Kingston Health Sciences Centre (KHSC), 3Department of Medicine, Queen's University, Kingston, ON, Canada

Introduction: The growing number of genes associated with myeloid cancers necessitates high-throughput testing. We performed targeted next-generation sequencing (NGS) using the Oncomine™ Myeloid (Thermo Fisher) panel (OMP) to capture these variants and assessed their clinical impact. Methods: As of 2018/04, OMP has been offered as a validated clinical assay at KHSC. Prior to this, patients were consented at the time of blood or marrow collection. Amplicon libraries targeting 40 DNA genes and 29 RNA fusion drivers were sequenced using Ion Torrent NGS technologies. Benign or likely benign genetic variants were excluded. Actionability of NGS results was assessed and defined as those aiding in achieving/clarifying diagnoses, informing prognoses and/or clinical management. Results: As of 2019/04, we have sequenced 131 nucleic acid samples from 121 unique patients (mean 63.9 y). Of these samples, the suspected or known cases of MDS represented 42%, 32% AML, 14% MPN, 5% MDS/MPN, and 7% other hematological disorders. We reported 200 variants in 66% of samples. The most frequently mutated genes included TET2 (13%), SRSF2 (9%), RUNX1 (8%), SF3B1 (7%), and ASXL1 (7%). Of detected variants, 72% were actionable, where 50% facilitated or clarified diagnoses, 36% affected prognoses, and 31% had the potential to influence clinical management. Conclusions: In the majority of patients, OMP molecular profiling revealed actionable results. OMP represents a promising strategy to capture important molecular features of myeloid malignancies. Supported by: The Ontario Institute for Cancer Research (OICR) through an Ontario Molecular Pathology Research Network (OMPRN) grant, and a Southeastern Ontario Academic Medical Organization (SEAMO) innovation grant.

8. **POLY(I:C)-MEDIATED DEATH OF HUMAN PROSTATE CANCER CELL LINES IS INDUCED BY IL-27 TREATMENT.** Olena Kourko1, Robin Smyth1, Daniela Cino1, Kyle Seaver1, Carlene Petes1, Sally Eo1, Sam Basta1, and Katrina Gee1 1Department of Biomedical and Molecular Sciences, Queen's University, Kingston ON

IL-27, an immunomodulatory cytokine, has been shown to have anti-cancer properties leading to interest in evaluating its therapeutic potential. Exhibiting certain overlapping properties with type I and type II interferons (IFNs), IL-27 impacts cancer cell viability and immune cell activity. Known to modulate TLR expression, we investigated whether IL-27 affects TLR3-mediated death in prostate cancer cells. Using DU145 and PC3 cell lines as models, we assessed effects of both IL-27 and IFN-γ on TLR3-mediated cell death. Our results demonstrate that when IL-27 or IFN-γ are added with TLR3 agonist poly(I:C), IFN-β expression increases concurrently with prostate cancer cell death. IL-27 and IFN-γ enhanced TLR3 expression, suggesting a mechanism for sensitization to cell death. Furthermore, PC3 cells were more sensitive to IL-27/poly(I:C)-induced cell death compared to DU145 cells. This correlated with higher production of IFN-β and IP-10 as opposed to IL-6 in response to treatment of PC3 cells compared to DU145 cells. Taken together, our results highlight a potential role for IL-27 in the treatment of prostate cancer. Research funded by Prostate Cancer Fight Foundation and the Ride for Dad

9. **INVESTIGATING THE STAT1 ASSOCIATED IMMUNE CHECKPOINT GENE EXPRESSION AND SPATIAL PROFILES OF ADAPTIVE IMMUNE CELLS IN HIGH-GRADE SEROUS OVARIAN CANCER.** Deyang Li1,4, Thiago Vidotto1, Noor Shakfa1,4, Afrakoma-Afriyie Asante1,4, Nichole Peterson2, Manon de Ladurantaye3, Anne-Marie Mes-Masson3, Julie-Ann Francis2, Madhuri Koti1,2, 4 1Department of Biomedical and Molecular Sciences, Queen's University 2Department of Obstetrics and Gynecology, Queen's University. Centre de recherche du Centre hospitalier de l'Université de Montréal (CRCHUM)/Institut du cancer de Montréal and Université de Montréal, Montreal, QC, Canada. 4Division of Cancer Biology and Genetics, Cancer Research Institute, Queen's University

   Resistance to platinum chemotherapy, leading to an incurable disease after recurrence, occurs in the majority of patients with high-grade serous ovarian cancer (HGSC). In a cohort of 734 HGSC patient tumours, we previously demonstrated that interferon-induced Signal Transducer and Activator of Transcription (STAT1) in combination with CD8+ tumour infiltrating lymphocyte (TIL) density are predictive of chemotherapy response. The goal of the current study was to determine STAT1 associated transcriptomic alterations and spatial profiles of select immune cell populations in chemosensitive and resistant HGSC tumours. In a cohort of 184 pre-treatment HGSC tumours, we performed multiplex immunofluorescence to determine the expression of PD-L1, PD-1, IDO1 immune checkpoints, and the density of CD8+ T cells, Mx1+ cells, FoxP3+ T regulatory TILs, CD68+ macrophages, and CD163+ M2 macrophages. A pre-selected subset of 43 sensitive (high STAT1 protein) and 17 resistant (low STAT1 protein) tumours that were previously characterized for STAT1 expression and CD8+ TIL density, were subjected to sequencing of RNA. STAT1 expression significantly correlated with immunomodulatory genes, including both immune checkpoints and activators in chemosensitive and resistant tumours. Findings were independently validated in a cohort of 379 HGSC tumour RNA-Seq profiles from The Cancer Genome Atlas Network ovarian cancer dataset. This study provides further evidence for the complex roles of STAT1 in the tumour microenvironment and evidence for potential adaptive immune resistance in pre-treatment HGSC tumours. Supporting Agency: Canadian Institutes of Health Research (CIHR) Lab: Cancer Research Institute 313

10. **POST-TRANSLATIONAL MODIFICATION OF PROGRAMMED DEATH LIGAND (PD-L1) IN TRIPLE NEGATIVE BREAST CANCER**. Min Ling, Dr. Xiaolong Yang, Department of Pathology and Molecular Medicine, Queen's University Kingston, Ontario Canada

    The programmed death ligand-1 (PD-L1) is a cell surface protein expressed on a variety of antigen presenting cells for the purpose of controlling immune response. Triple negative breast cancer has the most abundant tumor infiltrating lymphocytes (TILs) and PD-L1 is found highly upregulated in TNBC, allowing blockade immune checkpoint therapy a potentially effective treatment. However, the overall response rate is less than 20-30%. Over the past years, the literature that implicated regulation of PD-L1 was primarily on transcription levels. For this reason, my project aims to further characterize post-translational modification of PD-L1 in efforts to better target it in TNBC immunotherapy. Based on preliminary data from an affinity purification proteomic screen using PD-L1 as bait, PD-L1 was shown to have novel interacting tyrosine kinases. PD-L1 was phosphorylated by three of these novel interacting kinases in vitro, which may suggest PD-L1 phosphorylation as a novel post-translational modification. This PTM may account for PD-L1 upregulation and activation in TNBC immune evasion and tumorigenesis, which is a potential target in immunotherapy-resistant TNBC. This work was supported by grants from the Canadian Institute of Health Research (CIHR#119325, 148629), and the Canadian Cancer Society (CRS)/Canadian Breast Cancer Foundation (CBCF) to XY.

11. **ROLE OF THE PROGRAMMED DEATH LIGAND 1 (PD-L1) IMMUNE CHECKPOINT IN THE ACQUISITION OF DRUG RESISTANCE IN TUMOUR CELLS**. Minassian L1,2, Sanwalka D1,2, Macdonald-Goodfellow S1,2, Ghaffari A1,2, Craig A, Siemens1,2,3 DR, Graham CH1,2,3 1) Biomedical and Molecular Sciences, Queen's University, Kingston, Ontario, Canada. 2) Cancer Research Institute, Queen's University, Kingston, Ontario, Canada. 3) Department of Urology, Kingston General Hospital, Kingston, Ontario, Canada.

    Most studies on the Programmed Death 1 (PD-1)/Programmed Death Ligand 1 (PD-L1) immune checkpoint have focused on elucidating the signalling mechanisms leading to inactivation of immune effectors. There is evidence that signalling by the PD-1/PD-L1 immune checkpoint may be bidirectional, and we have shown that reverse signalling by PD-1/PD-L1 leads to activation of oncogenic pathways as well as resistance to chemotherapeutic agents in tumour cells. Autophagy is a well-established mechanism of chemoresistance in cancer cells. Hence, we hypothesized that PD-1/PD-L1 signaling induces chemoresistance in tumor cells by up-regulating autophagic pathways. Immunoblot analysis demonstrated that exposure of human breast cancer cells to recombinant PD-1 (rPD-1) resulted in a time-dependent increase in LC3-II as well as Beclin-1, two important mediators of autophagy. Treatment with rPD-1 also resulted in increased recruitment of LC3-II to the autophagic membrane. Moreover, imaging studies using live breast cancer cells expressing GFP-tagged LC3 revealed a time-dependent increase in autophagosome formation following administration of rPD-1. Using inhibitors of autophagy, we have shown that drug resistance induced by reverse PD-1/PD-L1 signalling is causally linked to increased autophagy. In addition, our studies indicate a role for extracellular signal-related kinase (ERK) signalling in PD-1/PD-L1 induced autophagy. These studies provide a rationale for the use of PD-1/PD-L1 immune checkpoint blockers and autophagy inhibitors as potential chemosensitizers in cancer therapy. (Supported by CIHR)

12. **EFFECTS OF MEN2 MUTATIONS ON RET RECEPTOR LOCALIZATION AND FUNCTION**. Eduardo Reyes-Alvarez, Brandy Hyndman, Eric Lian, and Lois M. Mulligan. Department of Biomedical and Molecular Sciences, Queen's University, Ontario Canada. Department of Pathology and Molecular Medicine, Cancer Research Institute Division of Cancer Biology and Genetics, Queen's University, Ontario Canada.

    The RET receptor tyrosine kinase is essential for proliferation, migration and differentiation of cells in the genitourinary system and neuroendocrine tissues. In patients with the cancer syndrome Multiple Endocrine Neoplasia type 2 (MEN2), point mutations that constitutively activate RET lead to tumorigenesis. Here, we evaluate MEN2 RET subcellular localization, trafficking, and downstream signaling compared to wild-type (WT) receptors. We evaluated the localization of MEN2 RET with markers of internalization and trafficking. We found altered localization of MEN2 RET receptors to lipid raft membrane domains, suggesting potential differences in localization and interactions at the membrane. We found that MEN2 RET interacts with clathrin-mediated endocytosis adaptors and localizes with early endosomal markers in the absence of ligand, suggesting that these mutant receptors are constitutively trafficked through the cell. However, unlike activated WT RET, we did not see MEN2 RET constitutive localization to recycling endosomes, suggesting different trafficking through endosomal compartments. Our data suggest that MEN2 RET decreases the phosphorylation of ERK1/2 and S6 compared to WT RET but these signals increase with ligand stimulation. This suggests that constitutively active MEN2 RET receptors are still responsive to ligand stimulation but differ in their abilities to activate signaling pathways compared to WT RET. These findings, in combination with future studies, will help to identify the key altered mechanisms that could be more specifically targeted to treat MEN2-associated tumorigenesis. (Supported by CIHR (LMM), Mitacs (ERA) and CONACYT (ERA))

13. **CAN THE GENOTYPE OF HIGH-GRADE SEROUS OVARIAN CANCER CELLS AFFECT THEIR RESPONSE TO CHEMO-IMMUNOTHERAPY?** Noor Shakfa1,2, Elizabeth Lightbody1,2, Afrakoma Afriyie-Asante1,2, Vinicius Kannen1,2, Madhuri Koti1,2,3 Department of Biomedical and Molecular Sciences, Queen's University, Kingston, ON, Canada, Division of Cancer Biology and Genetics, Queen's Cancer Research Institute, Kingston, ON, Canada, Department of Obstetrics & Gynaecology, Queen's University, Kingston, ON, Canada.

High grade serous ovarian carcinoma (HGSC) is the most prevalent form of ovarian cancer and most lethal gynecologic malignancy. Relapse is seen in >70% of cases, therefore alternate treatment options need to be explored. The tumor immune microenvironment (TIME) can be broadly divided into a "hot" state, characterized by higher expression of interferon genes and CD8+ tumor infiltrating lymphocytes (TILs), or conversely for a "cold" state. BRCA1 mutations have previously been shown to impart an immunologically "hot" phenotype and higher sensitivity to chemotherapy. In contrast, loss of PTEN is associated with poor outcomes and chemoresistence in HGSC. We hypothesize that HGSC tumors with loss of PTEN expression can be converted into an immunologically hot state via activation of the Stimulator of Interferon Genes (STING) pathway in combination with carboplatin chemotherapy in our well-established ID8 syngeneic mouse model of HGSC. We will characterize the TIME of tumors generated from ID8 Trp53-/-; Brca1-/- cells, and those from ID8 Trp53-/-; Pten-/- cells. The response of these distinct cells and tumors to combination carboplatin and STING agonist therapy will be determined. Our preliminary results demonstrate that treatment with STING pathway activator significantly increases chemosensitivity and overall survival of mice implanted with ID8 Trp53-/-; Pten-/- cells. This study will potentially establish cancer cell intrinsic oncogenic events that could guide HGSC patient selection for combination chemo-immunotherapy. Supporting Agency: Canadian Institutes of Health Research (CIHR) Lab: Cancer Research Institute 313

14. **ELUCIDATING ISOFORM SPECIFIC ROLES FOR CALPAIN-1 AND CALPAIN-2 IN BREAST CANCER.** Ivan Shapovalov, James MacLeod, Yan Gao, and Peter Greer. Department of Pathology and Molecular Medicine, Queen's University, Division of Cancer Biology and Genetics, Queen's Cancer Research Institute, Kingston, Ontario, Canada

Breast cancer is one of the most frequently diagnosed cancers worldwide. Despite progress in the early diagnosis and treatment, breast cancer still often reaches stages III and IV, when the expected overall survival significantly decreases. Clinical analyses have shown that high expression of calpain-1 and calpain-2, prototypic members of a calcium-dependent subfamily of cysteine proteases, correlates with poor survival in breast cancer. Calpains act through proteolytic cleavage of their substrates; many of which regulate actin reorganization, cell survival signaling, invadopodial dynamics and resistance to chemotherapeutics. Calpain-1 and calpain-2 are ubiquitously expressed heterodimers consisting of catalytic subunits CAPN1 and CAPN2, respectively, and a common CAPNS1 regulatory subunit. We hypothesize that calpain-1 and calpain-2 play isoform-specific essential roles in breast tumor growth, metastasis and resistance to specific chemotherapeutics. We developed a complete isogenic panel of MDA-MB-231 triple negative breast cancer cell lines which are CRISPR-Cas9 knocked out for individual CAPNS1, CAPN1 or CAPN2 genes, and then rescued by transduction with GFP-expressing lentiviral vectors encoding corresponding Myc-epitope tagged recombinant proteins (both wild type and catalytically inactive in the case of CAPN1 and CAPN2), or empty vector. This panel of cells is being used to elucidate the individual and combined roles of calpain-1 and calpain-2 on in vitro migration, invasion, and sensitivity to paclitaxel, doxorubicin, and docetaxel; and in vivo tumor growth, metastasis and drug sensitivity.

