



Ottawa Hospital  
**Research Institute**  
**Institut de recherche**  
de l'Hôpital d'Ottawa

# 2014 RESEARCH DAY



## Program and Abstracts

Thursday, November 13, 2014  
7:30 a.m. – 5:05 p.m.

St. Elias Centre  
750 Ridgewood Ave.  
Ottawa, ON

# Sponsors

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## DRS Construction



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## Research Day Committee

The Ottawa Hospital Research Institute would like to express its appreciation to members of the Research Day Committee for their dedication and hard work in organizing this event, and to the volunteers, whose assistance we could not do without.

Dr. Fraser Scott (Chair)  
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Dr. Anouk Fortin  
Ms. Alessandra Pasut  
Dr. Bill Stanford

Dr. Jay Baltz  
Dr. Jim Dimitroulakos  
Dr. Ian Lorimer  
Dr. Tim Ramsay  
Dr. Duncan Stewart

Jane Canniff  
Dr. Dean Fergusson  
Paddy Moore  
Dr. Luc Sabourin  
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## Volunteers

Greg Canham

Melanie Genereaux

Wayne Lowe

## WELCOME TO RESEARCH DAY

I look forward to this event every year.

Here we celebrate and showcase the outstanding work of our young researchers. Their insight, passion and commitment to our pursuit of scientific excellence are all critical to our success as one of Canada's top research hospitals — continuing to rank 3rd in terms of CIHR funding and in the top five for total research revenues.

This day is meant to foster and encourage interaction in a collegial environment for our trainees – to give them experience communicating their ideas clearly and effectively. Even if you are not involved in the judging of posters or oral presentations, I hope you will ask questions of our trainees. As well as providing this learning opportunity, today is a great chance for us all to learn about the exciting research projects taking place across the Ottawa Hospital Research Institute.

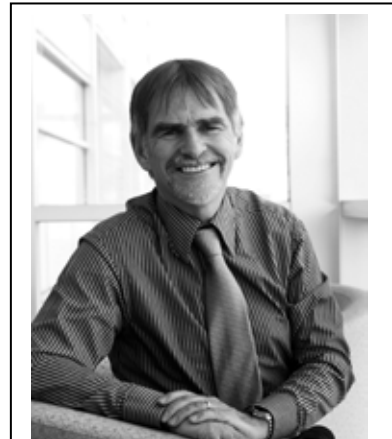
We are an organization with a broad scope of research endeavours, ranging from the lab to the clinic. To reflect this, today we are presenting two keynote lectures.

Dr. Robyn Tamblyn is internationally recognized for work on prescription drug use, its determinants and computerized interventions to improve drug safety. A leader in her field, she is studying the use of e-health technologies to support integrated care for chronic disease and is working to create technologies to monitor adverse events in populations. The title of her talk today is "The Use of Health Information Technologies: New opportunities for research and improvement in the safety and effectiveness of prescription drugs."

Dr. Daniel Drucker is a world-renowned pioneer in translational research and his groundbreaking science has led to the development of two new classes of therapies in the treatment of Type 2 diabetes, as well as a new therapy for patients with short bowel syndrome requiring parenteral nutrition, which are revolutionizing the treatment of these diseases. Today he will provide an overview of the effect of gut hormone receptors on the cardiovascular system and how they exert pharmacological actions on blood vessels and cardiomyocytes. His talk is titled "Gut Hormones Talk to the Heart: Mechanisms and clinical implications."

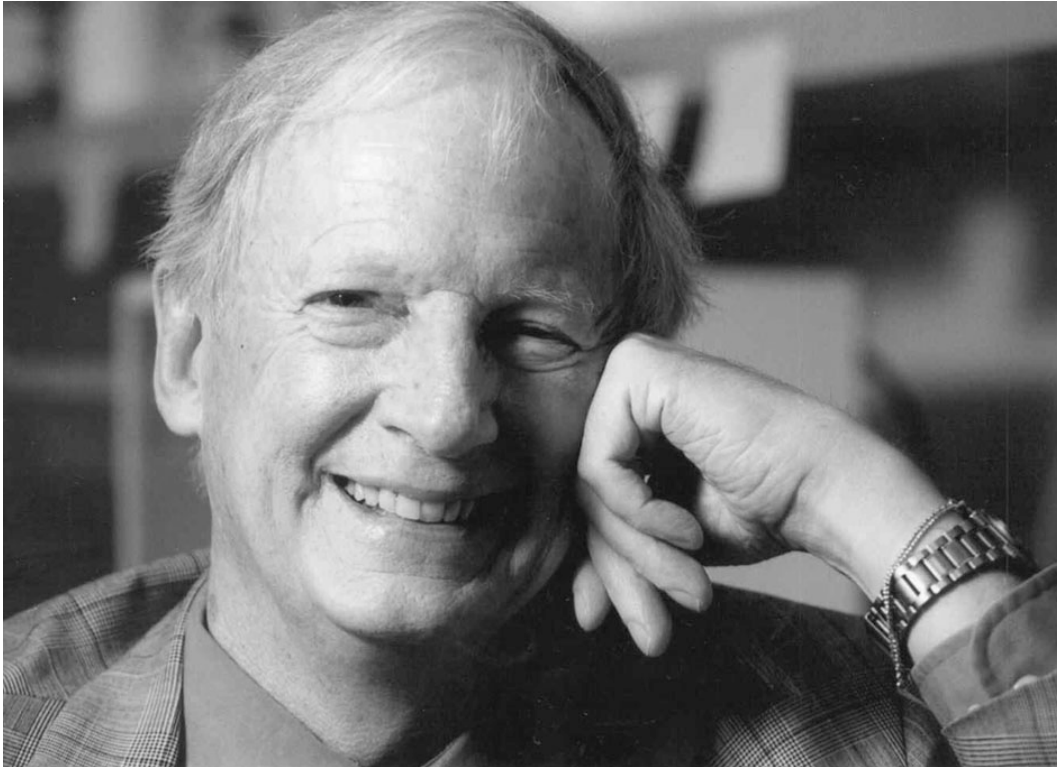
I would also like to draw your attention to the 4th Annual OHRI IMPACT Award. Standing for "Identification of Marketable Products, Applications and Commercializable Technologies," the IMPACT Award is designed to encourage our researchers to consider how their work could lead to innovations and to identify technologies, products or services that stem from their work. The IMPACT Award is part of our larger effort to create a culture that is proactive in translating research into benefits for Canadians. You will find the finalists' posters in the lobby.

On behalf of everyone at OHRI, I would like to thank all those involved in making this day happen, from our guest speakers to our presenters, judges, planning committee and volunteers. I would also like to thank the sponsors for helping to make today's event possible and encourage you to visit their booths.



Duncan Stewart, MD, FRCP  
 CEO & Scientific Director, Senior  
 Scientist in the Regenerative  
 Medicine Program,  
 Ottawa Hospital Research Institute  
 Vice-President, Research, The  
 Ottawa Hospital  
 Evelyne and Rowell Laisley Chair  
 Professor, Department of Medicine,  
 Faculty of Medicine, University of  
 Ottawa

## DR. J. DAVID GRIMES LECTURE



Dr. J. David Grimes, MD, FRSPC

This annual lecture is named in honour of Dr. J. David Grimes, founder of the Loeb Research Institute, which was the predecessor of the Ottawa Hospital Research Institute at the Civic Campus.

Dr. Grimes served as the institute's CEO and Scientific Director until he retired in 1997. He recruited and mentored many of Ottawa's leading health researchers. He also practiced neurology for more than 25 years, specializing in Parkinson's disease.

After a long and courageous battle with lung disease, Dr. Grimes passed away on May 9, 2001. A man of great vision and compassion, Dr. Grimes is missed by everyone who knew him. This annual lecture is just one of the ways his memory lives on at the Ottawa Hospital Research Institute. He is also remembered through the Dr. J. David Grimes Research Chair at the University of Ottawa and through the Grimes Career Achievement Award, which is awarded annually at The Ottawa Hospital Gala.

## 2014 DR. J. DAVID GRIMES LECTURE

*“Gut Hormones Talk to the Heart: Mechanisms and clinical implications”*

**Dr. Daniel Drucker**



Dr. Drucker received his MD from the University of Toronto in 1980, where he is currently Professor of Medicine. He holds a Canada Research Chair in Regulatory Peptides and the Banting and Best Diabetes Centre-Novo Nordisk Chair in Incretin Biology. His laboratory is based in the Lunenfeld-Tanenbaum Research Institute at Mt. Sinai Hospital in Toronto and studies a family of hormones produced in the pancreas, gastrointestinal tract and brain. These hormones regulate our appetite, the absorption of nutrients from the food we eat and the conversion of those nutrients to energy. His lab is looking for compounds that mimic and enhance the ability of these naturally occurring hormones to regulate these functions.

## 2014 KEYNOTE LECTURE

*“The Use of Health Information Technologies: New opportunities for research and improvement in the safety and effectiveness of prescription drugs”*

**Dr. Robyn Tamblyn**



Dr. Robyn Tamblyn is a Professor at McGill University in the departments of Medicine, and Epidemiology and Biostatistics. She is a James McGill Chair and the Scientific Director of the Clinical and Health Informatics Research Group at McGill University. Dr Tamblyn's ground-breaking research on educational outcomes has elucidated important relationships between health professional training, licensure and practice that have subsequently guided credentialing policies. She has also been awarded the CHSRF KT award for her research in improving the use of medication and the ACFAS Bombardier award for innovation in the development of a computerized drug management system. In January 2011, she became the Scientific Director of CIHR's Institute of Health Services and Policy Research.

## DR. GOODMAN COHEN SUMMER STUDENT AWARDS

Every year, the Ottawa Hospital Research Institute holds the summer student seminar series, which gives students at the institute the opportunity to present their research to other students. This year, 56 students participated from throughout the institute, ranging from high-school students to newly graduated Bachelor's students. Awards are given for the best presentations, based on both peer and coordinator evaluations. The students then submit a written paper and the top students are awarded the Dr. Goodman Cohen Summer Student Award. This year they competed in two categories: Senior (returning students) and Junior (new students).

Dr. Jay Baltz, Associate Scientific Director responsible for Trainees, would like to thank and recognize Drs. Jennifer Collins and Mehdi Shafa for their excellent job running the summer student program this year.

### 2014 Winners of the Dr. Goodman Cohen Summer Student Award

#### *Senior Award*

**David Bellamy** (Dr. John Bell)

"Accelerated natural selection-based screening for amiRNAs that enhance oncolytic virus efficacy in pancreatic cancer models"

#### *Junior Award*

**Kathleen Atkins** (Dr. Bernard Thébaud)

"Resident lung mesenchymal stromal cells are perturbed in experimental neonatal lung disease"

### Dr. Goodman Cohen



The Dr. Goodman Cohen Summer Student Awards are made possible by generous donations made in the memory of Dr. Goodman (Goody) Cohen, one of Ottawa's first and finest cardiologists.

Born in 1922, Dr. Cohen grew up in a tiny rural mining town located between Glace Bay and Sidney in Nova Scotia. The youngest of seven siblings (five boys and two girls), Dr. Cohen was the only one in this family to attend university, starting his post-secondary education at Mount Allison University in Sackville, New Brunswick. He went on to graduate from McGill Medical School in the early 1950s before doing post-graduate work at Harvard and Johns Hopkins universities. He met

his future wife, Rita Lambert, a nurse, while training at Massachusetts General Hospital. They settled in Ottawa where they raised three children.

Dr. Cohen, known as Goody, practiced cardiology for almost 35 years, until 1989 when he was diagnosed with cancer. He died in January 1990. Widely known as a kind and caring physician to thousands of patients over the years, he was also highly respected as a clear and forthright professor of medicine.

## RESEARCH TRAINEE SALARY AWARDS



See more of our trainees' photos on the inside back cover

Gregory Addicks  
Khalid Al-Zahrani  
Marc Thomas Avey  
Kyla Baron (Garbuio)  
Vikie Bedard  
Laura Boland  
Chadwick Boulay  
Steven Bugiel  
Stephanie Burke  
Lauren Carter  
Natasha Chang  
Ketul Chaudhary  
Shazia Hira Chaudhry  
William Chen  
Jennifer Collins  
Cecilia Costiniuk  
Sarah Cummings  
Wilfred Dang  
Marc-Olivier Deguise  
Sarah Dick  
Mary-Anne Doyle  
Nicolas Dumont  
Debra Eagles  
Jason El Bilali  
Mehdi Eshraghi  
Laura Evgin

Hervé Faralli  
Zach Ferraro  
Nicole Forbes  
Vanessa Garcia  
Steven Hawken  
Kendra Hodgkinson  
Crystal Holly  
Danton Ivanochko  
Lisa Marie Julian  
Rebecca Kalinger  
Brian Keller  
Daniel Kobewka  
Samantha Kornfeld  
Melanie Lacaria  
Manoj Lalu  
Luke Lavallée  
Caroline Lefebvre  
Yuefeng Li  
Jung Jin Lim  
Patricia Azevedo Lima  
Dandan Liu  
Christina Ly  
Anisha Lynch-Godrei  
Hannah Mazier  
Taylor McClatchie  
Amber Molnar

Anne-Laure Nivet  
Ryan O'Meara  
Alessandra Pasut  
Charis Putinski  
Nischal Ranganath  
Naomi Read  
Samantha Richard  
Pascale Robineau-Charette  
Paul Ronksley  
Dominic Roy  
Teslin Sandstrom  
Mohammed Selman  
Mehdi Shafa  
Melissa Snyder  
Roger Stanev  
Colin Suen  
Lee-Hwa Tai  
Peter Tanuseputro  
Bruno Trindale  
Anne Tsampalieros  
Hideaki Tsuyoshi  
Nhung (Rose) Vuong  
Yu Xin (Will) Wang  
Carmen Wong  
Yan Xu



## **OHRI RESEARCH DAY PROGRAM**

November 13, 2014, St. Elias Centre, Ottawa

**7:30 AM REGISTRATION / POSTER SETUP / CONTINENTAL BREAKFAST**

**8:15 AM OPENING REMARKS**

Dr. Jack Kitts, Dr. Bernard Jasmin, Dr. Duncan Stewart, Dr. Fraser Scott

**8:30 AM Immunobiology, Metabolism and Neurological Function**

(Talks: 9 minutes plus 3 minutes discussion)

*Moderators: Christopher Patrick and Mehdi Eshraghi*

- **Stephanie Burke** (Angela Crawley Group) Generalized CD8+ T cell impairment in HCV mono- and HIV-HCV co-infection and pronounced impairment in the liver
- **Lynley Pound** (Fraser Scott Group) Cathelicidin antimicrobial peptide: a novel promoter of insulin secretion and islet regeneration
- **Chadwick Boulay** (Adam Sachs Group) Single-trial dorsolateral prefrontal cortex neural trajectories predict intended saccade direction
- **Julianna Tomlinson** (Michael Schlossmacher Group) Whole skull microscopy visualizes alpha-synuclein and tau expression in olfactory epithelium: Implications for testing the pathogenesis of Parkinson's and Alzheimer's

**9:20 AM Patient-oriented Research**

(Talks: 9 minutes plus 3 minutes discussion)

*Moderators: Mathieu Chalifoux and Michael Chasse*

- **Steven Hawken** (Kumanan Wilson Group) Seasonal variation in rates of emergency room visits and acute admissions following routine infant vaccinations in Ontario
- **Jelena Ivanovic** (Andrew Seely Group) Development and implementation of the Thoracic Surgery Quality Monitoring, Information Management, Clinical Documentation System for continuous, point-of-care reporting and evaluation of post-operative adverse events
- **Catalina Hernandez** (Mark Clemons Group) How do breast cancer patients define "optimal control" of chemotherapy induced nausea and vomiting?
- **Peter Tanuseputro** (Douglas Manuel Group) The health care cost of dying: A population-based examination across health care sectors for Ontarians in their last year of life

**10:10 AM REFRESHMENT BREAK (15 minutes)**

**10:25 AM POSTER VIEWING / JUDGING OF POSTERS PRESENTED BY PhD, MSc, 4<sup>th</sup> YEAR HONOURS, CO-OP STUDENTS AND IMPACT AWARD FINALISTS (60 minutes)**

**11:25 AM KEYNOTE LECTURE (35 minutes plus 10 minutes discussion)**

***The Use of Health Information Technologies: New opportunities for research and improvement in the safety and effectiveness of prescription drugs***

**Dr. Robyn Tamblyn**, Professor, Department of Medicine and Department of Epidemiology and Biostatistics, McGill University, Faculty of Medicine; Medical Scientist, McGill University Health Centre Research Institute

*Moderator: Dr. Dean Fergusson*

**12:10 PM BUFFET LUNCH (60 minutes)**



**1:10 PM Cancer: Improving Therapeutic Responses**

(Talks: 9 minutes plus 3 minutes discussion)

*Moderators: Curtis McCloskey and Dominic Roy*

- **Ramya Krishnan** (Jean-Simon Diallo Group) Enhancement of oncolytic viral efficacy by novel drug candidates
- **Laura Evgin** (John Bell Group) Overcoming vaccinia virus neutralization in immune humans and macaques with complement inhibition
- **Bao Kong** (Benjamin Tsang Group) The role of p53 in regulating mitochondrial dynamics and chemoresistance in gynaecological cancer cells
- **Aissa Benyoucef** (Marjorie Brand Group) UTX inhibition as selective epigenetic therapy against TAL1-driven T cell acute lymphoblastic leukemia

**2:00 PM POSTER VIEWING / JUDGING OF POSTERS PRESENTED BY POSTDOCTORAL FELLOWS, CLINICAL FELLOWS, RESEARCH ASSOCIATES, RESIDENTS and MEDICAL STUDENTS (60 minutes)**

**3:00 PM Stem Cell Biology and Regenerative Medicine**

Talks: 9 minutes plus 3 minutes discussion)

*Moderators: Will Wang and Lisa Julian*

- **Natasha Chang** (Michael Rudnicki Group) p38-gamma MAPK regulation of Carm1 promotes satellite stem cell self-renewal via symmetric expansion
- **Jennifer Collins** (Bernard Thébaud Group) In vivo hyperoxia alters the potency and gene expression profile of CD146+ endogenous lung mesenchymal stromal cells
- **Parameswaran Ramachandran** (Theodore Perkins Group) Regression-based multiscale decomposition of the ChIP-Seq signal reveals novel quantifying factors
- **Kulwant Singh** (Jeffrey Dilworth Group) MSK1-mediated phosphorylation of Kap1 is a key determinate of MyoD function during skeletal muscle differentiation

**3:50 PM REFRESHMENT BREAK (15 minutes)**

**4:05 PM DR. J. DAVID GRIMES LECTURE (35 minutes plus 10 minutes discussion)**

***Gut Hormones Talk to the Heart: Mechanisms and clinical implications***

**Dr. Daniel Drucker**, Professor of Medicine, University of Toronto, Senior Scientist, Lunenfeld Tanenbaum Research Institute Mt. Sinai Hospital

*Moderator: Dr. Duncan Stewart*

**4:50 PM POSTER / ORAL PRESENTATION AWARDS AND CLOSING REMARKS**

*Moderators: Drs. Duncan Stewart, Jim Dimitroulakos and Fraser Scott*

**5:05 PM RECEPTION AND CASH BAR**

## ORAL PRESENTATIONS

### Immunobiology, Metabolism and Neurological Function (8:30 to 9:20)

Moderators: Christopher Patrick and Mehdi Eshraghi

#### 1-1 Generalized CD8+ T cell impairment in HCV mono- and HIV-HCV co-infection and pronounced impairment in the liver

Stephanie C. Burke<sup>1,2</sup>, Lorna Carrasco-Medina<sup>2</sup>, Winston Karges<sup>1,2</sup>, Curtis L. Cooper<sup>2,3,4</sup>, Angela M. Crawley<sup>1,2</sup>

1. Department of Biochemistry, Microbiology and Immunology, University of Ottawa, Ottawa, Canada.
2. Chronic Disease Program, Ottawa Hospital Research Institute, Ottawa, Canada.
3. Department of Epidemiology and Community Medicine, University of Ottawa, Ottawa, Canada
4. Division of Infectious Diseases, Ottawa Hospital-General Campus, Ottawa, Canada.

**BACKGROUND:** Effective immune responses against liver tropic hepatitis C virus (HCV) are dependent on HCV-specific CD8+ T cell (CTL) activity, which is impaired during chronic infection in blood and liver. In addition, generalized CD8+ T cell dysfunction has been observed. The mechanism of this CD8+ T cell impairment in HCV and other chronic viral infections, such as HIV, is not well understood. In HIV infection, reduced interleukin-7 (IL-7) receptor alpha (IL-7Ra, CD127) expression and decreased IL-7 activity contribute to CD8+ T cell impairment. It is hypothesized that IL-7 activity of CD8+ T cells is reduced in chronic HCV infection, particularly in HIV-HCV co-infection.

**RESULTS:** Included in this study were chronically HCV mono-infected, treatment naïve individuals, HIV-HCV co-infected individuals with untreated HCV infection but treated HIV-infection (HAART, <50 copies HIV RNA/microliter for >1yr), and HIV-/HCV- healthy controls. In blood-derived (bd)-CD8+ T cells isolated from HCV+ individuals there was a decreased proportion of naïve T cells (TN) compared to controls. While there were no differences in CD127 expression in bulk CD8+ T-cells between groups, in HCV infection central memory T cells (TCM) expressed lower levels of CD127 than controls. In HCV infection, the expression of IL-7-induced phosphorylated STAT5 (pSTAT5) in bd-CD8+ T cells, particularly in TN and TCM cells, was lower than controls. Decreased baseline Bcl-2 expression and reduced induction in response to IL-7 was also observed. IL-7-mediated cell proliferation was unchanged in HCV mono-infection. Inter-individual analysis found that intrahepatic CD8+ T cells isolated by liver biopsy from HCV+ individuals did not activate STAT5 with cytokine stimulation and baseline Bcl-2 levels were lower relative to bd-CD8+ T cells. In HIV-HCV co-infected individuals, there was a greater proportion of effector memory (TEM) cells in bd-CD8+ T cells than observed in controls or HCV mono-infection, and no difference in CD127 expression. The degree of IL-7 impairment in co-infection was similar to that observed in HCV mono-infection, with the exception of significantly reduced IL-7-mediated proliferation.