15. **ROLE OF INNATE IMMUNE MEMORY IN RESPONSE TO BCG IMMUNOTHERAPY OF BLADDER CANCER**.
William Tran, Jean-François Paré, Charles Graham

Bladder cancer is the fifth most common cancer and is associated with a high rate of morbidity. Currently, the gold standard treatment for treating non-muscle invasive bladder cancer (NMIBC) is using adjuvant intravesical injection of Bacillus Calmette-Guérin (BCG) after tumour resection. However, over half of NMIBC patients subsequently experience recurrence, and the mechanisms by which this treatment works remain unclear. There is emerging evidence that trained immunity, aka innate immune memory, plays an important role in mediating BCG efficacy. This study investigated the extent of trained immunity within circulating monocytes by analyzing the potential biomarkers lactate, HMGB1 and miR-155-5p, as well as their association with clinical outcomes. Our findings indicate that plasma samples do not provide a significant pattern on lactate or miR-155-p levels. However, a pattern of reduced plasma HMGB1 secretion at post-BCG compared to pre-BCG treatment was found in all recurrent patients, indicating potential support for this hypothesis. Finally, in purified monocytes, miR-155-5p expression–known to be upregulated during inflammatory states–increased after LPS addition, but basal levels did not increase after two BCG treatments. Therefore, our results suggest HMGB1 as a better hallmark of trained immunity to be secreted in plasma that may have a predictive value as to the success of BCG immunotherapy in NMIBC. Research supported by SEAMO

16. **TARGETING THE HIPPO PATHWAY FOR TRIPLE-NEGATIVE BREAST CANCER THERAPY.** Liqing Wu; Xiaolong Yang. Department of Pathology & Molecular Medicine, Queen's University Kingston, Ontario Canada

Breast cancer (BC) is the most frequently diagnosed cancer among females, accounting for 25% of all cancer cases worldwide. Among all the subtypes of BC, triple negative breast cancer (TNBC) is considered one of the most-deadly killer in a global world, featured by its increasing incidence and poor prognosis, which demands viable targeted therapy urgently. Recently the Hippo signaling pathway has emerged as an important cellular network mediating tumorigenesis, drug resistance and cancer metastasis. Current studies revealed that the core Hippo components are involved in the tumorigenesis and metastasis of BC and TNBC. As such, there have been concerted efforts by the scientific community to understand the relationship between Hippo pathway and TNBC, and to develop therapeutics targeting this process. In my thesis project, I hypothesize that the Hippo pathway, especially the Hippo output YAP/TAZ is a druggable target for TNBC therapy. To test these hypotheses, I have developed the following Experimental Aims: Establishment of cell lines monitoring the levels of the Hippo pathway components Screen for small molecules regulating the stability of the Hippo pathway components in TNBC Characterization of small molecule targeting Hippo pathway in TNBC cells and xenograft animal models. Support by Canadian Institute of Health Research (CIHR#119325, 148629), Canadian Cancer Society (CRS)/Canadian Breast Cancer Foundation (CBCF)

17. **THE ROLE OF VON WILLEBRAND FACTOR IN THE PATHOGENESIS OF DEEP VEIN THROMBOSIS.** Choi SJ, Swystun L, Michels A, Dwyer C, Nesbitt K, and Lillicrap D. Department of Pathology and Molecular Medicine, Queen's University, Kingston ON, Canada

Venous thromboembolism (VTE), comprised of deep vein thrombosis (DVT) and pulmonary embolism, is the leading cause of preventable deaths worldwide. VTE poses a concern for many individuals with associated risk factors such as cancer and sepsis. We aim to characterize the role of the procoagulant protein, von Willebrand factor (VWF), in mediating DVT pathogenesis. Lipopolysaccharide (LPS) was administered to healthy mice and circulating peripheral blood levels of VWF were measured at 6 different time points post-treatment. Consistent with the literature, the LPS-treated mice showed significantly higher VWF levels at all time points compared to controls. Currently, the effects of LPS on thrombosis is being evaluated in a murine stenosis model of venous thrombosis. Preliminary observations suggested that even 3 hours post-stenosis, LPS-treated mice have higher rates of thrombosis compared to controls. Further immunochemistry analysis will evaluate the molecular and cellular vessel wall interactions prior to thrombus formation and the composition and sizes of the resulting thrombi. The effects of LPS on VWF activity and multimer size will be measured by factor VIII and collagen binding assays, respectively. Finally, human endothelial cells in a flow chamber in vitro will be used to characterize how VWF is anchored to the endothelial surface under acute inflammation and turbulent flow, to potentiate thrombosis. We hypothesize that acute inflammation and turbulent flow will enhance DVT development through increased VWF expression. (Supported by the Heart and Stroke Foundation of Canada)

18. **TYROSINE-PROTEIN KINASE SRC REGULATES Kv1.5 CHANNEL ACTIVITY AND MEMBRANE EXPRESSION THROUGH INTERACTION WITH THE N-TERMINUS OF THE CHANNEL**. Taylore Dodd, Tingzhong Wang and Shetuan Zhang. Department of Biomedical and Molecular Sciences, Queen's University Kingston, ON, Canada.

Kv1.5 is a voltage-gated potassium channel that generates the ultra-rapid delayed rectifier potassium current (IKur) important in the repolarization of the atrial action potential. Malfunction of the Kv1.5 channel often results in atrial fibrillation (AFib). A reduction in Kv1.5 current (IKv1.5) occurs upon activation of the endogenous tyrosine-protein kinase Src. The Src SH3 domain binds to proline-rich motifs located within the N-terminus of Kv1.5. Disruption of these binding motifs has been involved in the development of familial AFib. The mechanism underlying the reduction of IKv1.5 upon Src activation has not yet been established and the relationship between Kv1.5 and Src is poorly understood. Therefore, the present study aims to further elucidate the mechanism behind IKv1.5 reduction. The hypothesis that Src regulates Kv1.5 activity by altering the density of mature membrane-localized channels was tested using whole-cell voltage clamp and Western blot analysis. We demonstrate that Src tonically inhibits Kv1.5 activity and decreases the density of mature membrane-localized channels. Kv1.5 channels possessing mutations within the Src binding motifs were also investigated and it was determined that each binding motif contributes to the Kv1.5-Src relationship, however, the binding of Src to an individual motif is sufficiently effective. Our findings indicate that Src regulates Kv1.5 through an interaction with the N-terminal binding motifs and suggests that the inhibition of forward trafficking may be involved in the underlying mechanism. (Supported by the Heart and Stroke foundation of Canada and The Canadian Institutes of Health Research).

19. **EXAMINING THE IMPACT OF ENDOTHELIAL BONE MORPHOGENETIC PROTEIN RECEPTOR 2 LOSS ON INTERLEUKIN-15 SIGNALING AND THE PATHOGENESIS OF PULMONARY ARTERIAL HYPERTENSION.** L. Rhiannon Hilton1,2, Matthew Rätsep1,3, Patricia Lima1, Melissa Mitchell1 and Mark. Ormiston1,2,3,4 1 Queen's Cardiopulmonary Unit, Queen's University, Kingston, Canada. 2 Department of Biomedical and Molecular Sciences, Queen's University, Kingston, Canada. 3 Department of Medicine, Queen's University, Kingston, Canada 4 Department of Surgery, Queen's University, Kingston, Canada

Rationale: Pulmonary arterial hypertension (PAH) is a disease of pathological vascular remodelling associated with mutations in BMPR2, the gene encoding the bone morphogenetic protein receptor type 2, and immune dysfunction. Natural killer (NK) cells, a subset of immune cells, have been suggested to play a role in the pathogenesis of PAH due to their capacity to influence vascular remodeling. A reduction in NK cell number and function has been described in the lungs of PAH rodent models and in patients. This is accompanied by reduced levels of interleukin-15 (IL-15), a regulator of NK homeostasis, in complex with its α-type receptor (IL-15Rα). The current objective is to examine the impact of BMPR2 loss in the pulmonary endothelium on IL-15 signaling and NK cell homeostasis. Methods: Gene expression, secreted protein levels, sub-cellular localization and surface presentation of IL-15 and IL-15Rα were assessed in human pulmonary artery endothelial cells (HPAEC) with or without BMPR2 silencing. Results: Loss of BMPR2 in HPAECs significantly reduced secreted IL-15Rα, and reduced surface presentation by 15%. It is also associated with differential intracellular localization of both IL-15 and IL-15Rα. Conclusion: We have identified a novel link between BMPR2 loss and altered NK homeostatic IL-15 signaling, which may influence disease development. Ongoing work includes using IL-15 knockout rats to assess the impact of impaired IL-15 signalling on vascular remodelling in rat models of PAH. This work is funded by the Canadian Institute for Health Research.

20. **ZONE 0/1 FROZEN ELEPHANT TRUNK WITH WHOLE BODY PERFUSION IN AORTIC DISSECTION**. Stephanie Jiang, Syed Hassan, Darrin Payne M.D., Andrew Hamilton M.D., Dimitri Petsikas M.D., Gianluigi Bisleri M.D. Department of Surgery, Kingston Health Sciences Centre, Ontario Canada.

The frozen elephant trunk (FET) procedure has emerged as a strategy to facilitate complex aortic arch disease in one stage. Potential technical limitations are represented by the need to perform aortic arch replacements often in zone 3, leading to a higher risk of paraplegia. Thus, we utilized a novel approach aimed at a proximal stent deployment in zone 0 or 1, combined with a multi-branch vascular graft while ensuring whole body perfusion (WBP) throughout the procedure. Three patients were diagnosed with Type A aortic dissection (AD) and underwent this FET procedure. Two patients received a combination of the Evita Open hybrid stent graft (28mm, Jotec, Hechingen, Germany) and the Lupiae multi-branch vascular prosthesis (28mm, Vascutek Terumo, Inchinnan, UK). One received the Thoraflex hybrid graft (36mm, Vascutek, Scotland) and the Lupiae multi-branch vascular prosthesis (32mm, Vascutek Terumo, Inchinnan, UK). Two stents were deployed in zone 0, and one was deployed in zone 1. All patients were put on WBP for the duration of the procedure to reduce hypoperfusion of distal structures. No major complications were reported. Post-operative stays were uneventful. We found that zone 0 and 1 deployment mitigates occlusion of intercostal vessels and potentially lowers the risk of paraplegia. The Lupiae graft allowed for a more proximal stent release and improved take-off angles of the supra-aortic branches, permitting reconstruction to better suit the patient's anatomy.

21. **INTRAOPERATIVE HIGH-DENSITY MAPPING DURING SIMULTANEOUS HYBRID ABLATION FOR THE TREATMENT OF ATRIAL FIBRILLATION.** Camila Mayorga Palacios, Syed M. Ali Hassan, Benedict Glover, Damien Redfearn, Andreas Enriques, Gianluigi Bisleri. Division of Cardiac Surgery, Queen's University, Kingston, ON, Canada

Long-standing persistent atrial fibrillation(LsP-AF) is characterized by a complex substrate. In the setting of simultaneous hybrid ablation, 3D high-density automated voltage mapping enables endo-epicardial substrate identification and guided modification. These voltage maps can potentially aid us in defining the substrate of LsP-AF and gain a better understanding of this complex disease. Fourteen patients (mean age:63.4±10.4yrs) with LsP-AF underwent a simultaneous hybrid ablation procedure with thoracoscopic epicardial ablation and transcatheter endocardial ablation: four patients were female, four patients had prior transcatheter ablations, the mean LVEF was 60±4.6% and the mean LA volume index was 37±17.4 ml/m2. All patients underwent baseline endocardial voltage mapping and the last 10 patients underwent combined endo-epicardial mapping. Advanced electrophysiological mapping during simultaneous hybrid ablation of LsP-AF allowed us to identify significant differences in the endocardial and epicardial substrates and revealed epicardial breakthroughs despite the presence of endocardial isolation in patients who had undergone previous failed transcatheter ablations. These findings highlight the need to improve the understanding of the baseline substrate characteristics as well as potential mechanisms of failure such as lack of transmurality. Further investigation on a larger series of patients is warranted to confirm these preliminary findings.

22. **BMPR2 LOSS CAUSES A PROLIFERATIVE SHIFT IN THE ENDOTHELIAL RESPONSE TO BMP9**. Anne L. Theilmann1, Lindsey G. Hawke1, L. Rhiannon Hilton1, Mara K.M. Whitford1, Jodi L. Mackeil1, Kimberly J. Dunham-Snary2, Paula D. James2, Donald H. Maurice1, Stephen L. Archer2, Mark L. Ormiston1,2,3 1 Department of Biomedical and Molecular Sciences, Queen's University, Kingston, Canada
2 Department of Medicine, Queen's University, Kingston, Canada .3 Department of Surgery, Queen's University, Kingston, Canada

Objective: Pulmonary arterial hypertension (PAH) is a disease of proliferative vascular occlusion that is linked to heterozygous mutations in BMPR2, the gene encoding the bone morphogenetic protein (BMP) type II receptor (BMPR-II). The endothelial-selective BMPR-II ligand, BMP9, has been shown to reverse disease in animal models of PAH and suppress the proliferation of healthy endothelial cells. However, the impact of BMPR2 loss on the antiproliferative actions of BMP9 has yet to be assessed in endothelial cells from PAH patients. Approach: BMP9 responses were assessed in blood outgrowth endothelial cells (BOECs) from PAH patients and controls, as well as human pulmonary artery endothelial cells (HPAECs) with or without BMPR2 silencing. Mouse pulmonary endothelial cells (MPECs), bearing a homozygous or heterozygous deletion of Bmpr2, were isolated from endothelial-conditional knockout mice (Bmpr2EC-/-), which were also assessed in vivo for altered retinal angiogenesis following BMP9 administration. Results: BMP9 suppressed proliferation in control endothelial cells, but increased proliferation in PAH patient BOECs, BMPR2 silenced HPAECs and Bmpr2EC-/- MPECs. This proliferative shift was not linked to altered metabolic activity or interactions with canonical TGF$\beta$ signaling, but was associated with the prolonged induction of the canonical BMP targets ID1 and ID2. Conclusion: Loss of BMPR2 results in a shift of the endothelial BMP9 response towards enhanced proliferation. This finding has potential implications for the clinical translation of BMP9 in PAH treatment. This abstract was funded by the Canadian Institutes of Health Research and the Ontario Thoracic Society.

23. **PAPILLARY MUSCLE RELOCATION WITH A MULTI-LOOP TECHNIQUE AS AN ADJUNCT FOR TREATMENT OF ISCHEMIC MITRAL REGURGITATION.** Maria Theresa Dela Cruz Servito BHSc,1 Lawrence Torkan BSc,1,2 Gianluigi Bisleri MD, FRCSC.1 1 Department of Surgery, Queen's University School of Medicine, Kingston ON, Canada. 2 Department of Mechanical and Materials Engineering, Queen's University, Kingston ON, Canada

Ischemic Mitral Regurgitation (IMR) is primarily due to left ventricular remodelling leading to annular dilatation and poor leaflet coaptation of the mitral valve (MV). Papillary muscle relocation (PMR) has been introduced as an adjunct for the treatment of IMR. Here, we present an innovative modification of the original technique for PMR using a multi-loop technique. Six patients underwent PMR and ring annuloplasty for the treatment of IMR (Table 1). Neo-chords were adjusted based on adequate leaflet coaptation, which was evaluated with a water test. The procedure was considered successful if TEE revealed mild or no mitral regurgitation. All operations were successful, as all patients exhibited none or mild mitral regurgitation immediately after the procedure. The mean post-operative length of hospital stay was 9 ± 2 days. At a mean follow-up of 108 ± 8 days, all patients were free from recurrent mitral regurgitation and none have required re-intervention. This technique is innovative, as the use of pledgets alleviates the tension imbued by the neo-chord on the papillary muscle. Additionally, the multi-loop reduces the number of sutures and pledgets that are passed through the papillary muscle. This approach can simplify the papillary muscle relocation technique while ensuring an extensive stabilization of the posterior mitral valve annulus. Further studies are warranted to confirm the long term outcomes of this preliminary findings and long-term outcomes.

24. **TOWARDS INTRAOPERATIVE USE OF AMBIENT MASS SPECTROMETRY IMAGING FOR CARDIAC TISSUE.** Randy E. Ellis1,2,3, Alice M.L Santilli2, Diane E. Tomalty1, John F. Rudan3,4, Martin Kaufmann1,4, Gianluigi Bisleri4. 1Department of Biomedical and Molecular Sciences, Queen's University; 2School of Computing, Queen's University; 3Human Mobility Research Centre, Queen's University, 4Department of Surgery, Queen's University

For patients undergoing cardiac surgery, atrial fibrillation (AF) is a serious cardiac arrhythmia that develops in up to 40% of cases. AF is associated with increased patient morbidity and mortality. Substantial gaps remain in our understanding of AF pathogenesis and perpetuation, particularly at the molecular level. Desorption electrospray ionization mass spectrometry (DESI-MS) is a metabolomics approach that is useful for understanding altered biological pathways, and uncovering novel diagnostic biomarkers through focused analysis of small molecules. It is hypothesized that DESI-MS analysis is a feasible method for the assessment of pathologic cardiac tissue, and will yield valuable metabolomic information. Right atrial appendages were harvested from 10 patients undergoing cardiac surgery, half of these patients had a history of AF, the other half did not. Samples were cryosectioned, DESI-MS images were acquired, and tissue sections were stained with H&E. Non-negative matrix factorization was used to estimate ion signatures which were used to visualize the data. Ion signatures were composed of a list of metabolites that dominated the mass spectra in each image. All tissue samples were spatially homogenous, with no clear patterns of metabolite distribution. DESI-MS was able to detect different metabolic profiles between AF and non-AF tissue, including differences in fatty acid and phospholipid expression, though no statistically significant patterns of metabolite distribution that distinguished AF from non-AF tissue were identifiable in this small sample set. This is the first reported use of DESI-MS to assess cardiac tissue.