**CONCLUSIONS:** Generalized CD8+ T cell impairment in HCV infection is characterized by impaired cytokine-mediated signaling and survival, independent of mCD127 expression, and is more pronounced in the liver. Preliminary observations suggest similar impairment in HCV mono- and HIV-HCV co-infection. Identifying the cause of and mechanisms underlying generalized CD8+ T cell impairment in HCV infection can facilitate the design of novel cytokine-directed immune therapies, and may provide insight on improving the health status of those chronically infected with HCV.

#### 1-2 Cathelicidin antimicrobial peptide: a novel promoter of insulin secretion and islet regeneration

Lynley Pound<sup>1</sup>, Chandra Eberhard<sup>1</sup>, Christopher Patrick<sup>1</sup>, Gen-Sheng Wang<sup>1</sup> and Fraser Scott<sup>1,2,3</sup>

1. Chronic Disease Program, Ottawa Hospital Research Institute
2. Medicine, University of Ottawa
3. Biochemistry, Microbiology and Immunology, University of Ottawa

**Background:**

Cathelicidin antimicrobial peptide (CAMP) is a naturally-occurring secreted peptide expressed in the gut with pleiotropic roles in immunomodulation, wound healing and cell growth. We have previously demonstrated that Camp expression is up-regulated in the gut when type 1 diabetes (T1D)-prone BBdp rats are protected from diabetes development. Unexpectedly, we recently identified CAMP expression in the pancreatic  $\beta$ -cell, a previously unreported finding.

**Objective:**

We sought to characterize the novel roles of CAMP in the pancreatic islet. In particular, we evaluated the role of CAMP in intracellular calcium mobilization, islet hormone secretion and paracrine signalling and islet regeneration. In addition, we identified changes in CAMP expression associated with diabetes susceptibility.

**Methods:**

Changes in Camp gene and CAMP protein expression were identified by qRT-PCR and western blotting, respectively. Dispersed islets were loaded with an intracellular calcium indicator, FURA-2AM, to assess calcium mobilization following treatment with the CAMP peptide. Glucose-stimulated insulin secretion and glucagon secretion were evaluated under high or low glucose conditions, respectively, in the presence or absence of the CAMP peptide. Finally, a cohort of BBdp rats was treated daily for one week with either the CAMP peptide or saline control. Duct-associated insulin+ cells were quantified to assess  $\beta$ -cell regeneration.

**Results:**

Camp gene expression is down-regulated in the islets of young diabetes-prone BBdp rats compared to control BBc rats prior to the onset of insulinitis, indicating a role in susceptibility to T1D. CAMP treatment of dispersed islets from both BBc and BBdp rats resulted in a significant increase in intracellular calcium levels. This effect was both delayed and blunted in the absence of extracellular calcium, indicating a role for CAMP in the mobilization of both extracellular and intracellular calcium stores. Consistent with the critical role of calcium mobilization in islet hormone secretion, CAMP treatment similarly promoted both insulin and glucagon secretion from islets isolated from BBc and BBdp rats. Finally, daily treatment with the CAMP peptide in vivo resulted in enhanced  $\beta$ -cell neogenesis.

**Conclusions:**

Our data indicate a novel role for CAMP in pancreatic islet function and regeneration. Importantly, by stimulating both insulin and glucagon secretion, CAMP may be promoting islet paracrine signalling thereby enhancing overall islet function and glucoregulation. Furthermore, changes in expression in diabetes-prone rats indicate that CAMP may be playing a critical role in T1D susceptibility. Taken together, these findings strongly suggest that CAMP could be a novel therapeutic target for the treatment and/or prevention of diabetes.

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**1-3 Single-trial dorsolateral prefrontal cortex neural trajectories predict intended saccade direction**

**Chadwick B Boulay**<sup>1,2</sup>, Florian Pieper<sup>3</sup>, Matthew Leavitt<sup>3</sup>, Julio Martinez-Trujillo<sup>3</sup>, Adam J Sachs<sup>1,2,3</sup>

1. Neurosciences, Ottawa Hospital Research Institute
2. Department of Surgery, University of Ottawa
3. Department of Physiology, McGill University

**Background:** Brain-computer interfaces (BCIs) translate brain signals into computer control signals. We use BCIs to induce and guide adaptive plasticity or to provide an alternative communication pathway for individuals with motor disabilities due to central nervous system trauma or disease. An effective communication BCI requires a brain signal that encodes intent and a decoder capable of extracting intent from single-trial activity. Prefrontal cortex (PFC) is involved in decision-making processes and the generation of intent but very few studies have examined its potential as a signal source for BCI.

**Objective:** As a first step in the development of a PFC BCI, we set out to determine if we could decode simple behaviours from single-trial PFC neuronal activity and what decoder techniques are ideally suited for this task.

**Methods:** We recorded neuronal spiking activity and field potentials from microelectrode arrays implanted in the dorsolateral PFC (dIPFC) of two adult macaques (*Macaca fascicularis*) while they made visually guided saccades to 8 visual targets. We decoded intended saccade direction from single-trial dIPFC activity using several different feature extraction techniques – including Gaussian process factor analysis (GPFA; Yu et al., 2009) that makes explicit use of single-trial time courses – combined with several different machine-learning (ML) algorithms.

**Results:** We decoded single-trial saccade direction with up to 95% accuracy (chance accuracy = 12.5%). GPFA yielded the best decoder accuracy of all feature extraction techniques. Different ML algorithms had little impact on decoder performance; regularized linear discriminant analysis (rLDA) was the fastest.

**Conclusions:** The results of this study demonstrate that it is possible to decode intended saccade direction from single-trial dIPFC activity. Further, GPFA was the best feature extraction technique suggesting that dIPFC activation time courses vary across trials because they reflect dynamic internal decision processes rather than stimulus-driven responses. We are now using GPFA and rLDA to investigate how PFC neuronal activity encodes contextual goals and how these encodings change during contextual learning.

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1-4 **Whole-skull microscopy visualizes alpha-synuclein and tau expression in olfactory epithelium: implications for testing the pathogenesis of Parkinson's and Alzheimer's**

Julianna J. Tomlinson<sup>1</sup>, Louise Pelletier<sup>2</sup>, Li Dong<sup>2</sup>, Fanyi Meng<sup>1</sup>, Megan Fitzpatrick<sup>1,3</sup>, Irene Harmsen<sup>1</sup>, Emma Grigor<sup>1</sup>, Steffany C. Bennett<sup>4</sup>, John Woulfe<sup>2,3</sup>, Earl Brown<sup>1,4</sup>, Diane Lagace<sup>3</sup>, Michael G. Schlossmacher<sup>1,3,5</sup>

1. Program in Neuroscience, Ottawa Hospital Research Institute,
2. Department of Pathology and Laboratory Medicine, The Ottawa Hospital
3. Department of Cellular & Molecular Medicine,
4. Biochemistry, Microbiology and Immunology, Faculty of Medicine,
5. Department of Medicine (Neurology), The Ottawa Hospital; University of Ottawa; Ottawa, Ontario, Canada;

**Background:**

Hyposmia occurs early in several neurodegenerative disorders. The role of the nasal epithelium in the pathogenesis of Parkinson disease and Alzheimer's has not yet been studied in part due to difficulty visualizing olfactory and respiratory epithelia.

**Objectives:**

To visualize the nasal epithelium and other structures of intact skulls together with the brain in mice to study interactions between neurodegeneration-associated genes and microbial pathogens.

**Methods:**

After optimizing a formic acid-based method that decalcifies bone and enhances select antigen accessibility, we processed intact mouse heads into 4 micrometer-thin sections for multiple microscopy applications.

**Results:**

Using a standardized protocol for 'holocranomicroscopy', we made 5 observations: 1) Expression of alpha-synuclein and tau in olfactory neurons of the nasal cavity; 2) Correlation between human SNCA allele numbers and alpha-synuclein levels throughout the cranium of PAC1-transgenic mice. There, 4 SNCA alleles induced abnormalities in movements, cognition and odor processing; 3) Formation of thioflavin T-positive amyloid-beta plaques in intracranial portions of the olfactory nerve in mutant human APP-cDNA-transgenic mice; 4) The tracking of infection following inoculation with a respiratory-enteric-orphan virus from the nasal cavity into cranial nerves-1 and -5 and on to encephalitis; and 5) Improved sensitivity in monitoring intracranial neurogenesis after BrdU administration.

**Conclusion:**

Holocranomicroscopy allows for the routine visualization of skull and soft tissue components in intact mouse heads including nasal epithelia, glands, cranial nerves, vessels, meninges and brain. Our protocol permits the experimental study of interactions between human susceptibility alleles and naturally occurring pathogens to explore the etiologies of sporadic Parkinson disease ("Braak hypothesis") and late-onset Alzheimer disease.

## Patient-oriented Research (9:20 to 10:10)

*Moderators: Mathieu Chalifoux and Michael Chasse*

2-1 **Seasonal Variation in Rates of Emergency Room Visits and Acute Admissions Following Routine Infant Vaccinations in Ontario**

Steven Hawken<sup>1,2</sup>, Robin Ducharme<sup>1,2</sup>, Kumanan Wilson<sup>1,2,3</sup>

1. ICES uOttawa
2. OHRI Clinical Epidemiology
3. Faculty of Medicine, University of Ottawa

**Background:** A number of previous observational studies have suggested that the incidence of immune-mediated diseases (such as type I diabetes, multiple sclerosis, inflammatory bowel disease) follow a seasonal pattern, such that specific birth months are associated with higher or lower incidences of disease.

**Objective:** To determine if birth month has an effect on the incidence of adverse events following 2- and 12-month recommended vaccinations.

**Methods:** We included children born in Ontario between April 1st 2002 and March 31st 2010 who received the diphtheria, tetanus, pertussis, inactivated poliovirus and Haemophilus influenzae type b (DTaP-IPV-Hib) vaccine recommended at 2 months and/or the measles, mumps, and rubella vaccine recommended at 12 months. Using health administrative databases, we conducted a population-based retrospective cohort study and employed a self-controlled case series analysis approach. We calculated the

relative incidence (RI) of hospitalizations and emergency room visits within a pre-specified risk period compared to a control period following vaccination. We measured the effect of birth month using relative incidence ratios (RIRs) to compare the RI for infants born in each month to that for the month having the lowest RI.

Results: For the 2-month vaccination we observed the lowest and highest RIs for infants born in October and April, respectively. The RIR (95% CI) for April compared to October was 2.06 (1.59 to 2.67,  $p < 0.0001$ ), consistent with a strong seasonal effect. For the 12-month vaccination, November births had the lowest RI, whereas August births had the highest. The RIR (95% CI) for August compared to November was 1.52 (1.30 to 1.77,  $p < 0.0001$ ).

Conclusions: Our findings suggest that susceptibility to adverse events following vaccination follows a seasonal pattern. The pattern we observed closely corresponds to seasonal patterns reported for other immune-mediated chronic diseases, such as multiple sclerosis and type I diabetes. Further study will be important to elucidate potential biological and/or behavioral explanations for the seasonal effect we observed.

## 2-2 Development and implementation of the Thoracic Surgery Quality Monitoring, Information Management, Clinical Documentation System for continuous, point-of-care reporting and evaluation of post-operative adverse events

Jelena Ivanovic, MSc;1,2,3 Caitlin Anstee, BA;3 Christian Finley, MD, MPH, FRCSC;4

Sebastien Gilbert, MD, FRCSC;2,3 Donna E. Maziak, MDCM, MSc, FRCSC, FACS;1,2,3 Farid M. Shamji, MBBS, FRCSC;3

R. Sudhir Sundareshan, MD, FRCSC, FACS;1,3 P. James Villeneuve, MDCM, PhD, FRCSC;3 Tim Ramsay, PhD;1,2

and Andrew J.E. Seely, MD, PhD, FRCSC.1,2,3

1. Clinical Epidemiology Program, Ottawa Hospital Research Institute, 501 Smyth Road, Ottawa, Canada
2. Department of Epidemiology and Community Medicine, University of Ottawa, 451 Smyth Road, Ottawa, Canada
3. Division of Thoracic Surgery, Department of Surgery, The Ottawa Hospital, 501 Smyth Road, Ottawa, Canada
4. Division of Thoracic Surgery, Department of Surgery, St. Joseph's Healthcare, 2757 King Street East, Hamilton, Canada

**BACKGROUND:** Objective reporting of postoperative adverse events (AEs) is the basis of surgical quality assurance. In 2008, we developed a standardized classification to identify presence and severity of Thoracic Morbidity & Mortality (TM&M). However, paper-based forms that were originally used to support AE reporting were cumbersome and inefficient, with potential for data entry error.

**OBJECTIVE:** To develop an interactive, portable method to record and report AEs, integrated with process of care delivery, the Thoracic Surgery Quality Monitoring, Information Management, and Clinical Documentation (TSQIC) system; and to describe the implementation and evolution of both the TM&M classification and the TSQIC system, and how they have afforded powerful quality assessment and improvement opportunities.

**METHODS:** The TM&M classification was developed in accordance to the Clavien-Dindo classification of AEs ([www.ottawatmm.org](http://www.ottawatmm.org)). Daily recording of all thoracic operative procedures and all AEs is performed by residents, along with weekly review by staff. Monthly data review, discussions, and iterative software refinements led to an evolution of the web-based, iPad-optimized TSQIC system allowing for real-time reporting, monitoring, and analyses of surgical volume and quality ([www.tsqic.org](http://www.tsqic.org)).

**RESULTS:** From 01/2008-09/2014, 3335 patients (mean age 61 years, range 14-97) underwent 4226 thoracic surgical procedures, of which 1614 (48.4%) patients had at least one complication. Complications were assigned a severity grade from I-V. Grade I (n=201; 4.8%) and II (n=828; 19.6%) complications require no therapy or pharmacologic intervention only. Grade III (n=420; 9.9%) and IV (n=116; 2.7%) complications require surgical intervention or life support. Grade V (n=46; 1.1%) complications result in patient death. Major complication rates (grades III-IV) for lobectomy, pneumonectomy, and esophagectomy were 16.5% (n=130/790), 37.5% (n=27/72), and 69.6% (n=112/161); mortality rates were 1.3% (n=10/790), 5.6% (n=4/72), and 3.7% (n=6/161). The most common complications by system were pleural (n=432; 10.2%), cardiovascular (n=291; 6.9%), and pulmonary (n=275; 6.5%) for all cases. Prolonged air leak (n=164; 11.6%) and atrial fibrillation (n=132; 9.4%) were identified as the most common complications after pulmonary resection. 30.5% (n=493) of AEs led to prolonged length of stay, and 10.6% (n=172) led to hospital readmission; 2.1% (n=35) of AEs were unrelated to a surgical procedure. We have incorporated automated reporting of AEs into M&M rounds, risk-adjusted individual surgeon performance feedback regarding AEs, and continuous quality improvement seminars.

**CONCLUSIONS:** The TM&M classification offers prospective, standardized, and reliable definitions to accurately report all postoperative AEs, while the complementary web-based software application, TSQIC, provides an effective method for data entry, and review.

## 2-3 How do breast cancer patients define “optimal control” of chemotherapy induced nausea and vomiting?

**Catalina Hernandez Torres**<sup>1</sup>, Sasha Mazzarello<sup>1</sup>, George Dranitsaris<sup>3</sup>, Terry Ng<sup>1</sup>, Brian Hutton<sup>2</sup>, Stephanie Smith<sup>2</sup>, Amy Munro<sup>1</sup>, Carmel Jacobs<sup>1</sup>, Mark Clemons<sup>1</sup>

1. Division of Medical Oncology and Department of Medicine, University of Ottawa.
2. Ottawa Hospital Research Institute.
3. Statistical Consultant. Toronto, ON.

**Background:** A major issue for comparing randomized trials of anti-emetic regimens for chemotherapy induced nausea and vomiting (CINV) is the large number of outcomes reported. Many of these endpoints fail to directly assess nausea. We are unaware of any studies evaluating clinical trial endpoints for CINV studies from the patients' perspective.

**Objective:** To determine optimal definition of CINV control from breast cancer patients' perspective.

**Methods:** Patients with early stage breast cancer who had received anthracycline-cyclophosphamide based chemotherapy were surveyed about their experiences of CINV and their perception of different CINV assessment tools and end points.

**Results:** Of 201 patients approached, 168 (83%) completed the survey. Patients consistently ranked nausea over vomiting as a “most feared side effect from chemotherapy”. Despite the use of multi-agent anti-emetic regimens 71% experienced nausea and 26% patients experienced vomiting. Only 57% of those patients with any nausea or vomiting took any rescue medication and only then when the nausea was severe. Most (76%) patients believed that the primary end point of anti-emetic trials should include the absence of both nausea and vomiting. Patients felt the most important time points for evaluating CINV was during the acute period (i.e. first 24 hours) after chemotherapy (27%), the delayed period (i.e. days 2 to 5) (23%) and the overall period (i.e. days 1-5) (44%).

**Conclusions:** Patients strongly favoured a CINV end point that includes the absence of both nausea and vomiting. “Use of rescue medication”, which is currently used as a surrogate measure for nausea control in many trials, is inappropriate as it significantly underestimates nausea.

## 2-4 The health care cost of dying: A population-based examination across health care sectors for Ontarians in their last year of life

**Peter Tanuseputro**<sup>1,2,3</sup>, Walter P Wodchis<sup>3,4</sup>, Rob Fowler<sup>5</sup>, Peter Walker<sup>1</sup>, Yu Qing Bai<sup>4</sup>, Mathieu Chalifoux<sup>2,3</sup>, Susan E Bronskill<sup>3,4</sup>, Douglas Manuel<sup>1,2,3</sup>

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2. Clinical Epidemiology Program, Ottawa Hospital Research Institute;
3. Population Health and Primary Care, Institute for Clinical Evaluative Sciences;
4. Institute for Health Policy, Management & Evaluation, University of Toronto
5. Department of Medicine and Department of Critical Care Medicine, Sunnybrook Hospital, the University of Toronto.
6. Department of Medicine, University of Ottawa

**Background:** Coordinated and appropriate health care across sectors is an ongoing challenge, especially at the end-of-life. Population-level data on end-of-life health care use and cost, however, are seldom reported across a comprehensive array of sectors. Such data will identify the level of care being provided and areas where care can be optimized.

- Objectives:**
1. Describe overall health care cost across health care sectors in the last year of life.
  2. Describe the socio-demographic characteristics of those using each health care sector at the end-of-life.
  3. Describe the cost curves for each health sector as the population approaches death

**Methods:** This retrospective cohort study identified all deaths in Ontario from April 1, 2010 to March 31, 2013. Using population-based health administrative databases, we examined health care use and cost of in the last year of life.

**Results:** Among 264,755 decedents, the average health care cost in the last year of life was \$53,661 (Q1-Q3: \$19,568-\$66,875). The total annual cost of \$4.7 billion represents approximately 10% of all government-funded health care. Inpatient care, incurred by 75% of decedents, contributed 42.9% of total costs (\$30,872 per user). Physician services, medications/devices, laboratories, and emergency rooms combined to less than 20% of total cost. About one-quarter used long-term-care and 60% used home care (\$34,381 and \$7,347 per user, respectively). Total cost did not vary by sex or neighborhood income quintile, but were less among rural residents. Costs rose sharply in the last 120 days prior to death, predominantly for inpatient care.

**Conclusions:** This analysis adds new information about the breadth of end-of-life health care, which consumes a large proportion of Ontario's health care budget. The cost of inpatient care and long-term care are substantial. Introducing interventions that reduce institutional care will likely improve the patient dying experience and reduce costs.

## Cancer: Improving Therapeutic Responses (1:10 to 2:00)

Moderators: *Curtis McCloskey and Dominic Roy*

### 3-1 Enhancement of oncolytic viral efficacy by novel drug candidates

**Ramya Krishnan**<sup>1,2</sup>, Mark Dornan<sup>3</sup>, Colin Davis<sup>1,2</sup>, Mohammed Selman<sup>1,2</sup>, Andrew Chen<sup>1</sup>, Nader El Sayes<sup>1</sup>, Paula Ou<sup>1</sup>, Rozanne Arulanandam<sup>1,2</sup>, Andrew Macklin<sup>4</sup>, Christophe Pardin<sup>3</sup>, Cory Batenchuk<sup>1,2</sup>, Vanessa Garcia<sup>1,2</sup>, Christina Moi<sup>3</sup>, Penny Le<sup>3</sup>, Kerkeslin Keillor<sup>3</sup>, John C. Bell<sup>1,2</sup>, Jeff Smith<sup>4,5</sup>, Christopher N. Boddy<sup>3</sup>, Jean-Simon Diallo<sup>1,2</sup>

1. Centre for Innovative Cancer Research, Ottawa Hospital Research Institute
2. Department of Medicine, University of Ottawa
3. Department of Chemistry, University of Ottawa
4. Department of Chemistry, Carleton University
5. Institute of Biochemistry, Carleton University

Background: Pre-clinical and clinical studies have shown that oncolytic virus (OV) therapy is safe, well tolerated and effective in a broad range of cancers. Still, resistance in a subset of tumors highlights areas for improvement in oncolytic virus based therapeutics. Combining OV therapy and drug therapy is a promising strategy to selectively enhance OV-mediated tumour cell death. To this end, we have previously identified the synthetic compound Viral Sensitizer 1 (VSe1) that enhanced the spread of oncolytic vesicular stomatitis virus (VSV?51) in resistant cancer cell lines up to a 1000-fold, resulting in synergistic cell killing and improved efficacy in vitro and in vivo. While we know VSe1 suppresses the ability of cancer cells to defend against viral infection, its mechanism of action and biological targets are unknown.

Objectives:

1. Characterize the structure-activity-relationship of VSe1
2. Identify VSe1 analogues with improved pharmacological and pharmacokinetic properties.

Methods: To study the structure activity relationship (SAR) of VSe1, enhancement of VSV?51 expressing firefly luciferase by VSe1 analogues was assessed by a high-throughput in vitro assay in resistant 786-0 (human renal carcinoma) cells. In vivo safety of VSe1 analogues was assessed in a maximum-tolerated dose (MTD) study in Balb/C mice.

Results: In vitro assays and a rational approach in the design of VSe1 analogues allowed us to identify functional groups that can be modified without hampering activity. Interestingly, some analogues possess improved properties such as potency, stability and in vivo tolerability.

Conclusion: We were able to modify VSe1 to generate analogues with more attractive pharmacological and physicochemical properties. Future work will aim to elucidate the mechanism of action of VSe1 and continue the evaluation of the in vivo efficacy of VSe1 analogues.