## Health Policy, Population health, and Epidemiology

**25. THE ASSOCIATION BETWEEN HIGH DIAGNOSTIC TESTING AND THE SPECIALIST DIAGNOSTIC INTERVAL IN SYMPTOMATIC BREAST CANCER PATIENTS.** Lucas Cohen, Colleen Webber, Andrea Eisen, and Patti Groome. Department of Public Health Sciences, Division of Cancer Care and Epidemiology, Queen's Cancer Research Institute

Background: Breast cancer is the most commonly diagnosed cancer in Canadian women. A protracted time to diagnosis is associated with increased patient anxiety, later stage diagnosis, and decreased survival. To date no research has explored the impact of high testing (beyond best practice guidelines) on the length of time from first specialist visit to diagnosis (specialist diagnostic interval). Methods: A population-based, cross-sectional design was used to study symptomatic breast cancer patients diagnosed in Ontario from 2007 through 2015 (n=42,020). The study population was derived from an existing database created from linked administrative health care databases housed at ICES. Patients were assigned high-testing status if they received at least three mammograms, three ultrasounds, two MRIs, or three biopsies during their diagnostic interval. The relationship between high diagnostic testing and the specialist diagnostic interval was evaluated using multivariable quantile regression models. Number of days of testing, disease, and sociodemographic variables were included in the model to control for potential confounding. Results: The median specialist diagnostic interval was 20 days (IQR: 8-42 days). After controlling for covariates, high diagnostic testing was associated with a 46.3 day increase in the median specialist diagnostic interval ($p<0.0001$). Conclusion/Future Directions: Understanding the strength of the association between high diagnostic testing and the length of the breast cancer specialist diagnostic interval will help policy makers and clinicians understand the importance of guideline adherence.

**26. SLEEP, MELATONIN, AND CIRCADIAN GENE METHYLATION AS POTENTIAL PATHWAYS BETWEEN SHIFT WORK AND BREAST CANCER RISK**. Felske LR, Tranmer J, Ritonja J, and Aronson KJ. Department of Public Heath Sciences, Queen's University Kingston, Ontario, Canada

Shiftwork-related circadian disruption was identified as a "probable" carcinogen by the International Agency for Research on Cancer in 2007, with most studies on breast cancer. However, there remains uncertainty about the biological mechanisms connecting shiftwork and breast cancer. Sleep patterns before, during, and after working night shifts, may contribute to disrupted circadian rhythm. Biomarkers of this disruption may be melatonin and circadian gene methylation. This study aims to: 1) compare sleep patterns and melatonin levels; and, 2) explore sleep patterns and circadian gene methylation among pre-menopausal shift workers compared to day workers. A cross-sectional pilot study (n=96) will be conducted among female pre-menopausal hospital employees who participated in a cross-sectional study in 2011-2014 (n=331). Participants have provided information on age, demographics, lifestyle behaviors, and past shift work, as well as all urine voids in a 48-hour period for measurement of melatonin. New information will include determination of chronotype by a validated questionnaire, assessment of sleep efficiency, duration, and timing using triaxial accelerometers worn over an 8-day period, and collection of a 5 mL blood sample to measure circadian gene methylation. These objectives are logical steps toward discovering mechanisms in the pathway connecting shiftwork to breast cancer risk. The long-term goals are to inform workers and contribute to future workplace policies, cancer prevention strategies and night work interventions. (Supporting agencies: Cancer Research Society, CIHR, WSIB Ontario)

27. **MODELLING THE PATHWAYS FROM SHIFT WORK TO CARDIOVASCULAR DISEASE RISK AMONG FEMALE HOSPITAL WORKERS.** Haley Golding1, Kristan Aronson1, Jennifer Ritonja1, and Joan Tranmer1,2. 1Department of Public Health Sciences and 2School of Nursing, Queen's University

Introduction: Evidence supports that long-term shift work including night work is associated with 1) circadian disruption, 2) sleep disturbances, 3) stress, and these in turn contribute to biologic changes in hormones and other biomarkers that increase CVD risk. However, limited information exists on the relative importance of these pathways or their potential interactions. Objective: To develop a multi-level model of the hypothesized causal mechanisms linking shift work to increased risk for CVD, considering all potential pathways. Method: Data from a cross-sectional study conducted among 331 day or rotating shift workers at Kingston Health Sciences Centre recruited between 2011 to 2014 will be used. Participants completed an interview, questionnaires, and clinical exam. Over an 8-day study period, participants wore triaxial accelerometers to measure movement during waking and sleeping hours, and also provided all urine over 48-hours from which melatonin and cortisol were measured. This study will test and refine a conceptual model, originally proposed by Knuttson and Boggild. The analysis will use structural equation modelling to determine associations between variables, and the relative importance of the various pathways and their interactions. Impact: This approach allows us to assess multiple causal pathways linking shift work to cardiometabolic risk, important knowledge that will help us better understand the CVD risks for working women in the healthcare sector.

28. **CONFOUNDING BY INDICATION, SURVIVAL AND SELECTION BIAS IN INFECTIVE ENDOCARDITIS RESEARCH**. Olivia Moir, MSc Epidemiology Student, Department of Public Health Sciences, Queen's University. Dr. Yingwei Peng, Department of Public Health Sciences, Faculty of Health Sciences, Queen's University. Dr. Susan B Brogly, Department of Surgery, Faculty of Health Sciences, Queen's University and ICES Queen's

Introduction: Infective endocarditis (IE) is a serious infection of the endocardium, the tissue that lines the inner chambers of the heart and the surface of the cardiac valves. Morbidity and mortality of IE is high and when left untreated it is usually fatal. The benefit of surgical (ST) vs. medical (MT) treatment on IE survival is unclear. Results of epidemiological studies have been mixed and methodological issues, including confounding by indication and survival bias, are common. Selection bias, inherent to the period specific hazard ratio, may explain why recent evidence has suggested ST increases short term but decreases long term mortality risk. Objectives: To explain confounding by indication, survival and selection bias in the context of IE literature. To estimate the causal effect of ST vs. MT on mortality and to assess whether selection bias accounts for any apparent long-term protective effect of ST on death. Methods This population-based retrospective cohort study used universal coverage healthcare data from Ontario, Canada. Discrete time hazards model using inverse probability of treatment weighting will be used to estimate the effect of ST vs. MT on mortality, assess the role of selection bias, and to control for the effect of time-dependent confounding.

## Inflammation, infection and immunity

29. **EXPLORING THE DIVERGENT EFFECTS OF HYPOXIA AND TGFB ON THE PHENOTYPIC CONVERSION AND ANGIOREGULATORY CAPACITY OF CIRCULATING PRIMARY NK CELLS**. Lindsey G. Hawke, Mara K.M. Whitford, Mark L. Ormiston. Queen's University Departments of Biomedical and Molecular Sciences, Medicine and Surgery, Kingston, Ontario, K7L 3N6, Canada

    Circulating Natural Killer (NK) cells are thought to convert to a tissue-resident, or type 1 innate lymphoid cell (ILC1)-like phenotype and begin producing angiogenic regulators in response to chronic stimuli by transforming growth factor-β (TGFβ) or hypoxia. However, the precise impact of these stimuli on these phenotypic changes, and whether their effect is combinatorial remains unclear. Culturing primary NK cells with chronic TGFβ induced phenotypic conversion was assessed based on the acquisition of CD9, CD103, and CD69. Culture with the NK cell survival factor interleukin-15 (IL-15) bolstered conversion while hypoxia had no impact. Instead, NK cells cultured at 1% or 4% oxygen began to produce vascular endothelial growth factor (VEGF) displaying a 2000-fold increase in gene expression by qPCR, a uniform upregulation by fluorescent in-situ hybridization on flow cytometry, and confocal microscopy, and NK cells began secreting 25-100pg/mL after 3-7 days. Again, no synergy was observed between exposure to hypoxic and TGFβ. Splice form specific qPCR indicated that NK cells predominantly produce classical VEGFA and not the possibly anti-angiogenic VEGFAb or VEGFAx forms which were also ruled out by ELISA. In conclusion, we have used human primary NK cells in vitro determine the effects of hypoxia and TGFβ are distinct from one another, where TGFβ induces an ILC1-like phenotype, and hypoxia initiates production of classical forms of VEGFA. (Supported by CIHR)

30. **TARGETING GENE NETWORKS OF SPINAL CORD INJURY PAIN**. Courtney A. Bannerman, Jihoon Choi, Julia P. Segal, Mitra Knezic, Scott Duggan, Qingling Duan, Nader Ghasemlou

    More than half of patients with a spinal cord injury (SCI) will suffer from chronic pain. However, there are few therapeutics available to patients suffering, with opioids commonly being prescribed. Using a microarray of peripheral blood mononuclear cells collected from spinal cord injury patients with and without chronic pain after injury we were able to determine specific targets and pathways that may play a role in the development and maintenance of pain. We plan to determine the role of these targets using a newly developed model of spinal cord injury in the mouse over a 6-week period as well as, their significance to immune cell activation through the use of behavioural and locomotor assays, qPCR, flow cytometry, and immunohistochemistry. Ultimately, this work will allow for the discovery of new, more specialized treatment options for patients and a better understanding of the mechanisms controlling spinal cord injury pain.

31. **INTERLEUKIN-27 ALTERS THE POLARIZATION AND RESPONSIVENESS OF HUMAN PMA-THP-1 MACROPHAGES**. Katelyn Gray, Olena Kourko, Natalya Odoardi, Katrina Gee. Department of Biomedical and Molecular Sciences

    In response to environmental stimuli, macrophages are capable of polarizing along a spectrum of inflammatory states, ranging from proinflammatory (M1) to anti-inflammatory (M2). These macrophages have distinct physiological functions but also contribute to disease manifestation and progression. It is thus important to investigate molecules that may modify the process of macrophage polarization. In the present study, the capacity of Interleukin (IL)-27 to influence macrophage polarization was investigated. Previous findings suggest that IL-27 has predominantly pro-inflammatory effects on myeloid cells, and therefore it was hypothesized that this cytokine would skew polarization towards an M1 phenotype. Interestingly, it was determined that IL-27 differentially modulated macrophage polarization. This effect was substantiated by the impact of IL-27 on the responses of polarized macrophages to stimulation with bacterial or viral components. IL-27 inhibited the responses of M1 macrophages but potentiated responses of the M2 macrophages. As a secondary objective, the ability of monophosphoryl lipid A to act as a M1 polarization agent was evaluated. This polarization generated an M1-like phenotype that was influenced by IL-27 in a manner that aligned with the observed context-dependent actions of this cytokine. These findings suggest that IL-27 may have a physiological role in immune homeostasis. Further, the differential effect of IL-27 may have clinical applications in cancer therapeutics, sepsis prophylaxis and vaccine adjuvant development. Future investigations into the therapeutic potential of IL-27 are warranted. Funding provided by NSERC.

**32. NOVEL TYROSINE KINASE INHIBITORS REGULATE INTESTINAL SMOOTH MUSCLE CELL GROWTH IN VITRO.** Jay Kataria, Sandra Lourenssen, and Michael Blennerhassett. Department of Medicine

In inflammatory bowel disease (IBD), chronic inflammation causes structural and functional alterations of the intestine that include rapid expansion of intestinal smooth muscle cells (ISMC) and extracellular matrix collagen deposition. Since there are minimal treatment options, we explored the effects of two multimodal tyrosine kinase inhibitors, nintedanib and pirfenidone, recently approved for idiopathic pulmonary fibrosis, a chronic lung condition resembling IBD. Methods: In vitro model systems of 3T3 fibroblasts or rat ISMC were assessed for response to serum or the mesenchymal growth factor PDGF-BB, evaluating growth responses by proliferation assay, and western blotting to assess type I collagen expression. Results: Both 3T3 fibroblasts and rat ISMC showed concentration-dependent proliferation in response to serum or PDGF application. Nintedanib inhibited growth at 5 μM ($p<0.05$, $n=5$) and reduced responses to baseline at 25 μM without cytotoxicity, while pirfenidone was ineffective at levels ≤ 5 mM. Western blotting for collagen expression identified a 125KD band for both cell types and suggested down-regulation with pirfenidone. Conclusion: Novel anti-fibrotic therapies for chronic pulmonary disease display distinctive effects on ISMC proliferation vs matrix production. These address molecular mechanisms of fibrosis that are in common with IBD, and so the translation of therapeutic approaches may be a promising treatment option.

**33. CONTROL OF SOMATOSENSATION BY NEUTROPHILS AND ENDOGENOUS OPIOIDS**. Mitra Knezic and Nader Ghasemlou. Department of Biomedical and Molecular Sciences, Queen's University Kingston, Ontario Canada.

Circadian rhythms describe the study of recurrent biological rhythms regulating the timing of processes such as hormone release and sleep cycle according to a 24-hour cycle. Previous unpublished work from the Ghasemlou lab has shown that thermal somatosensation follows a circadian rhythm, with lower heat sensitivity in naïve male C57BL/6J mice at night relative to during the day. Nociceptors involved in somatosensation are housed in peripheral structures called dorsal root ganglia (DRGs) and heat sensation is controlled through TRPV1 receptors present on these sensory neurons. TRPV1 activity, and therefore thermal sensitivity, is reduced following interactions between nociceptor μ-opioid receptors and endogenous opioids, which are known to be secreted by neutrophil immune cells. Neutrophils were manually quantified in DRGs collected from naïve male C57BL/6J mice at two time of day – 9AM and 9PM. Following quantification recruitment appears to follow a circadian rhythm and are present in significantly higher quantities at 9PM relative to 9AM. An endogenous opioid precursor, pro-opiomelanocortin (POMC), was qualitatively more highly expressed in tissue collected at night and is localized in neuron cell bodies and suspected smooth muscle tissue of vascular walls. POMC is cleaved by stress hormones to produce the endogenous opioid β-endorphin, which has been shown to act as a chemoattractant for neutrophils. These results suggest that neutrophils and endogenous opioid production may be involved in reducing thermal somatosensation at night. (Supported by an NSERC Discovery Grant).

34. **IL-27 DIFFERENTIALLY MODULATES TLR7 AND TLR8 RESPONSIVENESS IN HUMAN MONOCYTES AND MACROPHAGES INDEPENDENTLY OF TYPE I IFN.** Natalya Odoardi[1], Olena Kourko[1], Carlene Petes[1], Katrina Gee[1]. [1]Department of Biomedical & Molecular Sciences, Queen's University, Kingston, Ontario, Canada, K7L 3N6.