### 3-2 OVERCOMING VACCINIA VIRUS NEUTRALIZATION IN IMMUNE HUMANS AND MACAQUES WITH COMPLEMENT INHIBITION

**Laura Evgin**<sup>1,2</sup>, Sergio Acuna<sup>3</sup>, Christiano Tanese de Souza<sup>1</sup>, Monique Marguerie<sup>1,2</sup>, Chantal Lemay<sup>1</sup>, Theresa Falls<sup>1</sup>, Carolina Ilkow<sup>1</sup>, Kelley Parato<sup>1</sup>, Caroline Breitbach<sup>7</sup>, David Kirn<sup>7</sup>, Harold Atkins<sup>1</sup>, Greg Stahl<sup>4</sup>, Joshua Thurman<sup>5</sup>, John Lambris<sup>6</sup>, Andrea McCart<sup>3</sup>, John Bell<sup>1,2</sup>

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2. Department of Biochemistry, Microbiology and Immunology, University of Ottawa, Ottawa, ON, Canada;
3. Division of Experimental Therapeutics, Toronto General Research Institute, Toronto, ON, Canada;
4. Center for Experimental Therapeutics and Reperfusion Injury, Harvard Institutes of Medicine, Boston, MA, USA;
5. Department of Medicine, University of Colorado, Denver CO, USA;
6. Department of Pathology and Laboratory Medicine, University of Pennsylvania, Philadelphia, PA, USA;
7. Jennerex Inc., San Francisco, CA, USA

Background: Systemic administration of oncolytic viruses (OVs) enables the simultaneous treatment of multiple tumour sites that may be dispersed or inaccessible by direct injection. Natural barriers in the blood including neutralizing antibodies and complement however will likely limit the ability to repeatedly administer OVs by the intravenous route.

Objective: The overarching objective is to develop a strategy to mitigate the effect of natural barriers on the delivery of OVs.

Methods: In vitro, we have examined the factors contributing to viral neutralization in the blood of healthy Vaccinia virus (VACV) naïve and immune donors using monoclonal antibodies and a peptide inhibitor of complement components. We also used Fischer rat and cynomolgus macaque models to investigate feasibility and safety of complement intervention strategies.



Results: Using antibody collected from healthy vaccinated donors and hyper-immune patients who had received oncolytic VACV treatment, we have found that the neutralizing activity of antibodies is strictly dependent upon the activation of complement. Inhibition of complement in naive or immune plasma with a targeted C3 inhibitor dramatically increased viral stability. In a Fischer rat model, complement depletion stabilized the virus in the blood of immunized hosts and correlated with improved delivery to mammary adenocarcinoma tumours. Unexpectedly, complement depletion also provided a dramatic enhancement of tumour infection when delivered by intra-tumoural injection in immunized animals. We demonstrated in a cynomolgus macaque model that short term complement inhibition increased the infectious titer in the blood in immune animals at early points after the infusion, and a prolonged of the time during which infectious virus was still detectable.

Conclusions: We have demonstrated in three species that anti-VACV antibody is complement fixing and dependent. Complement inhibition is a viable and safe strategy to increase the effective dose of oncolytic VACV in immune patients.

### 3-3 **The role of p53 in regulating mitochondrial dynamics and chemoresistance in gynaecological cancer cells**

**Bao Kong**<sup>1,2</sup>, Benjamin Tsang<sup>1,2</sup>

1. Chronic Disease Program, Ottawa Hospital Research Institute

2. Department of Obstetrics & Gynecology and Cellular & Molecular Medicine, and Interdisciplinary School of Health Sciences; University of Ottawa

Background: Cervical cancer (CECA) and ovarian cancer (OVCA) rank first and the third in the number of new cases diagnosed in gynecologic cancers and chemoresistance severely limits their treatment success. The underlying mechanism of chemoresistance is multi-factorial and partly due to defects in drug-induced apoptosis. Mitochondria are highly dynamic organelles. Mitochondria fusion and fission is required for most mitochondrial functions, and is also involved in the regulation of mitochondria-mediated apoptosis. Mitochondrial dynamics are controlled by a series of molecules including fusion protein (Opa1 and its protease Oma1) and fission protein (Drp1 and Fis1). Tumor suppressor Prohibitin 1 (Phb1) regulates Oma1-mediated Opa1 processing, however, the mechanism is not known. CDDP-induced, p53-mediated mitochondrial cell death is a determinant of chemosensitivity in gynecologic cancer cells. However, the mechanism involved is not clear.

Objective: Our overall objective is to increase the current understanding on the regulation of mitochondrial dynamics and its role in chemoresistance in OVCA and CECA. Specifically, we will examine the mechanism by which p53 regulates mitochondrial dynamics and the possible participation of long form Opa1 (L-Opa1) processing in these gynaecological cancer cells.

Methods: Chemosensitive and chemoresistant ovarian (OVCA) and cervical (CECA) cancer cells were treated with cisplatin (CDDP). Mitochondrial dynamics and protein contents were assessed by immunofluorescence and Western blot, respectively. The requirement of p53 for mitochondrial dynamics was examined by siRNA or cDNA. The protein interaction was examined by immunoprecipitation.

Results: CDDP induces Oma1 increase, L-Opa1 processing and mitochondrial fission in chemosensitive but not in chemoresistant cells. Silencing p53 expression attenuated CDDP-induced Oma1 and Opa1 content, mitochondrial dynamics changes and apoptosis in chemosensitive CECA cells, while reconstitution of p53 in p53 mutant or null chemoresistant OVCA cells induced Oma1, L-Opa1 and mitochondrial dynamics change irrespective of the presence of CDDP. Prohibitin 1 (Phb1) dissociates from Opa1-Phb1 complex and binds phosphorylated p53 (serine 15) in response to CDDP in chemosensitive but not chemoresistant CECA cells.

Conclusion: Our study suggests the mechanism that p53 regulates L-Opa1 processing and mitochondrial fission induced by CDDP in chemosensitive cells, while this pathway is prohibited in chemoresistant cells. Dysregulated mitochondrial dynamics may in part be involved in the pathophysiology of CDDP resistance. Understanding the defection of mitochondrial dynamics in chemoresistance may provide novel therapeutic strategies for cancer.

### 3-4 **UTX inhibition as selective epigenetic therapy against TAL1-driven T cell acute lymphoblastic leukemia**

**Aissa Benyoucef**<sup>1,2</sup>, Carmen G. Pali<sup>1</sup>, Alphonse Chu<sup>1</sup>, Fengtao Dai<sup>1</sup>, Patricia Rakopoulos<sup>1</sup>, Herve Faralli<sup>1,2</sup>, Maxwell Sunohara<sup>1,2</sup>, F. Jeffrey Dilworth<sup>1,2</sup>, Marjorie Brand<sup>1,2,3</sup>

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Abstract:

T cell acute lymphoblastic leukemia (T-ALL) is an aggressive cancer of thymocytes [1, 2]. The transcription factor TAL1 (T-cell acute lymphocytic leukemia protein 1) (also named SCL) [3] is aberrantly expressed in 40%–60% of T-ALL cases [4, 5] and it has been

shown that TAL1 expression in T-cell progenitors causes increased proliferation, arrested differentiation, and aberrant survival that leads to the expansion of fully transformed leukemic cells [6-8]. Clinically, TAL1-positive T-ALL patients have a particularly bad prognosis [9, 10] with only 50% of patients surviving 5 years after treatment. Therefore, there is an urgent need to develop new therapeutics against this disease. To get insight into the mechanism of TAL1-regulated transcription in a leukemic context, we performed an extensive proteomic study to identify cofactors that interact with TAL1. Here, we report for the first time that TAL1 associates with the epigenetic enzyme UTX, which is normally involved in regulating the expression of developmentally regulated genes through removal of the repressive histone H3 lysine 27 trimethyl (H3K27me3) modification [11-12]. Furthermore, by using a combination of knockdown studies and CHIP-sequencing, we demonstrate that TAL1 recruits the epigenetic regulator UTX to the majority of its target genes to aberrantly activate an oncogenic gene expression program comprising genes involved in proliferation and anti-apoptosis. Based on these findings we have developed a novel therapeutic approach using an epigenetic drug that specifically inhibits the enzymatic activity of UTX. We demonstrate that this drug is highly efficient in eliminating human leukemic blasts both in cell culture, and in vivo in immunocompromised mice engrafted with TAL1-positive T-ALL primary cells from patients. This highly efficient strategy represents the first epigenetic therapeutic approach to treat TAL1-positive T-ALL patients.

## References:

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## Stem Cell Biology and Regenerative Medicine (3:00 to 3:50)

*Moderators: Will Wang and Lisa Julian*

### 4-1 p38-gamma MAPK regulation of Carm1 promotes satellite stem cell self-renewal via symmetric expansion

**Natasha C. Chang**<sup>1,2</sup>, Melanie Lacaria<sup>1,2</sup>, Michael A. Rudnicki<sup>1,2</sup>

1. Regenerative Medicine Program, Ottawa Hospital Research Institute
2. Department of Cellular and Molecular Medicine, University of Ottawa

Satellite cells, the adult stem cell of skeletal muscle, represent a heterogeneous population of both stem cells and committed myogenic progenitors. Satellite stem cells constitute one tenth of the satellite cell population and demonstrate superior engraftment capabilities compared to committed progenitors. During regeneration, satellite stem cells are responsible for maintaining the satellite cell pool through their ability to undergo self-renewal via both symmetric and asymmetric cell divisions. The intrinsic molecular mechanisms that underlie these cellular fate decisions, however, have remained elusive. The transcription factor Pax7 is required for specification of satellite cells and controls their entry into the myogenic program through its ability to target the myogenic regulatory factor Myf5. Transcription of Myf5 serves as an indicator of myogenic commitment and is regulated during asymmetric satellite cell division by the methyltransferase Carm1. Here we identify the MAP kinase p38-gamma as a regulatory kinase of Carm1. Specific depletion of p38-gamma in satellite cells revealed a preference for asymmetric cell division and inhibited symmetric satellite stem cell divisions. Moreover, in the absence of p38-gamma we observed enhanced Carm1/Pax7 associations. We conclude that in contrast to Carm1, which is necessary for asymmetric cell division, p38-gamma is required for satellite stem cell self-renewal via symmetric expansion. Along these lines, conditional inactivation of p38-gamma specifically in satellite cells in vivo resulted in a significant reduction in satellite cell number and impaired muscle regeneration following muscle injury. Ultimately, insight into the molecular pathways that regulate satellite stem cell self-renewal is essential for advancement of therapeutic strategies to treat muscle degeneration.

#### 4-2 **In vivo hyperoxia alters the potency and gene expression profile of CD146+ endogenous lung mesenchymal stromal cells**

**Jennifer J.P. Collins**<sup>1,2</sup>, Marissa A. Lithopoulos<sup>1,2</sup>, Claudia C. dos Santos<sup>3,4</sup>, Marius A. Möbius<sup>1,5</sup>, Arul Vadivel<sup>1</sup>, Shumei Zhong<sup>1</sup>, Bernard Thébaud<sup>1,2,6</sup>

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6. Children's Hospital of Eastern Ontario Research Institute, Ottawa, ON, Canada.

**Background:** Bronchopulmonary dysplasia (BPD), one of the most common adverse outcomes of extreme preterm birth, can be caused by oxygen-related lung injury and is characterized by an arrest in alveolar development. Mesenchymal stromal cells (MSCs), which have regenerative properties, are associated with BPD if found in the tracheal aspirates of preterm infants.