Anti-viral functions have been attributed to the heterodimer cytokine interleukin (IL)-27. IL-27 is produced by antigen presenting cells (APCs) as a result of Toll-like receptor (TLR) signaling. TLRs are important innate immune sensors which provide the first line of defense against invading pathogens. Specifically, TLR7 and TLR8 recognize single-stranded RNA (ssRNA) and are found within the endosome of immune cells. In myeloid cells, following ligand binding, a MyD88-dependent signalling cascade is initiated resulting in pro-inflammatory cytokine and type I interferon (IFN) production. Since IL-27 alters bacterial-sensing TLR expression on myeloid cells, it was of interest to investigate whether IL-27 can influence function of other TLRs. Given that IL-27 has been demonstrated to inhibit replication of ssRNA viruses, we examined the effects of IL-27 on expression and function of ssRNA-sensing TLR7 and TLR8. Analysis of IL-27-treated monocytes and macrophages revealed changes in mRNA and protein expression of TLR7 and TLR8. Treatment with IL-27 also enhanced TLR7- and TLR8-mediated NF-kB/AP-1 signaling and pro-inflammatory cytokine secretion. Since type I IFNs were not produced to detectable levels, this type I IFN deficient environment suggests the observed IL-27-mediated alternations in immune responses are type I IFN independent. Delineating the immunomodulatory role of IL-27 on TLR7 and TLR8 responses will provide insight into how its mechanistic actions can be applied to improve viral vaccine development and novel adjuvant research and design. *N.O. is supported by funding from the NSERC Alexander Graham Bell CGS-M Scholarship and wishes to be considered for an oral presentation or poster award.*

35. **ABERRANT INFLAMMATION IN RAT PREGNANCY CONTRIBUTES TO RISK FACTORS ASSOCIATED WITH CARDIOVASCULAR DISEASE AND PREGNANCY COMPLICATIONS IN SUBSEQUENT GENERATIONS**. Nicole Protopapas, Takafumi Ushida, Tiziana Cotechini, Shannyn K. Macdonald-Goodfellow, Charles H. Graham. Department of Biomedical and Molecular Sciences. Queen's University, Kington, Ontario Canada

Preeclampsia (PE) and intrauterine growth restriction (IUGR) are complications of pregnancy associated with an increased risk of cardiovascular disease (CVD) and in both mothers and offspring later in life. Common to the pathophysiology of these pregnancy complications is aberrant maternal inflammation. Using an established rat model of PE/IUGR in which pregnant rats are administered low-dose lipopolysaccharide (LPS) on gestational days (GD) 13.5-16.5, we sought to examine the association between aberrant maternal inflammation and the increased risk of disease in offspring. Physiologic parameters associated with CVD were assessed in offspring born to LPS- and control-treated dams at 24 weeks of age, including echocardiography, glucose tolerance, organ histone modifications and cardiac-growth related gene expression. Female offspring were mated and fetal weight was assessed on GD 17.5 to determine whether IUGR persists across generations. Our results reveal that pups born to LPS-exposed dams were growth-restricted and later exhibited sex-specific mild systolic dysfunction, increased cardiac growth-related gene expression and abnormal glucose tolerance. Histone modifications in target organs persisted for at least 24 weeks in both sexes. Fetal weights measured on GD 17.5 from second-generation LPS-treated dams were significantly reduced compared to their control counterparts. Our findings provide evidence for a cross-generational effect of aberrant maternal inflammation on the increased risk of CVD and pregnancy complications in offspring. This work was supported by a grant from the Canadian Institutes of Health Research.

**36. THE CONTRIBUTION OF DENDRITIC CELLS TO MECHANISMS OF INFLAMMATORY PAIN.** Madeline Robinson[1], Pascale Patenaude[1], Jaqueline Raymondi Silva[1], Nader Ghasemlou[1,2]. [1]Department of Biomedical and Molecular Sciences, [2]Department of Anesthesiology and Perioperative Medicine

Tissue injury, pathogenic infection, and chronic immune diseases cause inflammatory pain hypersensitivity at sites of inflammation, a common medical condition of which patients report poor management. The specific contribution of cells within the innate and adaptive immune system to the generation of inflammatory pain hypersensitivity has yet to be established. Previous research indicates a $CD11b^{+}Ly6G^{-}$ myeloid cell subset mediates mechanical pain hypersensitivity following incisional wound injury in mice, a model of post-operative inflammatory pain. As several $CD11b^{+}Ly6G^{-}$ myeloid candidates have since been ruled out (mast cells, monocytes, macrophages, NK cells), this project evaluates the contribution of dendritic (DC) and Langerhans cells (LC) to peripheral inflammatory pain in an attempt to identify this key myeloid population. Three aims were used to address this goal: i) development and optimization of several techniques for the study of DCs and LCs in the skin, including skin-layer separation and DC/LC-specific flow cytometry; ii) optimization of DC and LC depletion using transgenic human diphtheria-toxin-receptor expressing mice; and iii) assessment nociceptor depletion's effect on DC and LC activation and proliferation following incisional wound injury. Findings indicate nociceptors may modulate $CD11b^{+}CD24^{-}$ dermal DC activation and cutaneous DC proliferation after injury. This preliminary work lays the foundation for future research evaluating the contribution of skin-resident DC subsets to inflammatory pain, with the aim of identifying new therapeutic targets for its treatment in wound inflammation. **Funding Sources**: The Canadian Institute of Health Research, The J.P. Bickell Foundation

**37. EVALUATING PROPHYLACTIC VACCINATION MODELS TO ASSESS TUMOURIMMUNE CELL INTERACTIONS FOLLOWING TUMOUR ENGRAFTMENT.** Kyle Seaver[2], Peter Greer[1] and Sam Basta[2]. [1]Division of Cancer Biology and Genetics, Cancer Research Institute, Department of Pathology and Molecular Medicine, Queen's University, Kingston, Ontario, Canada. [2]Department of Biomedical & Molecular Science Queen's University, Kingston, Ontario, Canada.

Conventional cytotoxic therapies, chemotherapy and radiotherapy, have been associated with toxicity, drug resistance, off target effects, and immune-cell depletion. Immunotherapies have become an attractive alternative to cytotoxic therapies, however their limitation is that cancer cells commonly develop immune escape mechanisms. This outlines the importance of early intervention, which is becoming a feasible option with the advancements in cancer diagnostics. The goal of this research is to evaluate how the use of preventative vaccination models can develop protection against tumourigenesis *in vivo*. Here, we demonstrate that a prophylactic vaccine consisting of dead tumour cells is able to increase survival by 37% 60 days post tumour challenge. With the addition of adjuvants to this vaccine, we were able to see percent survival increase to 100% 60 days post tumour challenge. With the development of this successful vaccination model, we next aimed to determine the mechanisms involved in producing this robust anti-tumour immune response. This is accomplished by evaluating the immune landscape before and after vaccination and again after tumour engraftment. By understanding these tumour-immune cell interactions this research provides novel information towards the development of anti-cancer therapies. *Research funded by Natural Sciences and Engineering Research Council of Canada (NSERC).*

**38. NEUROIMMUNE MECHANISMS UNDERLYING CIRCADIAN CONTROL OF PAIN IN A MOUSE MODEL OF MULTIPLE SCLEROSIS.** Julia Segal, Courtney Bannerman, Ian Gilron, Nader Ghasemlou. Department of Biomedical and Molecular Science

Over half of multiple sclerosis (MS) patients experience chronic pain, with many reporting higher pain intensity at night. I hypothesize that pain, clinical symptoms, and neuroimmune interactions also exhibit a circadian rhythm in experimental autoimmune encephalomyelitis (EAE), the mouse model of MS, and that the body's "master clock" modulates disease outcome. I will: i) phenotype circadian control of clinical signs of disease and pain; ii) identify the contribution of circadian pathways to disease; and iii) target mechanisms underlying these effects to alter disease pathophysiology. Preliminary circadian studies have found that EAE mice show a clear circadian change in pain, where mechanical sensitivity is increased during the day (when mice are dormant) and reduced at night (when mice are active). These results match those observed in MS patients, as well as in patients with other types of chronic neuropathic pain. I have now characterized immune cell influx into the spinal cord and dorsal root ganglia at three times of day (9am, 3pm, 9pm) and have identified several immune cell subsets whose changes in infiltration correlate with observed changes in pain in our mice. Our next goal is to identify neuroimmune and pain-related mediators showing greatest change over the course of the day. These mediators may provide the best opportunity to identify therapeutic targets for the treatment of chronic pain in EAE and MS. Supporting agencies: Multiple Sclerosis Society of Canada, National Multiple Sclerosis Society.

**39. LCMV-ARMSTRONG INFECTION DIFFERENTIALLY AFFECTS GM-CSF AND M-CSF DERIVED MACROPHAGE EARLY ACTIVATION SIGNALING PATHWAYS.** Evan Trus[1], Torki Alothaimeen[1], Katrina Gee[1], Sam Basta[1]
[1]Department of Biomedical and Molecular Sciences, Queen's University, Kingston, ON, Canada, K7L 3N6

Lymphocytic Choriomeningitis Virus (LCMV) is a segmented, ssRNA virus belonging to the *arenavirus* family. A common model for both chronic and acute infection due to its ability to infect a wide range of mammalian cells, LCMV has contributed to the understanding of the immune system in many ways. Upon infection, the macrophage forms a key defense against this virus. Research into the relationship between LCMV and macrophages has shown that macrophages are essential in the early control of LCMV infection and that TLR7 mediates inflammatory monocytosis after acute infection (Seiler *et al.* 1997 and Buechler *et al.* 2015). TLR7, an endosomal pattern recognition receptor specific for ssRNA has also been found essential for clearance of certain LCMV strains (Walsh *et al.* 2012). In this study we seek to define the effects of Armstrong LCMV infection on the activation of TLR7 signaling proteins at early timepoints in GM-CSF and M-CSF derived murine macrophages. We evaluated the phosphorylation state of the signaling proteins p38, ERK, and NF-kB via western blotting and the production of cytokines TNF-alpha, IL-10, IL-6, and IL-12p40 via ELISA. We show that in the early hours of Armstrong LCMV infection, signal protein phosphorylation and cytokine production differ between GM-CSF and M-CSF derived macrophages. We also show that LCMV is able to prevent further phosphorylation of signaling proteins by the TLR7 agonist R848. This study provides further insights on innate immune response by macrophages of different activation states and enhances the understanding of how LCMV and potentially other arenaviruses establish infection.

**40. NOVEL STRAIN-BASED ANALYSIS OF TISSUE MECHANICS INFORMS ABOUT CHANGES IN STRUCTURAL INTEGRITY WITHIN THE CORPUS CALLOSUM FOLLOWING EXPOSURE TO SUB-CONCUSSIVE IMPACTS**

**Allen A. Champagne,[1] BSc, BA,** Emile Peponoulas,[1] BSc, Itamar Terem,[2] BSc, Andrew Ross, MSc, Maryam Tayebi,[3] MSc, Yining Chen,[1] MSc, Nicole S. Coverdale,[1] PhD, Poul M. F. Nielsen,[3,6] PhD, Alan Wang,[3] PhD, Vickie Shim,[3] PhD, Samantha J. Holdsworth,[4] PhD, Douglas J. Cook,[1,5] MD, PhD. [1]Centre for Neuroscience Studies, Queen's University, Kingston, ON, Canada. [2]Department of Radiology, Stanford University, Stanford, CA, United-States of America. [3]Auckland Bioengineering Institute, University of Auckland, Auckland, New Zealand . [4]Departement of Anatomy and Medical Imaging & Centre for Brain Research, Faculty of Medical and Health Sciences, University of Auckland, Auckland, New Zealand. [5]Department of Surgery, Queen's University, Kingston, ON, Canada. [6]Department of Engineering Science, Faculty of Engineering, University of Auckland, Auckland, New Zealand

Increasing evidence for the cumulative effects of head trauma on structural integrity of the brain has emphasized the need to understand the relationship between tissue mechanic properties and injury susceptibility. In this study, diffusion tensor imaging (DTI), helmet accelerometers and amplified magnetic resonance imaging (aMRI) were combined to gather insight about the region-specific vulnerability of the corpus callosum (CC) to microstructural changes in white-matter integrity upon exposure to sub-concussive impacts in Canadian football players. Longitudinal decreases in fractional anisotropy were characterized in anterior and posterior regions of the for athletes sustaining more impacts to the head on a daily basis. Using these findings as a basis for investigation, a novel strain analysis of sub-voxel motion based on the biomechanical response of brain tissues to cardiac impulses was developed to show that differences in maximum strain (and thus possibly stiffness) along the tract may reveal a possible signature relationship between changes in white-matter integrity and tissue mechanical properties. In light of these findings, additional information about the viscoelastic behavior of WM tissues upon exposure to external forces may be imperative in elucidating the mechanisms responsible for region-specific differences in injury susceptibility observed, for instance, through changes in micro-structural integrity following exposure to sub-concussive collisions. This work was funded by the Southeastern Ontario Academic Medical Organization (SEAMO), the Ontario Graduate Scholarship (OGS), the University of Auckland FDRF strategic initiative fund and the Globalink Research Award (GRA) from Mitacs.

**41. IMPACT OF DOSE RESPONSE FOR THE GENE THERAPY TREATMENT OF AB-VARIANT GM2 GANGLIOSIDES IN A MOUSE MODEL USING SELF-COMPLIMENTARY ADENO-ASSOCIATED VIRUS SEROTYPE 9** Natalie M. Deschenes[1], K. Osmon[1], S. Kot[2], Z. Chen[3], A. Ryckman[1], B. Quinville[1], A. Jadav[1], M. Mitchell[3], S. J. Gray[4] and J. 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/Departments of Pediatrics, Queen's University, Kingston, Ontario, Canada, K7L 2V7; [4]Department. of Pediatrics, UT Southwestern Medical Center; Dallas, TX, USA

GM2 Gangliosidoses is a group of autosomal recessive neurodegenerative, lysosomal storage disorders. It results in rapid neurological decline and death by age 4, in its human infantile form; there is currently no cure. These disorders are characterized by the excessive accumulation of GM2 ganglioside within the cell's lysosome, eventually leading to cell death. In a properly functioning cell, the metabolism of GM2 ganglioside into GM3 ganglioside is mediated by the β-Hexosaminidase A (HexA) enzyme, and its essential cofactor, the GM2 activator (GM2A) protein. GM2 Gangliosidoses manifests as three forms: Tay-Sachs, Sandhoff and AB-Variant. AB-Variant, the rarest of the three forms, is characterized by a mutation in the *Gm2a* gene, resulting in a deficiency of GM2A protein. Being a monogenic disease, AB-Variant is an exceptional candidate for gene therapy treatment, which is a promising method for producing a one-time treatment strategy. Previously a single stranded Adeno-associated virus serotype 9 expressing *GM2A* showed efficacy in decreasing GM2 accumulation in short-term and long-term using intravenous route of delivery in our lab. This study uses a self-complementary (sc)AAV9-*GM2A* vector delivered via intrathecal route into a $Gm2a^{-/-}$ mouse model (which lives normal life) and assess the short-term therapeutic efficacy. A dose of $1.0x10^{11}$vg/kg or $0.5x10^{11}$vg/kg was administered via a lumbar puncture, at six weeks of age. Starting at eight weeks of age, mice underwent monthly behavioural testing to assess coordination, activity and strength. All mice were euthanized at twenty weeks of age, where blood and various organs were collected for further biochemical and molecular analysis. We hypothesized that (1) scAAV9 will have a better efficacy than the previously trialed single stranded vector; and (2) the higher dose will prove to be the most efficacious in reducing GM2 accumulation and having a wide vector biodistribution. The behavioural data does not show any statistical differences between any of the cohorts (treated vs. untreated and between doses), as brain damage is expectedly less due to modest build-up of GM2 noted at twenty weeks of age, thus, likely not having a large effect on behavioural phenotype. Ganglioside extraction assays showed a significant decrease in accumulation within the midbrain of mice that received the medium dose treatment. Consistent with this data was the vector biodistribution, which displayed the highest quantity of vector genomes per mouse genome within mice that received the higher dose, in both the liver and midbrain. It is promising to see a wide vector distribution and a reduction in GM2 accumulation which lays a solid foundation towards clinical gene therapy of AB variant GM2 Gangliosidosis. Supporting Agencies: GlycoNet, UT Southwestern Medical Center, Kingston General Hospital and Queen's University

**42. GM2 GANGLIOSIDES: BIOMARKERS OF DISEASE PROGRESSION IN A SANDHOFF MOUSE MODEL.** Deirdre Hindmarch[1] & Jagdeep Walia [1,2]. [1] Centre for Neuroscience Studies, Queen's University, Kingston, Ontario, Canada
[2] KGH Research Institute

Sandhoff Disease (SD) is a rare genetic disorder which results in progressive neurodegeneration and death. SD is caused by a build up of GM2 Gangliosides, a lipid which is stored in the lysosome of neurons. It can occur in infantile, juvenile and adult forms; in the severe, infantile form, death occurs by the age of 4. It is well understood that this lipid build-up is the result of the mutation of the *HEXB* gene, and therefore the b-hexosaminidase A enzyme for which it codes; however, better characterization of the disease pathology is needed. Therefore, using a Hexb -/- knockout (KO) mouse model of SD, sections from the midbrain and the cerebellum, and serum and urine samples were taken from KO (n=10) and heterozygous (HET) mice (n=10) at three time points (5, 10 and 16-weeks), to assess brain changes in gene expression and metabolite changes in urine and serum, over the progression of the disease compared to unaffected age-matched controls. A recent microarray study (Ogawa et.al., 2018) revealed the upregulation of immune-related genes, and the downregulation of myelin genes in a Sandhoff mice, at 4 weeks old. Here, we have validated six of these genes (wfdc17, Lyz2, Ccl3, Ugt8a, Fa2h and Mog) in brain sections from the midbrain and cerebellum of Sandhoff mice compared to Hets, at three discrete time points and demonstrate that there are significant ($p$<0.05) and robust brain immune changes over the course of the disease. The characterization of these biomarkers will allow for better monitoring of preclinical therapies for SD.

**43. MAPPING PSILOCYBIN-ASSISTED THERAPY: A SCOPING REVIEW.** Ioudovski, Paul; McKeown, Sandra; Goldie, Craig; Dumont, Eric; Ron, Shore. Department of Biomedical and Molecular Science, Queen's University, Kingston, Ontario, Canada.

The potential of psilocybin as a therapeutic tool for treating mood and self-regulatory disorders has emerged in the last two decades. However, there is a significant gap in the literature regarding the efficacy of psilocybin-assisted therapy. A scoping review was conducted to determine the program variables leading to optimal psilocybin therapy outcomes. The secondary goal was determining the current trends in literature and identifying knowledge gaps. MEDLINE, Embase, PsycINFO, and Cochrane Central Register of Controlled Trials were searched using keywords and database-specific subject headings. Two independent reviewers conducted screenings and data extractions. 36 articles were identified from 1097 after all screenings and data extractions. From these 36, eight studies with original cohorts were identified. There were 175 patients identified across all eight studies treated for six different disorders: tobacco addiction, alcohol addiction, neurosis, obsessive-compulsive disorder, treatment-resistant depression, and end-of-life anxiety related to cancer diagnosis. Out of all eight studies, half were randomized control trials and the other half were open-label trials. Psilocybin therapies requires a larger sample size and a broader and more equal distribution of presenting conditions to increase the generalizability of results. More randomized control trials are also needed to increase the validity of results.

**44. USING EYE TRACKING TO IDENTIFY BIOMARKERS OF EATING DISORDER IN ADOLESCENTS.** Ryan H. Kirkpatrick, Linda Booij, Sarosh Khalid-Khan, and Douglas Munoz. Centre for Neuroscience Studies, Queen's University, Kingston, Ontario, Canada.

Of all psychiatric diagnoses, eating disorders (EDs) have the highest mortality rate and while 2.7% of adolescents meet ED criteria, up to 30% of adolescents fully or partially relapse following treatment. Therefore, there is a need to identify biomarkers of EDs to allow for early detection. Saccadic eye movements have been identified as biomarkers in various psychiatric diagnoses including schizophrenia and depression. This study aims to determine whether saccadic eye movements present a biomarker of EDs in adolescents. Patients (12-18 years of age) were recruited from an outpatient ED treatment clinic and control participants were recruited from the community. All patients met DSM 5 criteria for an ED. All participants completed a well-validated interleaved pro-/anti-saccade task, a free viewing task and clinical questionnaires. The free viewing task consisted of approximately 20 minutes of uninstructed viewing of short video clips with length varying between 2 and 8 seconds, and video clips of food related stimuli were randomly included. Preliminary results indicated a faster reaction time and more anticipatory (<90 ms after stimulus onset) saccades in patients compared to controls on the interleaved pro-/anti-saccade task. Patients had different scan-path behaviours when food stimuli were presented during the free viewing task. Overall, cognitive control of saccadic eye movements appears to differ between adolescents with an ED and controls suggesting their efficacy as a behavioural biomarker for an ED. (Supported by an Ontario Graduate Scholarship and the Canadian Institutes of Health Research).

**45. TARGETING NECROPTOSIS VS APOPTOSIS IN ISCHEMIA-INDUCED MYENTERIC NEURON DEATH.** Savio Cyril Kocherry, Sandra Lourenssen, & Michael Blennerhassett. Department of Medicine, Queen's University.

**Background:** Animal models of Inflammatory Bowel Disease (IBD) show neuron loss in inflamed regions that is associated with ischemia. We explored the role of programmed cell death to develop interventions that might preserve the enteric nervous system (ENS). **Methods:** A primary co-culture model of the ENS was developed using myenteric neurons, glia and smooth muscle from the intestine of neonatal rats. The metabolic inhibitor DNP or hypoxia (1% $O_2$), were combined with inhibitors of apoptosis or necroptosis, and neuron survival was determined. **Results:** DNP (0.75 mM; n=12) caused selective neuron loss vs control ($p<0.05$), but neither inhibition of caspases (ZVAD; apoptotic pathway) or RIP3Kinase (GSK-872; necroptosis) was effective. However, the combination of zVAD+GSK blocked neuron loss effectively ($p<0.05$), suggesting a combination of apoptotic and necroptotic pathways. In contrast, these inhibitors were not effective against 1% $O_2$, where a similar neuron loss occurred (n=7). **Conclusion:** DNP and hypoxia are effective models for study of inflammation-induced neuronal death. While these models display an unexpected and complex profile of programmed cell death, a combination of selective targeting approaches can promote survival. Future study will define insult-specific aspects of neuronal cell death, and explore strategies for testing in vivo. (Supported by NSERC).

**46. ISOLATING ENDOGENOUS MOLECULAR TRIGGER OF SPREADING DEPOLARIZATION RELEASED BY NEURAL TISSUE UNDER ABRUPT HYPERTHERMIA.** Kelly Lee, Nikita Ollen-Bittle, R.D. Andrew, A.Y. Jin**.** Center for Neuroscience Studies, Queen's University, Kingston ON, Canada.

Spreading depolarization (SD) is a common mechanism of insult across different types of acute neural injury and secondary brain damage from the subsequent fever. It is defined by the $Na^+/K^+$ pump failure and the wave of lost transmembrane potential as the inward, cationic current far exceeds that of the outward one in a positive feedback manner. The excitotoxicity from the persistent energy depletion translates into cerebral edema and the expansion of the necrotic core, where therapeutic opportunity is lost. However, while SD continues to be associated with poor functional outcome and higher mortality, the suspected existence of a molecular trigger remains elusive. In support of an endogenous, molecular SD activator (SD*a*) that initiates the cascade of ischemic injury, SD was induced in naïve rat brain slices superfused with aCSF previously incubating 8 coronal slices and raised to a febrile temperature of 40℃ from 35℃. The current study aims to isolate the SD*a* by analyzing the aCSF superfusate solution by high pressure liquid chromatography (HPLC) and the slices undergoing SD by Matrix Assisted Laser Desorption/Ionization – Mass Spectrometry (MALDI-MS).

**47. EXPRESSION OF NA+/K+-ATPASE ISOFORMS IN HIGHER AND LOWER BRAIN REGIONS FOLLOWING FOCAL ISCHEMIA IN MICE: A PRELIMINARY ANALYSIS.** Chloe Lowry, Brian Bennett, R. David Andrew**.** Centre for Neuroscience Studies, Queen's University, Kingston, ON, Canada

Clinically and experimentally, higher gray matter is more susceptible to acute ischemic injury than lower gray matter. Discovering the mechanisms which contribute to the brainstem's resilience may inform targets for improved survival of higher brain neurons. As failure of the Na+/K+-ATPase is a key event following ischemia, we hypothesize that differential regional susceptibility of the brain to ischemia might be explained in part by variable expression of Na+/K+-ATPase isoforms, which differ in pumping efficiency under low energy conditions. Our mRNA expression analyses in mice have shown that under basal conditions, the ischemia-vulnerable alpha1 isoform is on average 2.2x higher than alpha3 in neocortex, whereas the ischemia-resistant alpha3 isoform is on average 2x higher than alpha1 in brainstem. Parallel protein expression analyses are consistent with these findings. Preliminary data from mice undergoing a 30-minute middle cerebral artery occlusion (MCAo) shows that 24-hours post-stroke, mRNA expression of alpha1 decreases significantly in the ischemic compared to control hemisphere. We are currently following this up with analysis of alpha1 and alpha3 mRNA and protein levels in various higher and lower brain regions of mice undergoing MCAo. We suspect alpha1 expression will decrease and alpha3 will increase following stroke, particularly in the neocortex. Understanding how Na+/K+-ATPase isoforms may differ in their expression in response to metabolic stress will yield insights into how such differences protect neurons during ischemia.

**48. MORPHOMETRIC AND SPINE DENSITY ANALYSIS OF PYRAMIDAL NEURONS IN A MOUSE MODEL OF SPORADIC ALZHEIMER'S DISEASE.** R. H. Mehder, M. Yoon, B. M. Bennett, R.D. Andrew. Department of Biomedical and Molecular Sciences, Queen`s University, Kingston, ON, Canada K7L3N6

The study of late-onset Alzheimer`s disease (LOAD) has been hindered by the lack of animal models. We have developed an oxidative stress-based model of LOAD based on gene deletion of aldehyde dehydrogenase 2 (Aldh2). These knockout (KO) mice exhibit a progressive decline in recognition and spatial memory, and in other AD-like pathologies. To determine if altered neuronal structure can account for the observed memory deficits, dendritic morphology of one-year-old KO and WT mice was compared using branched structured analysis and Sholl analysis of dorsal (dCA1) and ventral (vCA1) hippocampal pyramidal neurons (PNs), and the overlying PNs of layer V $1^{o}$ visual neocortex (V1). We also assessed dCA1 PNs of 6 month old animals. In one year old animals, morphology and complexity of dCA1 (but NOT vCA1) PNs from KO mice showed significant reductions in apical and basal dendritic length, and significantly fewer dendrite intersections, ends, and nodes. In 6 month old animals, significant reductions in these parameters were only observed in basal dCA1 PNs. The spine density along dCA1 apical and basal dendrites were reduced compared to WT controls in both age groups. V1 dendritic complexity was slightly but significantly reduced in KO vs WT. These data indicate that dCA1 dendritic complexity and spine density are reduced in KO mice whereas vCA1 dendrites appear unaltered. It is likely that this is the specific structural basis for the cognitive deficits seen in our LOAD model, given the central role of the dorsal hippocampus in both recognition and spatial memory. (supported by Saudi Cultural Bureau, an NSERC grant to Dr. Andrew and a CIHR grant to Dr. Bennett)

**49. INVESTIGATING A POTENTIAL ACTIVATOR OF SPREADING DEPOLARIZATION RELEASED BY ISCHEMICALLY STRESSED GRAY MATTER.** N.K. Ollen-Bittle, K.H. Lee, M.S. Fisher, R.D. Oleschuk, A.Y. Jin, R.D. Andrew. Centre for Neuroscience Studies, Queen's University Kingston, Ontario Canada.

Stroke, head trauma and cardiac arrest result in ischemic insult to the brain and cause failure of the $Na^{+/}K^{+}$ pump within minutes wherever blood flow is completely compromised. Following failure of the $Na^{+/}K^{+}$ pump, gray matter undergoes a phenomenon referred to as spreading depolarization (SD). Without reperfusion, SD can devastate neurons in its wake; however, in partially perfused tissue SD can recur and expand neuronal injury over ensuing hours and days. The molecular events linking ischemia, pump failure and SD are not well understood. Previous work in our lab has demonstrated superfusate from 8 live coronal rat brain slices incubated in oxygen/glucose deprived saline (OGD, $35^{o}$C, 10 min), then rebalanced with $O_2$ and glucose and superfused over a naïve slice at $35^{o}$C can trigger SD genesis. We propose that an SD *activator* (SD*a*) released from OGD-exposed slices induces SD in the non-stressed slice. We are analysing the incubate of the 8 slices using liquid chromatography and mass spectroscopy as well as analyzing brain slices undergoing SD with MALDI to further characterize a hypothesized SD*a*. Release of an SD*a* by compromised gray matter could mediate both the initiation and propagation of SD.

**50. INVESTIGATION INTO THE CORRECTION OF GM2 GANGLIOSIDOSIS - IMPACT OF THE AGE OF ADMINISTRATION OF AAV9 MURINE AND HUMAN BICISTRONIC HEXOSAMINIDASE VECTORS IN SANDHOFF MICE.** Karlaina J. L. Osmon[1], Natalie M. Deschenes[1], Eminet Bogale[2], Shalini Kot[2], Zhilin Chen[3], Melissa Mitchell[2], Clifford Heindel[4], John G. Keimel[4], William F Kaemmerer[4], Steven J. Gray[5], 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/Departments of Pediatrics, Queen's University, Kingston, Ontario, Canada, K7L 2V7; 4 New Hope Research Foundation, North Oaks, Minnesota, USA 5 Department of Pediatrics, University of Texas Southwestern Medical Center, Texas, United States.

GM2 Gangliosidoses are severe neurodegenerative diseases caused by a Hexosaminidase A (HexA) enzyme deficiency leading to the neurotoxic accumulation of GM2 gangliosides (GM2). The HexA enzyme is comprised of an α- and β- subunit, and complexes with the GM2 activator protein to breakdown GM2. Mutations in the HEXA, HEXB, or GM2APcause deficiencies in the α-, β- subunit, or GM2AP, giving rise to Tay Sachs Disease (TSD), Sandhoff Disease (SD), or the AB-variant disease, respectively. The infantile form of GM2 Gangliosidosis are fatal by 4 years old, and there are no current treatments. Here, we evaluated the impact of the age of administration of gene therapy. AAV9 vectors containing either a human bicistronic (hB-A), a murine bicistronic (mb-a), or a murine hexb only (mhexb) transgene were administered intravenously to SD mice at 0 weeks of age (at 2.5E11vg/mouse or ~2.5E14vg/kg) or 2, 4 or 6 weeks of age (at 1E12vg/mouse, 1.88vg/mouse, and 2.5E12vg/mouse (respectively) or ~1.25E14vg/kg). Half of the mice in each cohort were euthanized at 16 weeks, equivalent to the humane endpoint for untreated controls. The rest were observed until their humane endpoint. Mice treated at 0, 2, 4, and 6 weeks with the hB-A vector survived to a median age of 59, 68.4, 43.6, and 43.8 weeks and mice treated with the mb-a vector survived to median age of 42.2, 76.8, 65.8, and 68.14 weeks, respectively. Mice treated with the mhexb vector at 0 and 6 weeks are currently surviving to a median age of 65.72 and 70.71 weeks respectively, with one mouse from the 0 week mhexb group still alive past 95 weeks of age. All the survival curves are significant vs vehicle controls ($p<0.001$). A significant increase in behavioural performance was observed at 16 weeks in all treatment groups across all ages of administration ($p<0.01$). Preliminary analyses showed slight increases of midbrain HexA activity across all treatment groups and ages of administration, significant decreases in GM2 storage in the hB-A and mb-a groups at all ages of administration ($p<0.001$), and constant hB-A biodistribution across the midbrain at all ages of administration. Anti-hB-A antibodies arose in mice receiving hB-A at 6 weeks of age ($p<0.01$). A cross-species antibody response was expected; the human bicistronic vector may not elicit an immune response in patients. Study limitations include the existence of an alternative sialidase pathway for GM2 catabolism in mice, the differential affinity of the subunits, the immature blood-brain barrier in mice at birth, and the possible interaction of the human HEXA enzyme with the murine GM2AP, each of which may have enhanced efficacy. Nevertheless, this preliminary data demonstrates that a bicistronic hexosaminidase AAV9 vector may be a viable option for the treatment of SD and TSD in humans.

51. **THE EFFECTS OF NEURONAL NITRIC OXIDE SYNTHASE AND APOPTOSIS ON NEURAL STEM CELL. PROLIFERATION WITHIN THE ADULT ENTERIC NERVOUS SYSTEM**. Catherine Parisien & Alan E. Lomax. Centre for Neuroscience Studies, Queen's University Kingston, Ontario, Canada.