**Objective:** To determine whether in vivo hyperoxia exposure perturbs gene expression in CD146+ endogenous lung MSCs in an oxygen-induced rat model of BPD.

**Methods:** Rat pups were exposed to 21% or 95% oxygen from postnatal day 0 to 10 and sacrificed on day 12. Lung MSCs were isolated by enzymatic digestion and FicolI-purification. CD146+ L-MSCs were isolated through magnetic bead selection and characterized according to the International Society for Cellular Therapy criteria. mRNA was extracted for microarray analysis, using the Affymetrix GeneChip and gene set enrichment analysis (GSEA) software. Epithelial repair potential was tested by scratch assay.

**Results:** Hyperoxia exposure decreased CD73 expression in CD146+ L-MSCs, and tended to lower differentiation and colony forming potential. Gene expression of the axonal guidance cue and CDC42 pathways increased after in vivo hyperoxia, whereas genes of the JAK/STAT pathway were decreased. CD146+ L-MSCs promoted epithelial wound healing, regardless of in vivo exposure.

**Conclusions:** In vivo hyperoxia exposure lowered CD73 and JAK/STAT expression, indicating decreased immune function, and changed the gene expression of vital alveolar development pathways. These changes in endogenous L-MSCs likely reflect their role in BPD pathogenesis, and must be further explored to understand the effectiveness of exogenous MSC therapy.

#### 4-3 **Regression-based multiscale decomposition of the ChIP-Seq signal reveals novel quantifying factors**

**Parameswaran Ramachandran**<sup>1,2</sup>, Theodore J Perkins<sup>1,2</sup>

1. Regenerative Medicine Program, Ottawa Hospital Research Institute
2. Department of Biochemistry, Microbiology and Immunology, University of Ottawa

**Background:** Although the nature of the "ChIP-seq signal" has been analyzed in previous studies, a clear quantification of the roles of factors such as mappability, chromatin accessibility, and notions of control at a genome-wide scale is missing from the literature.

**Objective and Methods:** Using a large number of ChIP-seq datasets from the ENCODE Consortium spanning multiple cell lines, we build a regression-based model that not only quantifies the components of the ChIP-seq signal but also analyzes it at multiple scales. The model is validated by correlating our binding-site predictions with motif densities. We then use the model to investigate the observed binding-expression associations reported in multiple previous studies.

**Results:** While some of these binding-expression associations may well be real, our results share the concerns raised by certain other studies and go a step further to suggest that many of these associations may actually be due to confounding factors such as chromatin accessibility.

**Conclusions:** Caution, therefore, should be exercised while interpreting ChIP-seq signals in highly expressed genomic regions. Overall, our model and related results reveal new insights that show a lot of promise towards designing better peak-calling methodologies in the near future.

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4-4 **MSK1-mediated phosphorylation of Kap1 is a key determinate of MyoD function during skeletal muscle differentiation**

**Kulwant Singh**<sup>1</sup>, Marco Cassano<sup>2</sup>, Evarist Planet<sup>2</sup>, Soji Sebastian<sup>1</sup>, Suk Min Jang<sup>2</sup>, Gurjeev Sohi<sup>1</sup>, Jinmi Choi<sup>3</sup>, Hong-Duk Youn<sup>3</sup>, Didier Trono<sup>2</sup>, and F. Jeffrey Dilworth<sup>1,4</sup>

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**Background**

The transcriptional activator MyoD serves as a master regulator of skeletal myogenesis. Often in partnership with Mef2, MyoD binds to the promoters of hundreds of muscle genes in proliferating myoblasts, yet activates these targets only upon receiving cues that launch differentiation. However, what regulates this off/on switch of MyoD function has been incompletely understood.

**Objective**

To identify the key regulators of MyoD responsible for this off/on switch in proliferating/differentiating myoblasts.

**Methods and Results**

Using proteomic and genomic approaches, we have identified KAP1/TRIM28 as a key regulator of MyoD function. In myoblasts, we found that KAP1 is present with MyoD and Mef2 at many muscle specific genes. While bound to these genes, we show that KAP1 acts as a scaffold to recruit not only co-activators such as p300 and LSD1, but also co-repressors such as G9a and HDAC1, with promoter silencing as net outcome. Upon differentiation, when the p38 MAPK signaling pathway activate the downstream Kinase MSK1, we observed that the KAP1 protein becomes phosphorylated, leading to release of the co-repressors from the scaffold thereby unleashing transcriptional activation by MyoD/Mef2 and their positive cofactors.

**Conclusions**

Our results reveal KAP1 as a previously unappreciated interpreter of cell signaling, which modulates the ability of MyoD to drive myogenesis.

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# POSTER PRESENTATIONS

## OHRI IMPACT Award

(Identification of Marketable Products, Applications and Commercializable Technologies)

- 1 **Enhancing neurogenesis for post-stroke recovery**  
**Melanie Lacaria**<sup>1</sup>, Sharlene Faulkes<sup>1</sup>, Diane Lagace<sup>2</sup>, Dale Corbett<sup>2</sup>, Michael Rudnicki<sup>1,2</sup>  
 1. Regenerative Medicine Program, Ottawa Hospital Research Institute  
 2. Cellular and Molecular Medicine, University of Ottawa
  
- 2 **Using mobile technologies to transform immunization practice**  
**Atkinson, K**<sup>1,2</sup>, Bell, C<sup>2</sup>, Wilson, K<sup>1,2,3</sup>  
 1. Clinical Epidemiology Program, Ottawa Hospital Research Institute  
 2. Sigvaria Mobile Technologies Inc.  
 3. Department of Medicine, University of Ottawa
  
- 3 **Oncolytic viruses as a potential approach to eliminate cells that constitute the HIV**  
**Nischal Ranganath**<sup>1</sup>, Sandra C. Côté<sup>1,2</sup>, Jonathan B. Angel<sup>1,2,3</sup>  
 1. Biochemistry, Microbiology & Immunology, University of Ottawa, Ottawa, ON, Canada.  
 2. Infectious Diseases, Ottawa Hospital Research Institute, Ottawa, ON, Canada.  
 3. Division of Infectious Diseases, Ottawa Hospital-General Campus, Ottawa, ON, Canada.

## Cancer Therapeutics Program

- 4 **The Role of Ldb1 in Neu-mediated Tumorigenesis**  
**Sarra M. Ahmed**<sup>1,2</sup>, Chris J. Storbeck<sup>1</sup>, Luc A. Sabourin<sup>1,2</sup>  
 1. Centre of Cancer Therapeutics, Ottawa Hospital Research Institute  
 2. Department of Cellular and Molecular Medicine, Faculty of Medicine, University of Ottawa

Background: The ste-20 like kinase (SLK) is activated downstream of HER2/ErbB2/Neu. Focal adhesion kinase (FAK) signaling is required for Neu-mediated SLK activation. SLK activity is required for focal adhesion turnover and cell migration downstream of FAK signaling. Previously, the transcriptional cofactor LIM binding domain protein 1 (Ldb1) has been identified as an important regulator of SLK kinase activity and cell migration. Specifically, Ldb1 deletion in MEF3T3 cells has been shown to be associated with a significant increase in SLK kinase activity, adhesion turnover and cell migration. Ldb1 global knockout leads to severe developmental defects and embryonic lethality.

Hypothesis: Conditional targeted deletion of the Ldb1 gene in the mammary gland is hypothesized to result in an increase in SLK kinase activity; which may contribute to the metastatic potential of Neu-driven tumorigenesis.

Objectives: To study (1) Ldb1 deletion effect on mammary gland development. (2) Ldb1 role in Neu-induced tumorigenesis. (3) Ldb1 deletion effect on SLK kinase activity and metastasis.

Methods/Results: Efficient Ldb1 knockout has no significant impact on mammary gland development, overall survival and tumor onset. Furthermore, Ldb1 knockout has no significant effect on the Ki-67 proliferative index and the apoptotic status of primary mammary tumors. Kinase assay analysis showed that Ldb1 knockout in vivo does not have a significant effect on SLK kinase activity. Scratch-wound healing assay followed by Immunofluorescence analysis demonstrated that SLK and Ldb1 do not co-localize in migrating Neu-induced primary tumour cells. The results from IF show that SLK staining is cytoplasmic whereas Ldb1 staining is nuclear in migrating primary tumor cells. Ldb1 knockout however is associated with a significant increase in cellular migration and invasion of Neu-induced primary tumor cells as shown in Boyden chamber migration and invasion assays, respectively.

Conclusion: It has been shown in this study that Ldb1 does not contribute to mammary gland development, Neu-mediated tumor

growth nor overall survival. The results from Boyden chamber migration and invasion assays demonstrate that Ldb1 has an anti-migratory and anti-invasive effects on primary tumor cells. Also, the role of Ldb1 in mediating cellular migration is SLK independent as shown in the results from IF and kinase assay. The fact that Ldb1 localizes to the nucleus of primary tumor migrating cells demonstrates that its effect on cellular migration is mediated by its role as a transcriptional cofactor. Assessing the role of Ldb1 in Neu-induced metastasis might offer therapeutic opportunity to limit the spread of metastatic breast cancers.

## 5 The role of PAX2 in the etiology and progression of ovarian cancer

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PAX2 is a transcription factor that is essential for development. Aberrant expression of PAX2 in adult tissues is associated with carcinogenesis and experimental evidence shows that PAX2 generally exhibits oncogenic properties. Although PAX2 is not expressed in normal ovaries, it is highly expressed in low malignant potential and low-grade epithelial ovarian tumors, suggesting that PAX2 induction in ovarian surface epithelium (OSE) may contribute to transformation. Herein, we provide evidence that expression of PAX2 in normal murine OSE cells enhances their proliferation and survival and, when combined with loss of P53, induces tumorigenicity. When PAX2 was expressed in murine ovarian cancer cells, it enhanced or inhibited their aggressiveness, depending on the model system. In OSE cells transformed by K-RAS and c-myc, PAX2 inhibited P53 and apoptotic induction and increased the level of pERK1/2 and COX-2, all of which are enhancers of tumor aggressiveness. However, in a murine model of high-grade serous ovarian cancer, PAX2 expression slowed tumor progression and improved animal survival. Mechanistic studies showed that increased Htra1 and decreased COX-2 are associated with PAX2 expression in those tumors. Thus, PAX2 may not act as a classical oncogene or tumor suppressor; rather, it modulates tumorigenesis differently, depending on the tumor context.

## 6 IL-12 Mediated NK Cell Recruitment Improves Anti-tumour Efficacy in an Oncolytic Infected Cell Vaccine

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**Background:** Oncolytic Maraba MG1 virus (OV) is our best clinical candidate and it was designed to selectively replicate in tumour cells, with the primary objective of directly lysing cancer cells. It is becoming increasingly clear, however, that OV infection results in a profound inflammatory reaction within the tumour, initiating innate and adaptive immune responses against it and that is critical for the therapeutic benefit. This anti-tumour immunity appears to be mediated predominantly by natural killer (NK) cell and cytotoxic T cells. Interleukin 12 (IL-12) is a cytokine that induces proliferation and cytotoxicity of both NK cells and T cells. Direct intra-tumoural administration of IL-12 has been shown to stimulate a potent antitumor immunity.

**Hypothesis:** We have hypothesized that a replication competent oncolytic virus expressing IL-12 would generate high intra-tumoural levels of IL-12, enhanced anti-tumoural immunity and result in improved cancer efficacy.