Inhibition of neurotransmission affects proliferation of neural stem cells (NSCs) within the adult central nervous system. While the adult enteric nervous system (ENS) contains NSCs, it was thought that they remained quiescent postnatally. It has recently been proposed that the adult ENS is in constant equilibrium between neuronal apoptosis and neurogenesis. We hypothesised that manipulating neurotransmission would lead to enteric NSCs becoming mitotically active and forming new neurons. Organotypic culture of myenteric plexus (MP) from mouse colon was performed for one week while inhibiting both neuronal nitric oxide synthase (nNOS) and caspase-3 mediated apoptosis. Neurons generated during culture from proliferating ENSCs were identified as cells that contained the proliferation marker 5-ethynyl-2-deoxyuridine (EdU) and the neuronal protein HuC/D. Neurons per ganglia were quantified by counting HuC/D-immunoreactive cells. Inhibition of nNOS with 7-nitroindazole (30μM) led to a ~250% increase in EdU-positive neurons, while there was no effect on the number of neurons per ganglia. Inhibition of Caspase-3 mediated apoptosis with zVAD-fmk (80μM) caused a ~50% increase in neurons per ganglia, with no increase in EdU positive neurons. Inhibiting both caspase-3 mediated apoptosis and nNOS led to a 200% increase in EdU-positive neurons, and a 100% increase in neurons per ganglia. These findings suggest that nitric oxide-induced suppression of neurogenesis and caspase-dependent neuronal apoptosis play important roles in maintaining the adult ENS.

52. **QUANTIFYING RAPID CORRECTIVE RESPONSES IN THE STROKE POPULATION USING THE UPPER LIMB.** Kayne Park and Stephen H. Scott. Centre for Neuroscience Studies, Queen's University, Kingston, Ontario, Canada

Daily activities require rapid processing of sensory information to guide and control motor actions. For example, playing a game of table tennis requires rapid visual and somatosensory feedback to use a paddle to successfully hit a ball to the other side of the table. Following stroke, activities that require fast feedback processing can be impaired. Clinical assessment commonly quantifies the ability of subjects to perform various motor actions. However, they do not specifically identify if the key problem is the ability to use sensory information to control action. We designed an interception task (Fast Feedback Interception Task (FFIT)) to quantify potential impairments in using visual and/or somatosensory feedback with the KINARM robot. This task required individuals to intercept a ball that moves towards them. On random trials, perturbations were applied through sudden changes in the spatial position of the ball or hand, sudden mechanical perturbations of the arm, or an abrupt change in the ball's colour instructing the subject to either intercept or avoid the moving ball. Performance was quantified in 18 individuals with stroke and 46 neurologically intact controls. Longer reaction times and larger spatial errors were evident for individuals with stroke compared to healthy control subjects. As well, some individuals with stroke showed distinct sensory impairments: impaired at using visual, but not proprioceptive feedback. Further, impairments were commonly observed in the ipsilesional arm. Supported by the Canadian Institutes of Health Research and the Ontario Research Fund.

**53. PROGRESS TOWARDS THE INDUCTION OF IMMUNE TOLERANCE TO THE HYBRID HEXM ENZYME FOLLOWING INTRAVENOUS GENE THERAPY IN A MOUSE MODEL OF SANDHOFF DISEASE.** Brianna M. Quinville[1], Shalini Kot[2], Zhilin Chen[3], Melissa Mitchell[2], Karlaina J.L. Osmon[1], Natalie M. Deschenes[1], Deirdre Hindmarch[1], John G. Keimel[4], William F. Kaemmerer[4], Steven J. Gray[5] 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 [5]Department of Pediatrics, University of Texas Southwestern Medical Center, Texas, USA

Immune responses towards therapeutic proteins are known to diminish the effectiveness of enzyme replacement therapies and gene therapies in CRIM negative subjects. Here we investigated methods for the induction of immune tolerance towards a protein foreign to mice following an AAV gene transfer. Previously we have reported both an antibody and interferon gamma T-cell response following this gene transfer in a mouse model of Sandhoff disease (SD). SD belongs to a group of neurological disorders known as GM2 gangliosidosis, which result from the excessive accumulation of GM2 ganglioside in neuronal cells. Normally, the β-hexosaminidase A (HexA) enzyme hydrolyzes the terminal N-Acetylgalactosamine amino-sugar from GM2 ganglioside and thereby allowing further lipid catabolization. HexA is a heterodimer comprised of two subunits, α and β, and deficiencies of either subunit can inhibit its function. A deficiency of the β subunit results in SD. A stable homodimer variant to human HexA, known as HexM, has been developed that effectively hydrolyzes GM2 *in vivo*. Gene transfer with the HexM encoding gene, *HEXM,* packaged within a self-complementary adeno-associated viral vector, serotype 9 (scAAV9), has shown increased lifespan in SD mouse model (*Hexb*$^{-/-}$). However, our study also showed that an immune response possibly impacted the long-term efficacy of the treatment. It was hypothesized that immunosuppression would allow tolerance development towards the expressed HexM enzyme. Immunosuppressants (IS) - Rapamycin (R) and Prednisone (P) were selected based on their known properties of reducing cytotoxic responses while increasing regulatory T-cell activity. In this study, ten cohorts of SD mice were given different combinations of R and/or P, short- and/or long-term, with the *scAAV9-HEXM* treatment. Daily IS administration, via oral gavage, began at 5 weeks of age, followed by an IV injection of *scAAV9-HEXM* at six weeks. Behavioural testing and blood collections were completed at monthly intervals. Serum, organs and splenocytes were collected for analysis of the transgene copy number, enzyme activity, histology, humoral and cellular immune response to HexM and humoral response to the AAV9 capsid. The long-term administration of both R and P showed a significant decrease seen in both T-cell response and antibodies towards the HexM enzyme long after the end of the IS regimen. Additionally, this cohort had a significantly higher long-term liver vector distribution compared to the other cohorts. While the administration of IS did significantly reduce the immune response to the HexM enzyme, here we also show there was a diminished, but not significant, effect on the immune response to the AAV9 capsid. The *scAAV9-HEXM* treatment in combination with R and P resulted in overall reduction of GM2 ganglioside accumulation, significantly higher enzyme activity, greater long-term vector biodistribution and persistent tolerance towards HexM well beyond the 13-week regimen of IS. The survival of the mice after vector treatment was notably increased up to 7 weeks longer when administered IS compared to mice without IS. The results of this study show the induction of tolerance towards a gene transfer expressed protein with the use of IS and may be applicable to other gene therapies.

**54. ASSESSMENT OF COGNITIVE PERFORMANCE IN DP(16)1/YEY/+ MOUSE MODEL OF DOWN SYNDROME.**
Negin Rezaie, Dr. Brian Bennett

Down syndrome (DS, trisomy 21) is the most common autosomal aneuploidy. DS greatly increases the risk of Alzheimer's disease (AD), and most individuals with DS develop AD neuropathology by age 40. Our objective was to determine whether the working and spatial memory impairments seen in AD and in AD mouse models are also present in the Dp(16)1/Yey/+ (Dp16) mouse model of DS. In this model, the entire Hsa21 orthologous region on Mmu16 (~119 genes), has been duplicated and added onto the distal portion of one of the endogenous Mmu16 chromosomes. We assessed recognition memory using the novel object recognition (NOR) task, spatial working memory by the Y-Maze task and spatial reference memory using the Morris Water Maze (MWM) task. Behavioural testing was performed bimonthly in Dp16 mice and age-matched WT littermates beginning at 2 months of age. In the NOR and Y-maze tasks, Dp16 mice demonstrated a progressive decrease in performance in both memory tasks compared to WT mice. In the MWM task, there were no differences in latency times for cued platform training between Dp16 and WT mice, whereas latency times for Dp16 mice were significantly longer in hidden platform trials. The Dp16 DS mouse model may thus represent a useful comparator to current AD mouse models for the assessment of pharmacological interventions designed to improve cognitive performance in humans with DS and/or AD.

**55. USING EYE TRACKING TO IDENTIFY SACCADE BIOMARKERS OF NEURODEGENERATIVE DISEASE.** Heidi C. Riek, Brian C. Coe, Don Brien, Sandra Black, Michael Borrie, Dar Dowlatshahi, Elizabeth Finger, Morris Freedman, David Grimes, Donna Kwan, Anthony Lang, Connie Marras, Mario Masellis, Gustavo Saposnik, Rick Swartz, Carmela Tartaglia, Lorne Zinman, the ONDRI Investigators and Douglas P. Munoz. Centre for Neuroscience Studies, Queen's University Kingston, Ontario, Canada.

The Ontario Neurodegeneration Research Initiative is investigating six neurodegenerative diseases to characterize neurodegeneration between and within the component patient groups: Alzheimer's disease (AD), mild cognitive impairment (MCI), Parkinson's disease (PD), amyotrophic lateral sclerosis (ALS), frontotemporal dementia (FTD), and vascular cognitive impairment (VCI). Cross-referencing eye tracking and clinical datasets can further this goal. As a first step, we examined saccade responses in all patient groups and age-matched controls to evaluate their viability as possible biomarkers. We used video-based eye tracking of patients (n=504, age 40-87) and healthy age-matched control subjects (n=133, age 50-93) performing a randomly interleaved pro- and anti-saccade task; the colour of a central fixation point conveyed the instruction for a prosaccade (look at peripheral target) or antisaccade (look away from peripheral target). We assessed parameters including task errors, reaction times, and their association with clinical parameters (e.g. MoCA score). Patients displayed age-controlled abnormalities on subsets of eye tracking parameters (e.g. increased antisaccade direction errors – erroneously looking at peripheral target; increased antisaccade reaction time) relative to controls; patterns of abnormality differed across disease groups (e.g. increased saccades away from fixation point in AD/MCI, FTD, and VCI only). These dramatic saccadic changes signify unique behavioural biomarkers for neurodegeneration that can powerfully inform novel diagnostic tools and treatments. (Supported by the Ontario Brain Institute)

**56. ALZHEIMER'S DISEASE BIOMARKERS IN CEREBROSPINAL FLUID OF NONHUMAN PRIMATES.** Emma Robertson[1], Susan Boehnke[1,2], Brittney Armitage-Brown[1], Robert Wither[1], Natalia Lyra e Silva[1], Andrew Winterborn[3], DJ Cook[1,4], Ron Levy[1,4], Fernanda De Felice[1,5], Douglas Munoz[1,2], [1]Centre for Neuroscience Studies, [2]Department of Biomedical and Molecular Sciences, [3]Animal Care Services, [4]Department of Surgery, [5]Department of Psychiatry, Queen's University

Alzheimer's disease (AD) pathology, such as amyloid plaques and neurofibrillary tangles, are present in humans before the onset of behavioural symptoms (Sperling et al., 2011). Markers for these pathological changes can be analyzed in cerebrospinal fluid (CSF). Changes in CSF levels of amyloid-β 1-40 (Aβ40), amyloid-β 1-42 (Aβ42), total tau proteins (tTau), phosphorylated tau (pTau), and neurofilament light (NFL) have been implicated as biomarkers of human AD. Here, we sought to determine levels of these biomarkers in a colony of naïve, control cynomolgus and rhesus macaque monkeys (n=30) to establish baseline values to compare to disease models. CSF samples were collected through lumbar punctures or a lumbar port. Baseline values of Aβ40, Aβ42, tTau, and pTau showed some inter-subject variability, but were similar between species and to published human values. Importantly, we found that repeating lumbar punctures at different time points elevated NFL (~200%) but did not elevate other biomarkers. We also tracked CSF biomarkers in a recently developed monkey model of AD (Forny-Germano et al., 2014, Batista et al., 2018). In monkeys receiving injections of Aβ oligomers, CSF AD biomarkers were elevated, but not in monkeys receiving vehicle injections. Thus, these changes in CSF may be reflective of developing AD-related pathology in the brain and will allow us to investigate disease progression, pathological mechanisms and test novel therapeutics. (Supported by CIHR, Brain Canada)

**57. NOVEL CHARACTERIZATION OF IMPACT BIOMECHANICS REVEALS DIFFERENCES ACROSS POSITIONAL GROUPS IN CANADIAN HIGH SCHOOL FOOTBALL PLAYERS.** Kaden Shearer,1 BSc, Allen A. Champagne,1 BSc, BA, Emile Peponoulas,1 BSc, Douglas J. Cook,1,2 MD, PhD**.** 1Centre for Neuroscience Studies, Queen's University, Kingston, ON, Canada**.** 2Department of Surgery, Queen's University, Kingston, ON, Canada

The study of impact biomechanics in contact sports has enhanced the current understanding of concussion mechanisms and the cumulative effects of sub-concussive impacts on brain health. However, biomechanical parameters in the literature tend to be restricted to linear acceleration and rotational velocity, thus, limiting a complete characterization of potentially injurious head impacts. Additionally, recent studies have shown that low threshold impacts may be at increased risk for false positives due to homogeneities in the kinematic profiles of true impacts and 'noise' (e.g. players dropping helmets). Taken together, these factors highlight the need to establish more comprehensive biometric parameters to quantify impacts to the head, particularly when relating neuroimaging findings to exposure. In this study, a novel characterization of head impacts is proposed to differentiate the mechanical loading events between positional groups in football athletes (n = 39). Differences in the frequency of impacts per-session, peak impact duration, and peak impact impulse - defined as an index for cumulative microtrauma per impact - were documented between the groups. However, no differences in jerk (i.e., rate of force development) were observed. These results suggest that different biomechanical properties may be underlying the signature kinetic profiles that define exposure to sub-concussive collisions. Moving forward, this approach may be applied to further elucidate the relationship between changes in neuroimaging biomarkers and repetitive head impacts. This work was funded by the Southeastern Ontario Academic Medical Organization (SEAMO).

**58. CEREBROVASCULAR PROTEINS INVOLVED IN AMYLOID B DISPOSITION IN A MOUSE MODEL OF SPORADIC ALZHEIMER'S DISEASE: A PRELIMINARY ASSESSMENT.** Kaitlyn A. Tresidder, Brian M. Bennett

We have developed an oxidative stress-based model of late onset Alzheimer's disease (AD) based on a gene deletion of aldehyde dehydrogenase 2 (*Aldh2*). These knockout (KO) mice exhibit age-related cognitive impairment and AD-like biochemical pathologies in both the brain and cerebral vasculature, including amyloid β (Aβ) deposition in cerebral microvessels. Our objective was to assess whether vascular oxidative stress alters cerebrovascular proteins involved in Aβ disposition. Using a mechanical dispersion and filtration technique, we obtained cerebral microvessels (CMVs) from 3, 6, 9, and 12-month old *Aldh2* KO mice and age-matched, wild type (WT) littermates. Immunoblot analysis of these CMV preparations indicated an absence of the neuronal and oligodendrocyte markers NeuN and Olig2, the presence of the astrocyte marker GFAP, and a strong signal for smooth muscle α-actin. Preliminary immunoblot analysis of the basal levels of a number of proteins involved in the formation (nicastrin), catabolism (neprilysin), or transport (LDL receptor-related protein 1, LRP1) of Aβ indicates few differences between KO and WT (n=2-4 per group) at any of the ages assessed. We are currently following this up with activity assays to determine whether there are differences in the function/activity of these proteins. These studies will serve to increase our understanding of the degree to which vascular oxidative stress contributes to the AD-like pathological changes observed in *Aldh2* KO mice.

**59. EVIDENCE FOR EFFECTS OF PHOENIXIN ON NEURONS OF THE PARAVENTRICULAR NUCLEUS.** Emma Walton and Alastair V. Ferguson. Centre for Neuroscience Studies, Canadian Institutes of Health Research

The paraventricular nucleus (PVN) of the hypothalamus has been implicated in autonomic regulation.
Through projections to the pituitary gland, median eminence and hindbrain, the PVN has been found to play a role in stress response. Findings have indicated that both phoenixin (PNX), a peptide involved in such responses, and its receptor, GPR173, are expressed throughout PVN. PNX sensitizes the pituitary gland to releasing hormones, as well as contributes to regulation of stress hormone release. Recently, specific stress-related effects of this peptide have been demonstrated in the nucleus of the solitary tract (NTS). In this study we investigated the effects of PNX on PVN neuronal activity using in vitro extracellular recordings. Recordings from a total of 820 neurons showed that 16% (n= 130) of cells were activated by bath administration of PNX, while 14% (n= 117) were inhibited. Remaining cells tested were classified as non-responsive. Furthermore, we observed that excitatory and inhibitory effects are maintained, but in lower proportions, when recordings are obtained in low-Ca2+/ high-Mg artificial cerebral spinal fluid (to block synaptic release of neurotransmitters and thus synaptic transmission), indicating that PNX has both direct and indirect effects on PVN neurons.
These results implicate PNX in autonomic functioning and potentially in the central regulation of stress.

## Patient Care and Nursing Research

**60. EXAMINING QUEEN'S UNIVERSITY NURSES' PERCEPTIONS OF HOSPITAL ORIENTATIONS AND THEIR TRANSITION TO PRACTICE.** Katherine Gregory, Marian Luctkar-Flude, and Kim Sears. Faculty of Health Sciences, School of Nursing, Queen's University Kingston, Ontario Canada.

Annually, new graduate nurses enter the workforce; each transitioning from the role of student to Registered Nurse (RN). These nurses have described feelings of stress and anxiety about "absolutely everything". To identify and address factors that facilitate and enhance successful transitions into the workforce, it is vital to understand the perceptions of these new graduates. While researchers have examined these experiences, the methodological limitations have left gaps where further analysis is required. The aim was to describe new graduate nurses' perceptions of their hospital orientation and subsequent transition, in their first two years of practice after graduating from the undergraduate nursing program at Queen's University. A qualitative methodology was utilized. Semi-structured, online interviews were conducted with eight RNs that graduated from Queen's University's nursing program and completed a hospital orientation within the past two years. Data analysis is ongoing. The findings will highlight the perceptions of new graduate nurses regarding: orientation programs, emotions, and implications of their transition into current practice. The results will aid in identifying and addressing some of the shortcomings experienced by new graduate nurses from Queen's University. This research may have significant implications for both nursing education and practice. These findings could impact the undergraduate nursing curriculum to better prepare nursing students for their transitions into practice and could also help to develop more encompassing orientation programs in Canadian hospitals.

## Protein Structure and Function

**61. UNDERSTANDING THE UNIQUE MECHANISM OF MICROTUBULE LENGTH CONTROL BY KINESIN-8.** Byron Hunter, Matthieu Benoit, Ana Asenjo, Caitlin Doubleday, Daria Trofimova, Hernando Sosa, and John Allingham. Department of Biomedical and Molecular Sciences, Queen's University, Kingston, Ontario, Canada Department of Physiology and Biophysics, Albert Einstein College of Medicine, Bronx, New York, USA

Chromosome segregation hinges on proper assembly of the mitotic spindle that establishes bipolar attachment to each chromosome. In order to position segregated chromosomes at the centres of nascent daughter cells, the size of the spindle must be adjusted in relation to the changing cell dimensions. Kinesin-8 motor proteins regulate spindle size by 'walking' to the ends of microtubule filaments within the spindle, where they catalyze removal of tubulin subunits. A single region within the protein, known as the motor domain, appears to be responsible for both its microtubule walking and depolymerization activities, but the molecular mechanism allowing its dual functionality remains unclear. By determining the structure of the *Candida albicans* kinesin-8 (*Ca*Kip3) motor domain when bound and unbound to microtubules, we were able to observe a conformational change in a kinesin-8-specific structural subdomain that contacts the microtubule lattice. The effects of mutagenesis of this region suggest that it modulates the catalytic activity of the motor in accord with the shape of tubulin polymer it binds. We propose that on the straight tubulin lattice of a microtubule, this subdomain acquires a conformation that stimulates kinesin walking. Once the motor reaches the curved tubulin polymers found at microtubule ends, its conformation changes in a way that triggers microtubule depolymerization. These findings provide new mechanistic insights into a major regulatory factor of mitotic spindle positioning and scaling. (Supported by the Natural Sciences and Engineering Research Council of Canada)

**62. ENGINEERING A MULTI-FUNCTIONAL CAZYME COMPLEX WITH ENHANCED AGAROSE-DEGRADING PROPERTIES.** Keegan B. Turner-Wood, Julie Grondin, Benjamin Pluvinage, Alisdair B. Boraston, Holly L. Spencer, and Steve Smith**.** Department of Biomedical and Molecular Sciences, Queen's University Kingston, Ontario Canada. (Supported by NSERC)

Marine polysaccharides are an abundant source of chemical energy, representing a functionally limitless reservoir of renewable carbon. Accessing the energy trapped within these highly ordered crystalline structures is immensely difficult. The alternating α and β bonds that link together the sugar subunits each require a different mode of action to be cleaved. Many marine bacteria have evolved an array of specialized carbohydrate-active enzymes (CAZymes) able to specifically digest these recalcitrant structures. One example of an agarose digesting system is the four agarose-specific glycoside hydrolases (GH2, GH16, GH86, and GH117) expressed by *Bacteroides uniformis* to fully deconstruct the polysaccharide agarose. To optimize the synergistic hydrolytic activities of CAZymes targeting terrestrial polysaccharides a subset of bacteria incorporates their suite of enzymes on a single scaffold, a complex referred to as the cellulosome. To maximum the synergistic agarose-degrading activities of the four *B. uniformis* enzymes we have used cellulosome modules and recombinant protein engineering towards generated an agarose-degrading, multi-enzyme complex. Kinetic data gathered from these studies will shed light on the increased processivity associated with integrating enzymes into multi-enzyme complexes. A comprehensive understanding of molecular scaffolds and their associated enzyme complexes will also inform the generation of future biological nanomachines.

## Reproduction and Sexual Function

**63. EFFECT OF CARBON MONOXIDE ON VASCULAR ADAPTATIONS DURING PREGNANCY.** Megan A Dickson[1], Nichole Peterson[1], Karalyn E McRae[1], Jessica Pudwell[2], Chandrakant Tayade[1], Graeme N Smith[1,2]. [1]Department of Biomedical and Molecular Sciences, Kingston, ON, Canada; [2]Department of Obstetrics and Gynaecology, Kingston Health Sciences Centre, Kingston, ON, Canada

Introduction: Preeclampsia (PE) is characterized by abnormal placentation. The use of carbon monoxide (CO) as a therapeutic for PE is under investigation due to its hypothesized role of improving placental vascular function, however there is limited evidence of CO's impact on pregnancy specific adaptations. Methods: Female CD-1 mice were administered 250 ppm CO, or ambient air from gestational day (GD) 0.5 until sacrifice at GD10.5 or GD16.5 (n=5/treatment/time-point). A qRT-PCR array was used to determine expression of angiogenic/inflammatory related genes at GD10.5 and GD16.5 implantation sites. Multiplex cytokine assays were used to measure maternal plasma cytokine levels (GD0.5, GD5.5, GD10.5, GD16.5), and implantation site cytokine levels (GD10.5). Implantation sites (GD10.5, GD16.5) were stained for Ki67 (cellular proliferation), cytokeratin (trophoblast invasion) and DBA lectin (uNK cell abundance). The ΔΔCt method, ANOVA and Mann-Whitney U tests were used for analysis. Results: CO potentiated angiogenic pathways at the murine implantation sites on GD10.5, without altering the local or systemic cytokine profiles associated with pregnancy. Additionally, CO did not impact parameters of placental health including cellular proliferation, trophoblast invasion, or uNK cell abundance. Conclusions: Collectively, our studies provide the basis that that low dose CO appears to be safe during murine pregnancy. Future studies are required to validate safety and efficacy of CO as a potential therapeutic for vascular insufficiency diseases such as PE and IUGR. (Supported by CIHR/OGS)

**64. INVESTIGATION OF THE NEGATIVE IMPACT ON FEMALE SEXUAL FUNCTION AS A RESULT OF MIS-URETHRAL SLING AND LOOP ELECTROSURGICAL EXCISION PROCEDURES: INNERVATION STUDIES.** O Giovannetti, M Monaghan, MA Adams

Whether surgical procedures targeting abnormalities in female urogenital tissue and function can impact female sexual function (FSF) has not been sufficiently established. Implantation of a mid-urethral sling (MUS) to manage stress urinary incontinence, and the LEEP, to excise abnormal cervical cells, have been reported to negatively impact FSF. The function and role of the female periurethral space and the cervix in FSF has not been fully elucidated. Thus, our research seeks to characterize the morphological features of the periurethral tissue from fresh female cadavers and cervical tissue obtained from the hospital tissue repository. Immunohistochemical assessments were performed to determine the location and type of innervation, glandular structures and vasculature. Positive staining indicated the presence of sensory, sympathetic, and parasympathetic innervation. The qualitative analysis of serial tissue sections demonstrated colocalization and multiple innervation of blood vessels and large nerve bundles in the regions of surgical excision. Periurethral and cervical tissue in women contain richly innervated glandular, nervous and vascular elements that may include sensory nerves and have important functions in female sexual responses. Disruption of prostatic glandular tissue and innervation may be a root cause of the effects of MUS surgery and of LEEP on female sexual function. Department of Biomedical and Molecular Science, Queen's University.

**65. THE ORIGIN AND CHARACTERIZATION OF SURFACE-BORNE GLUTATHIONE-S-TRANSFERASE OMEGA 2 WITHIN MOUSE AND BOAR CAPACITATION.** Lauren E. Hamilton[1], Wei Xu[1], Michal Zigo[2], Jiude Mao[2], Peter Sutovsky[2,3], and Richard Oko[1]**.** [1]Department of Biomedical and Molecular Sciences, Queen's University, Kingston, Ontario, CA**.** [2]Division of Animal Sciences, College of Agriculture, Food and Natural Resources, University of Missouri, Colombia, Missouri, USA**.** [3]Department of Obstetrics, Gynecology and Women's Health, School of Medicine, University of Missouri, Colombia, Missouri, USA

In our pursuit to characterize the newly identified perinuclear theca resident, glutathione-s-transferase omega 2 (GSTO2) within mammalian spermatozoa, our findings revealed a secondary localization of the enzyme on the surface of the sperm plasmalemma. This novel localization of surface-borne GSTO2 led our group to investigate its origins and possible role during sperm capacitation, the morphological and physiological priming of mammalian spermatozoa that enables them to acquire the ability to fertilize the oocyte. Through surface protein biotinylation and indirect immunofluorescence, GSTO2 was identified on the plasmalemma of both mouse and boar spermatozoa. The immunohistochemical localization of GSTO2 in the epididymal epithelium suggests GSTO2 may be secreted into the epididymal lumen and transferred onto the plasma membrane. Functional inhibition studies demonstrate a dampening of tyrosine phosphorylation during in vitro capacitation and a significant decrease in the ability of spermatozoa to undergo the induced acrosome exocytosis reaction when the active site of GSTO2 is inhibited. Furthermore, a decrease in progressive motility during capacitation and a decrease in fertilization ability of GSTO-impaired spermatozoa during in vitro fertilization lends further support to a possible role for GSTO2 during the capacitation process. Overall, whilst the specific role of GSTO2 within capacitation is not fully understood, our findings suggest that it may participate in a regulatory role as mammalian spermatozoa acquire the ability to fertilize the oocyte. (This work was supported by the Canadian Institute of Health Research (84440) and the Natural Science and Engineering Research Council of Canada (RGPIN/05305) to RO, along with the Agriculture and Food Research Initiative Competitive Grant no. 2015-67015-23231 from the USDA National Institute of Food and Agriculture and seed funding from the Food for the 21st Century Program of the University of Missouri to PS.)

**66. ESTABLISHING AN EFFECTIVE PROTOCOL FOR THE SELECTIVE EXTRACTION OF NON-NUCLEAR CORE HISTONES FROM THE MOUSE PT.** Morgan Lion[1], Lauren Hamilton[1], Nicole Protopapas[1], Wei Xu[1], and Richard Oko[1]. [1]Department of Biomedical and Molecular Sciences, Queen's University, Kingston, ON, Canada

The perinuclear theca (PT) is a cytoskeletal structure surrounding the sperm nucleus that houses various proteins involved in fertilization. Utilizing Western Blots and immunohistochemistry, we have recently localized core histones within the mouse PT, predominantly to the post-acrosomal sheath and murid-specific perforatorium. Our main goal is to functionally characterize these proteins by using histone-depleted sperm for Intracytoplasmic Sperm Injection (ICSI) of mouse oocytes. Due to their non-nuclear localization, we hypothesize that PT-associated core histones stabilize male chromatin following decondensation of the sperm nucleus inside the ooplasm. To test this, it is necessary to establish a protocol to selectively extract core histones from the mouse PT. Thus, we compared the extractability and selectivity of three solubilizing agents: SDS, acid (HCl), and high salt (KCl). SDS-PAGE analysis of mouse sperm from all treatments revealed four bands which, by immunoblotting with anti-core histone antibodies, corresponded to calf thymus core histones (H3, H2B, H2A, H4) run in adjacent lanes. In all treatments, histone bands were found primarily in the supernatant with negligible banding remaining in the pellet. However, both SDS- and acid-treated sperm displayed substantial removal of PT constituent, PAWP, indicating non-selective extraction. Furthermore, HCl treatment resulted in altered head morphology and significantly reduced oocyte activation following ICSI. Conversely, KCl treatment appeared to be the most selective method of extraction and will be employed in upcoming functional fertilization assays. This work was supported by NSERC (RGPIN/192093).

**67. LOCALIZATION OF PLCZ1: A CANDIDATE SPERM-BORNE OOCYTE ACTIVATING FACTOR.** Ruben Warkentin, Nicole Protopapas, Lauren E. Hamilton, Wei Xu, Richard Oko. Department of Biomedical and Molecular Sciences, Queen's University, Kingston, Ontario, CA

The sperm-borne oocyte activating factor (SOAF) is a protein that causes the resumption of meiosis in oocyte, upon sperm-oocyte fusion. As a suspected resident of the perinuclear theca, the SOAF is proposed to reside within the post-acrosomal sheath – the first region to solubilize within the oocyte cytoplasm. There are currently two SOAF candidates, PLCZ1 and PAWP. However, the PLCZ1 mouse knockout model is currently the only SOAF candidate knockout to show a significant reduction in oocyte activation. PLCZ1 is a testis specific protein that has been shown to elicit intracellular calcium release, a requirement for oocyte activation. Despite that, the localization of PLCZ1 in spermatozoa is disputed and inconsistent in the literature. Moreover, PLCZ1 extractability is inconsistent between mammalian species, specifically between mouse and human spermatozoa. One explanation for the different localization patterns of PLCZ1 in the literature may be due to the presence of various PLCZ1 isoforms. The various isoforms may localize to different areas within spermatozoa, yet most antibodies used in the literature are specific to a sequence common to all antibodies. We aimed to address the various localization patterns found in the literature, by using antibodies that are designed to discriminate between PLCZ1 isoforms. Additionally, we aimed to compare the localization of PLCZ1 between mouse and human spermatozoa. (This work was supported by the Canadian Institute of Health Research (84440) and the Natural Science and Engineering Research Council of Canada (RGPIN/05305) to RO)

**68. HUMAN OVGP1 ENHANCES TYROSINE PHOSPHORYLATION OF PROTEINS IN THE FIBROUS SHEATH INVOLVING AKAP3 AND INCREASES SPERM-ZONA BINDING.** Yuewen Zhao and Frederick W. K. Kan. Department of Biomedical and Molecular Sciences, Queen's University, Kingston, Ontario, Canada.

**Purpose:** Oviduct-specific glycoprotein 1 (OVGP1) plays an important role in early events of mammalian fertilization. Recombinant human OVGP1 (rHuOVGP1), produced in our laboratory, has been shown to enhance tyrosine-phosphorylation of sperm proteins during capacitation and potentiate acrosome reaction. Our aims were to investigate if rHuOVGP1-enhanced tyrosine-phosphorylated (pY) proteins are components of specific structure(s) of human sperm and if rHuOVGP1 binds to oocytes and enhances sperm-egg binding. **Methods:** Immunofluorescence and confocal microscopy were utilized to examine localization of pY proteins, outer dense fiber (ODF), and A-Kinase Associated Protein 3 (AKAP3) in human sperm during capacitation. Western blot analysis and immunoprecipitation were employed to analyze protein levels of pY proteins and AKAP3. Immunofluorescence was performed to examine binding of rHuOVGP1 to human oocytes. The effect of rHuOVGP1 on sperm-zona binding was examined using hemi-zona assay. **Results:** pY proteins were detected mainly in the fibrous sheath (FS) surrounding the ODF with a weak immunoreaction in the neck and mid-piece. Western blot analysis revealed co-migration of the pY 105 kDa protein with AKAP3, which was confirmed by immunoprecipitation correlating immunofluorescent results of co-localization of pY proteins with AKAP3. rHuOVGP1 was found to bind specifically to the zona pellucida (ZP) of human oocytes. Prior incubation of sperm and/or ZP with rHuOVGP1 increased sperm-egg binding. **Conclusions:** 1) One of the major rHuOVGP1-enhanced pY proteins could be AKAP3 of the FS. 2) rHuOVGP1 is capable of binding to the ZP of human oocytes. 3) The presence of rHuOVGP1 in the medium enhances sperm-zona binding. 4) Supplement of rHuOVGP1 in *in vitro* fertilization media could be beneficial for enhancement of the fertilizing ability of human sperm.