**Methods and Results:** To enhance the immunostimulatory properties of MG1 virus, we used a reverse genetic technology to insert the murine interleukin-12 (IL-12) gene into the MG1 genome. We demonstrated that the virus has an equivalent in vitro cytotoxicity to the parental MG1 virus on a panel of murine and human cell lines. We also confirmed high levels of IL-12 expression in vitro and in vivo following infection with MG1-IL-12. Tumour infection with MG1-IL-12 virus results in a profound inflammatory reaction that initiates innate NK cell response that is critical for OV therapeutic benefit. MG1-IL-12 was able to induce NK cell IFN- $\gamma$  secretion in vivo by utilizing infected cell vaccine (ICV) platform. It also enhanced the ex-vivo cytotoxicity of human peripheral blood mononuclear cells (PBMCs) and mouse NK cells. In addition, it boosts the migratory capacity of NK cells in vitro and in vivo through the induction of IP-10 chemokine.

**Conclusions:** Our results demonstrated that MG1-IL-12-ICV has improved the tumour remission in CT26 peritoneal carcinomatosis model suggesting that MG1-IL-12 is a promising oncolytic immunotherapy with therapeutic efficacy secondary to IL-12 mediated NK migration and cytotoxicity.

## 7 Characterization of putative stem cells in fallopian tubes and their regulation by PAX2

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**Introduction:** Recent studies have provided evidence that oviductal epithelial cells (OVE) are a potential cell of origin for high-grade serous ovarian carcinoma. In addition to secretory and ciliated epithelial cells that express PAX2, undifferentiated stem-like cells expressing CD44 have been located in human fallopian tubes. Loss of PAX2 expression is recognized as an early event in epithelial transformation, but the role of PAX2 and the contribution of stem-like cells to cancer initiation are unknown. Thus, this study was designed to define the role of PAX2 in OVE cells and its response to ovulatory factors, characterizing specifically its potential involvement in the regulation of stem cell-like behaviours that may contribute to formation of cancer-initiating cells.

**Objective:** We aim to isolate and characterize mouse OVE cells and test the effects of inducing EMT and altering PAX2 levels on the "stemness" of these cells.

**Method:** Mouse OVE cells were established in culture and validated for PAX2 and PAX8 expression. OVE cells were treated with TGFβ1 and assessed for sphere forming capacity, acini formation, and stem cell gene expression using qPCR, flow cytometry and immunofluorescence. PAX2 expression was knocked down in OVE cells using shRNA and PAX2 expression was forced in ovarian surface epithelial cells (OSE), which do not normally express PAX2. Stem cell markers were analyzed in both of OVE cells with loss of PAX2 and in MOSE cells with PAX2 expression.

**Results:** OVE cells have a limited capacity to self-renew and form spheres when cultured in suspension, and they form acini-like structures in matrigel. Exposure to TGFβ increases the stemness characteristics, including expression of the stem cell markers, CD44 and Sca-1. This shift in phenotype is associated with suppression of PAX2 in OVE cells. In addition, PAX2 promotes the epithelial differentiation by inhibiting characteristics of stemness in OVE cells, particularly CD44 expression.

**Conclusion:** TGFβ1 enhances some stem cell characteristics through the suppression of PAX2, which we anticipate might render the cells more susceptible to transformation. PAX2 is an essential transcription factor for the epithelial differentiation of oviductal cells and loss of PAX2 decreases the stability of the differentiated state of OVE cells, and enhances their reversion to a more stem-like state.

## 8 The role of SLK in ErbB2-induced mammary tumorigenesis

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HER2/Neu/ErbB2 is an epidermal growth factor receptor, whose overexpression is implicated in approximately 30% of human breast cancers. The Ste-20 like kinase SLK, plays an important role in cell motility and migration. The role SLK plays in ErbB2-induced mammary tumorigenesis will be the focus of this fellowship application. Although there is an important link between ErbB2 and breast cancer invasion and metastasis, the mechanisms by which it contributes to these processes are not known. Recently, SLK has been shown to be activated by ErbB2 and required for heregulin chemotaxis, suggesting that migratory signals mediate SLK-dependant pathways. Therefore, we hypothesize that SLK is required for ErbB2-induced mammary tumorigenesis and metastasis in vivo and contributes to the increased invasiveness and metastatic potential of these tumours. Mammary tissue specific SLK-deficient mice are being used to study the effect of SLK in ErbB2-driven tumorigenesis, and analyzing the effect of SLK in ErbB2-induced metastasis and invasion. SLKfl/fl x NIC mice were generated and tumor onset is significantly delayed accompanied by an overall increase in survival as compared to NIC control mice. These tumors show an increase in epithelial markers accompanied by a decrease in mesenchymal markers suggestion a role for SLK in the epithelial-to-mesenchymal transition. As a result of this project, it is our goal to achieve a better understanding of the etiology of this significant cancer phenotype. These results will then hopefully lead to new targets for cancer therapies that will be more successful than those currently employed in treating this cancer, leading to increased quality of life and survival for cancer patients.

## 9 Oncolytic Synergy Through Dual Effects of Microtubule Disruption on Interferon Translation and Death by Virus-Induced Cytokines

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In this study, we show that several microtubule-destabilizing agents (MDAs) used for decades for treatment of cancer and other diseases also sensitize cancer cells to oncolytic rhabdoviruses and improve therapeutic outcomes in resistant murine cancer models. Drug-induced microtubule destabilization leads to superior viral spread in cancer cells by disrupting Type I IFN mRNA translation leading to decreased IFN protein expression and secretion. Furthermore, MDAs specifically promoted cancer cell death following stimulation by a subset of infection-induced cytokines, thereby increasing viral by-stander effects. This study reveals a previously unappreciated role for microtubule structures in the regulation of the innate cellular antiviral response and demonstrates that unexpected combinations of approved chemotherapeutics and novel biological agents can lead to improved therapeutic outcomes.

## 10 Recruitment and Activation of SLK at the leading edge of migrating cells requires Src family kinase activity and the LIM-only protein 4

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The Ste20-like kinase SLK plays a pivotal role in cell migration and focal adhesion turnover. We have previously shown that SLK kinase activity is regulated by the LIM domain-binding proteins Ldb1 and Ldb2. In addition to playing role in tumor initiation and progression, these adapter proteins have been demonstrated to interact with LMO4 in the organization of transcriptional complexes. Therefore, we have assessed the ability of LMO4 to also interact and regulate SLK activity. Our data show that LMO4 can directly bind to SLK and activate its kinase activity in vitro and in vivo. LMO4 can be co-precipitated with SLK following the induction of cell migration by scratch wounding and Cre-mediated deletion of LMO4 in conditional LMO4<sup>fl/fl</sup> fibroblasts inhibits cell migration and SLK activation. Surprisingly, deletion of LMO4 impairs Ldb1 and SLK recruitment to the leading edge of migrating cells. Supporting this, Src/Yes/Fyn-deficient cells (SYF) express very low levels of LMO4 and do not recruit SLK to the leading edge. Similarly, src-family kinase inhibition by PP2 impairs SLK recruitment to the leading edge of migrating fibroblasts. This suggests that both expression of LMO4 and the recruitment of SLK to the leading edge require c-src activity. Finally, re-expression of wildtype Myc-LMO4 in SYF cells, but not a mutant version, restores SLK localization and kinase activity. Overall, our data suggest that cell migration and activation of SLK by haptotactic signals requires its recruitment to the leading edge by LMO4 in a src-dependent manner. Furthermore, this establishes a novel cytosolic role for the transcriptional co-activator LMO4.

## 11 Generation of tumor-specific immune responses by oncolytic virotherapy

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**Background:** Oncolytic viruses are known to stimulate the immune system but the extent by which they revert tumor-induced immune suppression is not well characterized. By specifically replicating in tumor cells, they are believed to trigger immune activation through the release of tumor-specific antigens in the context of viral pathogen associated molecular patterns to allow for simultaneous TLR co-stimulation. It is already published that infection by oncolytic viruses, just like infection with other viruses, will activate NK cells and T cells. Also, various successful vaccination strategies using oncolytic viruses have already been described by our group and others, thus demonstrating the potential of oncolytic viruses as tumor-specific immune stimulators.

**Objective:** To demonstrate the general activation of immune cells as well as the reversion of tumor-driven immune suppression by treatment with vesicular stomatitis virus (VSV).

**Methods:** In this study, we used 4T1 mammary adenocarcinoma and CT26 colon carcinoma mice tumor models as well as flow cytometry, ELISPOT, qPCR and cytometric bead array.

**Results:** We observed a rapid and sustained upregulation of the pro-inflammatory cytokines IFN $\gamma$ , IL-6 and TNF $\alpha$  in the blood following treatment, demonstrating the well characterized general immune activation generated by the virus. Flow cytometry analysis showed a greater activation of dendritic cells, natural killer cells and T cells in the blood and the spleen of VSV-treated animals. Also, an ELISPOT assay allowed us to demonstrate the presence of an increased number of IFN $\gamma$ -secreting tumor-specific T cells following virus treatment. The results also show that the increased regulatory T cell numbers in tumor bearing mice versus naïve mice is reverted to basal levels following oncolytic virus treatment. As a further means to activate immune responses, oncolytic viruses can be engineered to encode immune-stimulating genes. We generated a panel of cytokine-expressing viruses and demonstrated the expression of the respective transgenes in the tumors of treated mice. Some of these viruses slowed tumor growth and prolonged survival in a resistant tumor model where the empty virus had no efficacy. The beneficial effects of these various transgenes were also demonstrated by the expansion of different cell populations as well as, in some cases, a greater

number of active tumor-specific T-cells and an increased CD8/CD4 ratio.

Conclusions: Taken together, these results show the great potential of oncolytic viruses as immune stimulators to generate a tumor-specific immune response as well as their potential in targeted gene therapy by expression of beneficial genes specifically within the tumor.

12 **Extracellular matrix-integrin  $\beta$ 1 signaling is a major mediator of epithelial-to-mesenchymal transition and contributes to prostate cancer invasion and metastasis**

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Within North America, prostate cancer is the most commonly diagnosed cancer in men with devastating implications deriving from metastasis, particularly those established in the bone. We have elucidated mechanisms by which prostate cancer undergoes metastasis with particular focus on the relationship between extracellular-matrix binding proteins, Integrin  $\beta$ 1 (ITGB1) and epithelial-to-mesenchymal transition (EMT). Previous results in the laboratory suggested that prostate tumor cells depleted of ITGB1 were unable to form colonies in soft agarose and were impaired in their invasive abilities in vitro. In order to determine the possible mechanisms by which ITGB1 controlled these phenotypes, we performed numerous genetic techniques including siRNA targeted depletion of ITGB1 and subsequent profiling of pathway specific message RNA by RTqPCR to monitor the effects ITGB1 depletion on factors known to control EMT which is also associated with cell invasion. We found that prostate tumor cells depleted of ITGB1 had altered expression of numerous genes associated with EMT or cell invasion, many of which have been validated by RT-qPCR and western blot. In particular, SLUG has been established as a target of ITGB1 regulation, in part via the ability of ITGB1 to regulate upstream factors associated with SLUG expression such as TGF $\beta$ 2. Of particular interest to prostate cancer bone metastasis, we further found that collagen I interacting with ITGB1 significantly upregulated this pathway, and given the known role of EMT in mediating resistance to anti-cancer agents, this finding could help elucidate novel targets for the development of new drugs to treat prostate cancer bone metastasis.