## Therapeutics and Toxicology

**69. DNA DAMAGE AND PERTURBED TOPOISOMERASE IIA AS A TARGET OF 1,4-BENZOQUINONE TOXICITY IN MURINE FETAL LIVER CELLS.** Trent H. Holmes[1] and Louise M. Winn[1]. [1]Department of Biomedical and Molecular Sciences, Queen's University Kingston, Ontario Canada

Recent studies suggest that maternal exposure to benzene during fetal development may lead to hepatic and hematopoietic toxicity in offspring. While the extent of fetal benzene toxicity is poorly understood, benzene is known to affect the critical DNA repair enzyme topoisomerase IIα (Topo2α), which is known to be associated with childhood leukemias. Here we describe the first study to examine the effects of the benzene metabolite benzoquinone (BQ) on the fetal version of Topo2α in cultured fetal liver cells taken from gestational day 14 CD-1 mice. Cultured fetal liver cells were exposed to BQ at a concentration of 12.5 µM, as this was shown to be non-cytotoxic. Fetal Topo2α activity was measured at 2, 12, and 24 hours following BQ exposures causing significantly decreased Topo2α activity at 24 hours. DNA-Topo2α covalent complexes detected at 24 hours following 12.5 µM exposure and 30 minutes exposure following 50 µM exposure indicated that BQ is a Topo2α poison in cultured fetal murine liver cells. Additionally, double-stranded DNA breaks, measured by levels of γH2AX, were significantly higher after 24 hours of 12.5 µM exposure, implying Topo2α is involved in DNA damage following BQ exposure. Lastly, 4-hydroxynonenal (4-HNE), a marker of oxidative stress, was not significantly increased in fetal liver cells following BQ exposure, showing no association between increased ROS and Topo2α inhibition, a continuously contested hypothesis. Further *in vivo* complementary experiments will confirm and characterize the role of fetal Topo2α in transplacental benzene toxicity. Support: CIHR

70. **CHARACTERIZATION OF THE CONTRIBUTION OF THE NF-KB-MEDIATED SIGNALING PATHWAY ON THE TERATOGENICITY OF VPA FOLLOWING IN VIVO EXPOSURE IN CD-1 MOUSE EMBRYOS.** Sidra Shafique1, Louise M. Winn1,2. Department of Biomedical and Molecular Sciences, Queen's University, Kingston1 School of Environmental Studies, Queen's University, Kingston2

Introduction: Valproic acid (VPA), a widely prescribed anti-epileptic drug and an effective treatment for bipolar disorder and neuropathic pain, results in developmental defects including neural tube defects and craniofacial anomalies. NF-kB, the heterodimer of subunits p65 and p50, regulates transcription and acts as an anti-apoptotic factor. NF-kB is phosphorylated by Pim1 into its active form and acts along with Stat3 to function as transcription factor. This study investigated the effects of in utero VPA exposure on NF-kB (p65 and p50 subunits), Pim1 and Stat3 mRNA and protein expression, during the critical period of neural tube closure (i.e. GD9) in CD-1 mouse embryos. Methods: Pregnant CD-1 mice were exposed to 400 mg/kg VPA or saline on gestational day (GD) 9 via subcutaneous injection. Embryos were harvested at 0, 1, 3, 6, 24 hours and GD13 following exposure. mRNA expression of NF-kB (p65, p105 subunits), Pim1 and Stat3 was measured by RT-qPCR. Analysis was done in whole embryos at GD9, control, open and closed neural tube (NT) at GD10 and heads of GD13 control, non-affected and exencephalic fetuses. Results: mRNA expression of p65, p105 and Pim1 was significantly ($p<0.05$) downregulated at the 3hrs time point following VPA exposure in GD9 embryos. No significant change was observed in any other group or in Stat3 mRNA levels. Protein analysis is ongoing on for the corresponding time points and tissues by Western Blotting. Conclusion: The data informs on the direct effect of VPA on the role of NF-kB (p65 and p50 subunits) and Pim1 on the developing mouse embryos. The rapid downregulation of p65 and p105 following the shortest exposure at the 3 hrs. time point indicates the importance of these transcription factors in the induction of apoptosis and failure of neural tube closure during mouse embryo neurulation.

71. **EXAMINATION OF DNA DOUBLE-STRAND BREAKS AND OXIDATIVE DNA DAMAGE FOLLOWING TREATMENT OF MICE WITH THE NUTRACEUTICAL SULFORAPHANE**. Kristen Zamperoni and Thomas E. Massey. Department of Biomedical and Molecular Sciences, Queen's University, Kingston, Ontario Canada.

Previously, we observed that *in vivo* treatment of mice with the nutraceutical isothiocyanate sulforaphane (SFN) increases nucleotide excision repair (NER) activity in the liver. However, the mechanism by which SFN upregulates NER is unknown. Despite exhibiting many beneficial effects, it has been suggested that SFN itself may be genotoxic and therefore, upregulation of NER following SFN administration could potentially be a homeostatic response to SFN-induced DNA damage. The current study examined whether *in vivo* SFN administration leads to DNA damage by assessing biomarkers of double-strand breaks (DSBs) and oxidative DNA damage. CD1 mice were treated with SFN (100mg/kg) or corn oil (0.2mL) via oral gavage. Organs were harvested 12- or 3-hours post-treatment and histone proteins and DNA were extracted. As an indicator of DSBs, levels of $\gamma$-H2A.X in histone extracts were assessed by immunoblotting. For oxidative DNA damage, levels of 8-OHdG in DNA were determined by colourimetric ELISA. SFN treatment did not significantly change $\gamma$-H2A.X or 8-OHdG levels in the liver, kidney, or brain compared to control at the timepoints investigated ($P>0.05$). However, a trend towards an increase in $\gamma$-H2A.X in the liver and a trend towards a decrease in 8-OHdG in the kidney at 12 hours were observed. Though findings of this study suggest SFN does not cause significant DNA damage, it is possible that damage may be observed at other timepoints. (Supported by the Natural Sciences and Engineering Research Council of Canada).

**72. GENOME-WIDE ASSOCIATION STUDY OF HUMAN MILK OLIGOSACCHARIDES AMONG LACTATING MOTHERS IN CANADIAN LONGITUDINAL BIRTH COHORT.** Amirthagowri Ambalavanan[1], Le Chang[1,2], Jihoon Choi[1], Amel Lamri[3], Bianca Robertson[4], Chloe Yonemitsu[4], Stuart E. Turvey[5,6], Piushkumar J. Mandhane[7], Allan B. Becker[8,9], Theo J. Moraes[10], Sonia S. Anand[11], Guillaume Paré[12], Diana L. Lefebvre[11], Malcolm R. Sears[11], Padmaja Subbarao[10], Lars Bode[4], Meghan B. Azad[8,9], Qingling Duan[1,2]

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Human milk oligosaccharides (HMOs) are complex carbohydrates found uniquely in human breastmilk, and are absent in most of the infant supplementary formulas. HMOs shape the growth of infant gut microbiota and contribute to immune system development. While specific genetic variants in fucosyltransferase (*FUT*) genes are known to determine maternal secretor status, it remains to be determined if other genetic factors modulate HMO concentrations in breastmilk. In our current study, we ascertained the genomics data (Illumina HumanCore Exome BeadChip) involving a subgroup of 980 Canadian mothers from the largest birth cohort (N=3455) in Canada known as the Canadian Healthy Infant Longitudinal Development (CHILD) study. Breastmilk samples were collected 3–4 months postpartum. A total of 19 HMOs were analyzed by high-performance liquid chromatography-mass spectrometry (HPLC–MS); secretor status was determined from the presence of 2'-fucosyllactose. We performed a genome-wide association study (GWAS) of maternal secretor status and of each HMO concentration, which included 5.8 million imputed variants (minor allele frequency > 0.05). We identified significant associations for multiple loci in chromosome 19 (encoding *FUT2*) with 16 HMO concentrations and maternal secretor status. Additionally, variants on chromosome 3 (downstream of *ST6GAL1*) were correlated with HMOs 6'-sialyllactose and disialyllacto-*N*-hexaose ($P = 1.51 \times 10^{-8}$). Our results suggest that genetic factors modulate the production of HMOs in breastmilk of lactating mothers. We speculate that exposure to specific HMOs protects against respiratory outcomes among infants.

**73. THE EFFECTS OF *IN UTERO* BENZENE EXPOSURE ON FETAL NF-κB CELL SIGNALLING IN CD-1 MICE.**

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*In utero* exposure to benzene, a known carcinogenic environmental toxicant, is associated with the development of childhood leukemia. We have previously demonstrated that *in utero* exposure to benzene in CD-1 mice can alter the expression of the transcription factor NF-κB, which regulates genes involved in cell proliferation and programmed cell death, however, a full characterization of benzene's effects on fetal NF-κB is still needed. Since NF-κB regulates genes involved in cell proliferation, inappropriate activation can result in the proliferation of damaged cells potentially leading to the development of leukemia. We hypothesize that benzene metabolism in the maternal liver leads to fetal changes in NF-κB signalling. To test this hypothesis, pregnant CD-1 mice were exposed to 200 mg/kg benzene or its vehicle control on gestational days 8, 10, 12, and 14. Dams were sacrificed at 2, 6, and 24 hours after the last benzene dose. Fetal livers were collected, and immunoblotting was done to assess changes in protein levels of phospho-p65, phospho-p38-MAPK, and inhibitor of NF-κB (IκB-α). Results show that after *in utero* benzene exposure, protein levels of phospho-p65 and phospho-p38-MAPK did not change significantly after benzene treatment but protein levels of IκB-α significantly increased 6 hours after the last benzene dose. However, there were no observed changes in the mRNA level of IκB-α. Future steps will assess the DNA binding activity of NF-κB as well as the protein levels of p50, the NF-κB subunit containing the DNA binding domain.Canadian Institutes of Health Research (CIHR)

**74. EXPLORING CHANGES IN THE FATHERS' FAMILIAL ROLE AND ITS ASSOCIATION WITH CHILD OUTCOMES IN MONGOLIA.** Lesley A. Pablo and Colleen Davison. Department of Public Health Sciences, Queen's University Kingston, Ontario Canada.

Over the last two decades, Mongolia experienced dramatic socioeconomic and climate changes that impacted nomadic livelihoods, gender roles, and family dynamics. There are concerns over the effects a decline in pastoral lifestyles, and the changing roles of fathers in some families, might be having on child outcomes. While some gender-focused research exists, there has been limited exploration of the child health and education effects of the changing roles of men in Mongolia. This cross-sectional study uses data for children aged 3-4 from UNICEF's Multiple Indicator Cluster Surveys conducted between 2000-2013. The father's role was measured using two indicators: father's household presence and father engagement with their children in six different activities (e.g. reading with their child or taking them outside, etc.). Child outcome measures include child illness and child pre-school attendance. Trend analysis was performed to explore fluctuations in father presence and engagement between 2000-2013. Multivariate regression modeling was employed to identify associations between father engagement and child outcomes. Father presence ranged from 78-83% ($P_{Trend}$ <.0001) and engagement from 40-49% ($P_{Trend}$=0.3299) between 2000-2013. Fluctuations appeared to coincide with major climate events and varied by region and urban/rural residence. In unadjusted analyses, father engagement was associated with pre-school attendance ($OR_{crude}$=1.12; 95% CI 1.04-1.20) but not with child illness ($OR_{crude}$=1.04; 95% CI 0.95-1.14). Father engagement was no longer associated with pre-school attendance after controlling for covariates ($OR_{adj}$ = 0.98; 95% CI 0.90-1.06). Results suggest that climate and socio-economic change has affected father's presence and engagement in many households, but in the 2013 data, suggests that father presence and engagement was not associated with child illness or child pre-school attendance outcomes.

**75. CHARACTERIZATION OF NEUTROPHIL INVOLVEMENT IN ENDOMETRIOSIS PATHOPHYSIOLOGY.** Lindsey K Symons[1], Jessica E Miller[1], Bruce A Lessey[2], Steven L Young[3] & Chandrakant Tayade[1] [1]Department of Biomedical and Molecular Sciences, Queen's University, Kingston, CAN [2]Department of Obstetrics and Gynecology, Greenville Health System, Greenville, USA [3]Department of Obstetrics and Gynecology, University of North Carolina, Chapel Hill, USA

Endometriosis is a chronic-inflammatory disease characterized by endometrial-like tissue lesions outside of the uterus. Given that neutrophils are elevated in endometriosis patients as well as the emerging role for these innate immune cells in chronic and sterile inflammation, we sought to characterize the role of neutrophils in endometriosis pathophysiology. Through immunohistochemical analysis, myeloperoxidase$^+$ neutrophils were found to significantly infiltrate patient endometriomas (n=12) compared to control endometrium (n=15). Patient lesions (n=5) also expressed significantly elevated levels of IL-8, a potent neutrophil chemoattractant, compared to control endometrium (n=6). To characterize neutrophil involvement *in vivo*, endometriosis was induced in female C57BL/6 mice (n=5) and mice were euthanized on days 1, 2, 3, 7, and 28 after surgery (five independent experiments). Flow cytometric analysis revealed that neutrophils ($CD11b^+Ly6G^+Ly6C^+$) significantly infiltrated the peritoneal environment 24-72 hours after induction of endometriosis compared to sham-operated controls. Neutrophil recruitment was associated with elevated local and systemic chemokines (G-CSF, CXCL1, CXCL2), inflammatory cytokines (IL-6, TNF-$\alpha$, IL-4) and pro-angiogenic factors (VEGF-A), as determined by multiplex cytokine analysis. Myeloperoxidase$^+$ neutrophils also significantly infiltrated murine lesions and remained present long term. Taken together, these data demonstrate that both patient and murine endometriotic lesions possess a microenvironment that may support neutrophil recruitment. Further *in vivo* neutrophil depletion studies are in progress to establish a cause-and-effect relationship between neutrophil recruitment, inflammation, angiogenesis, and lesion development. (Supported by the Canadian Institutes of Health Research)

**76. PLACENTAL MORPHOLOGY AND THE PREDICTION OF UNDERLYING CARDIOVASCULAR RISK FACTORS**. Aida Zaza1, Jessica Pudwell2, Shannon Bainbridge3, Kristin Connor4, Graeme N Smith1, 2 1Department of Biomedical and Molecular Sciences, Queen's University, 2Department of Obstetrics and Gynaecology, Queen's University, 3Department of Cellular and Molecular Medicine, University of Ottawa, 4Department of Health Sciences, Carleton University

Introduction: Pre-eclampsia (PE) is a hypertensive disorder that complicates 3-5% of pregnancies and is the leading cause of maternal and fetal morbidity worldwide. The purpose of this study is to investigate the ability of placental morphology to predict the presence of maternal cardiovascular risk (CVR) at six months postpartum in women who experienced PE. Methods/Results: A chart and biometric review was used to collect placental measurements, PE-related blood work, blood pressure measurements, and CVR information. Multivariate logistic regression was used to examine the relationship between placental measures and high lifetime risk of cardiovascular disease at six months postpartum. 186 women with PE who attended the Maternal Health Clinic met inclusion criteria. Mean placental weight was 503.2 ± 187.3g. Mean ratio of placental to birth weight was 18.2 ± 4.4%. In multivariate modelling that controlled for maternal age, increased pre-pregnancy BMI (OR=1.09, $p<0.01$), HELLP syndrome (OR=4.89, $p<0.01$), placental to birth weight ratio of 15.0-19.9% (OR=3.05, $p<0.05$) and <15.0% (OR=5.09, $p<0.01$) were associated with increased odds of high lifetime risk. Greater gestational age (OR=0.79, $p<0.001$) was associated with decreased odds of high lifetime risk. Conclusion: Preliminary data suggests an association between the ratio of placental to birth weight and CVR at six months postpartum. Placental measurements may be used to identify those who are likely to be at greatest risk and who would most benefit from risk screening preventive interventions. (Supported by CIHR).