13 **TGF $\beta$ 1 increases stem cell characteristics in the ovarian surface epithelium by inducing an epithelial to mesenchymal transition and increasing Ptgs2 expression in a Smad2/3 dependent manner**

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Background: Ovulation is the primary non-hereditary risk factor for ovarian cancer. The ovarian surface epithelium (OSE) is a monolayer of cells surrounding the ovary that is ruptured during ovulation; improper healing of this wound may lead to ovarian cancer. We have shown that the OSE contains a Stem Cell Antigen1 (SCA1) expressing population that exhibits stem/progenitor cell characteristics such as sphere formation. These stem cell characteristics are enhanced by Transforming Growth Factor  $\beta$ 1 (TGF $\beta$ 1), a protein found in the follicular fluid, and we hypothesize that this increased "stemness" may be linked to ovulatory wound repair.

Methods/Results: Treating OSE cells with TGF $\beta$ 1 induces an epithelial to mesenchymal transition (EMT) through an 8-fold upregulation of the transcription factor Snai1. In the presence of a Smad2/3 inhibitor, TGF $\beta$ 1 does not upregulate Snai1 or induce EMT, indicating these actions are dependent on Smad2/3. SNAI1 overexpression in OSE cells induces an EMT and increases sphere formation. To identify other TGF $\beta$ 1 target genes, a PCR array was performed to compare gene expression in normal OSE vs. TGF $\beta$ 1 treated OSE. The array identified an 8-fold increase in Ptgs2 mRNA with TGF $\beta$ 1 treatment. This upregulation was also dependent on Smad2/3. Treatment with the PTGS2 product PGE2 increased stem cell markers Sca1 and CD44 mRNA levels (1.4 and 2-fold respectively), similar to TGF $\beta$ 1 treatment (5 and 3-fold respectively).

Conclusion: These data suggest that at ovulation, TGF $\beta$ 1 increases the OSE stem cell population through the canonical Smad2/3 pathway to induce an EMT and increase Ptgs2 expression. Expansion of this stem cell population may promote ovulatory wound repair and dysregulation of this mechanism may underlie the initiation of ovarian cancer.

- 14 **Investigating the role of FGL2 in placental development and preeclampsia**  
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#### Background

Preeclampsia, a disease that affects 2-8% of all pregnancies, is thought to be caused, in part, by impaired trophoblast invasion during placental development. Establishing an immune balance during pregnancy is essential to this process, and there is evidence that Fibrinogen-like protein 2 (FGL2) is involved in the regulation of this balance.

Previous data in our lab has shown, in mice, that chromatin-remodelling protein SNF2L regulates FGL2 expression in the ovary. Because SNF2L is also expressed in other reproductive organs, we hypothesized that a similar regulatory relationship exists in the placenta. Therefore, mice with inactivated SNF2L (SNF2L Ex6DEL) are used to study the role of FGL2 in the placenta.

#### Objective

To investigate the role of FGL2 in placental development and preeclampsia.

#### Methods

Using SNF2L Ex6DEL mice, the impact of SNF2L inactivation on reproductive capacity will be evaluated. A potential relationship between SNF2L and FGL2 will be investigated. Using human trophoblast cell lines and placental explants and samples, the role of FGL2 in placental development will be investigated. A placenta-specific knockout of FGL2 in mouse blastocysts will allow for evaluation of the role of FGL2 in the development of preeclampsia.

#### Results and conclusions

Data to date suggest a modest impairment of reproductive capacity in SNF2L Ex6DEL mice. Trophoblast invasion occurs, in mice, around e12.5, which coincides with an observed peak in placental FGL2 expression, leading us to believe in its importance during invasion. This is reinforced by the finding that in vitro, FGL2 expression is higher in HTR-8/Svneo trophoblasts (invasive) than in BeWo trophoblasts (non-invasive). We observe significantly lower FGL2 expression at e12.5 in SNF2L Ex6DEL mice, suggesting that inactivation of SNF2L inhibits FGL2 activity in the placenta, leading to impaired trophoblast invasion. A significant decrease in fetal and placental weights are also seen at e12.5, but not later in pregnancy, suggesting that impaired invasion causes a delay in fetal and placental development.

Future work includes knockdown and overexpression of FGL2 in trophoblast cell lines and placenta-specific knockdown of FGL2 in mouse embryos.

- 15 **SLK-Mediated Breast Cancer Motility and Invasion through Transforming Growth Factor  $\beta$ -Induced Epithelial-to-Mesenchymal Transition**

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**Background:** Cancer metastasis is the cause of 90% of all cancer deaths in patients. In addition, 30% of breast cancer patients overexpressing an epidermal growth factor receptor called HER2 have been shown to present with a more invasive and metastatic form of cancer. Metastasis can be stimulated by a process called EMT (epithelial-to-mesenchymal transition), where epithelial cells located on the periphery of tumors transition into a migratory phenotype and break free into the body's blood and lymph systems. The Ste20-like kinase, SLK, has been highly implicated in the process of cell migration and has been shown to be involved in signaling pathways downstream of the HER2 receptor.

**Objective:** The goal of this study is to examine the relationship between SLK signaling and the EMT pathway.

**Methods:** Immunofluorescence analyses as well migration and invasion assays were used to elucidate the potential relationship between SLK and the EMT process. In addition, kinase assays were used to determine SLK response to EMT stimulation. We also look at genetic targets of EMT in an SLK-null system. Finally, we express dominant negative forms of possible upstream SLK targets to elucidate a signaling pathway.

**Results:** It was determined that in SLK knockdown conditions, there is a decrease in the cell's ability to progress into EMT, indicating that SLK activity is involved in downstream EMT signaling. In addition, complete loss of SLK results in a significant decrease in the migratory and invasive capacities of cells when EMT is induced using transforming growth factor  $\beta$ . Finally, SLK is shown to be downstream of CDC42 signaling implicated in the non-canonical EMT pathway.

**Conclusions:** Our results demonstrate that SLK either prevents cell migration, or prevents cells from transitioning into a mesenchymal phenotype. This study identifies SLK as a molecular target in TGF $\beta$ -induced epithelial-to-mesenchymal transition.

## 16 **Snf2h-mediated chromatin remodelling is essential for the maintenance of embryonic stem cell identity**

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### Background

Chromatin dynamics play a vital role in the establishment and maintenance of cell identity gene expression programs. Numerous histone modifiers, chromatin remodelling complexes, and structure protein complexes coordinate to regulate these dynamics. Snf2h is an ATP-dependent chromatin remodelling enzyme that is expressed fairly ubiquitously throughout the body. Its expression is essential for the viability of the peri-implantation embryo, and has also been shown to regulate stem cell populations in ovarian follicles and the developing cerebellum. While its importance is evident, its precise function in these cells is unclear.

### Objective

The aim of this project is to understand how Snf2h is recruited to coordinate unique gene expression programs. It is also of particular interest to understand global mechanisms governing cell type-specific recruitment of ubiquitous chromatin regulators.

### Methods

To understand the role of Snf2h in regulating cell identity, we established a mouse embryonic stem cell line from Snf2hfl/fl mice (Snf2hfl/fl-mESC). Knockout of Snf2h was accomplished through introduction of Cre recombinase or GFP by adenoviral infection (AdCre or AdGFP, respectively). Colony morphology was monitored and cells were screened for the expression of pluripotency factors. To assess genome-wide localization of Snf2h, we analyzed ChIP-seq datasets from mammary epithelial cells and neural progenitor cells. High-confidence peaks were called with a p value threshold of 1e-09. Common peaks were defined as peaks from each dataset whose alignments have a minimum 1bp overlap. Gene ontology term enrichment was calculated using DAVID (Database for Annotation, Visualization and Integrated Discovery).

### Results

Snf2hfl/fl-mESCs infected with AdCre form considerably fewer pluripotent colonies than those infected with the AdGFP vector control. Genotyping of these cells confirmed the presence of Cre-mediated recombination, and there was no difference in viability between cells infected with AdCre or AdGFP. This suggests Snf2h is not required for general survival, but may be important for the regulation of cell state. Analysis of ChIP-seq data showed that the localization of Snf2h is largely cell type-specific, and that it is enriched at genes associated with the source cell's identity.

### Conclusions

These data support our model of Snf2h regulating the mESC gene expression program, and its inactivation resulting in failure to maintain a pluripotent state. ChIP-seq analysis will be required to discover its regulatory targets. It will be important to understand the global mechanisms that govern cell type-specific recruitment of chromatin regulators. This knowledge will allow us to comprehend the aberrant epigenetic regulation of diseased states, such as cancer.

## 17 **Identifying Novel Mechanisms of Innate Viral Immunity**

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### BACKGROUND:

Oncolytic viruses are promising new cancer therapeutics that selectively infect and kill cancer cells without affecting normal tissues, thereby limiting side effects. However, as these novel agents advance through clinical trials, it is clear that while some patients show remarkable responses and even cures, most patient's cancers are resistant to oncolytic virotherapy and cannot be treated.

### OBJECTIVE:

We aim to understand the genetic and epigenetic differences that exist between cancers that are sensitive compared to those that are resistant to oncolytic virotherapy. This will allow for better prediction of oncolytic virotherapy outcomes, and possibly provide gene targets that can be manipulated using small molecules, with the aim of being able to consistently switch resistant cancers into a treatable state.

**METHODS:**

CT26-WT, a mouse colon carcinoma, is resistant to oncolytic vesicular stomatitis virus (oVSV). However, CT26-LacZ, a transgene-expressing subclone created from CT26-wildtype is in contrast highly sensitive to oVSV In vitro and In vivo. Using H3K4me2 Chip-Seq data we were able to quickly identify through transcription factor motif searches that the JAK-STAT pathway transcription factor ISGF3 was inactive in CT26 LacZ. Microarray analysis supports this data showing a significant dysregulation of transcription in the majority of downstream genes of ISGF3. Biochemical assays are now being performed tracing up the JAK-STAT pathway to determine the cause of this complete loss of signalling.

**RESULTS:**

Recent data suggest that the interferon receptor (IFNAR1/2) is being inhibited through a currently unrecognized mechanism causing complete inhibition of the JAK-STAT signalling cascade CT26 LacZ.

**CONCLUSIONS (Future Work):**

In future work we plan to validate our most recent findings to and to create a reproducible model In Vitro and In Vivo. Additionally, we aim to develop a small molecule to reproduce this phenotype in a therapeutically relevant manner. Finally, we aim to test various resistant and sensitive tumours to determine whether this target can be predictive as a marker for treatment outcome of oncolytic virus therapy.

**18 Use of Microtubule Destabilizers for Improving Oncolytic Virotherapy**

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**Background**

Vesicular stomatitis virus (VSV) is a negative-stranded RNA virus that is well suited as an oncolytic virus (OV) backbone as it does not undergo genetic recombination or integrate its genome into the host. Moreover, normal cells are resistant to VSV infection by innate response mechanisms that most tumour cells lack. VSVd51 contains an attenuating deletion in its matrix (M) protein gene, which plays a key role in virus-host cell interactions. This deletion restricts viral replication to cells with a defective interferon response, namely cancer cells. However, some tumors remain resistant to OV infection. Our group has previously shown that VSVd51 spread and replication can be enhanced in tumors when combined with microtubule-targeting (MT) agents. Despite the use of MT agents in cancer therapy, their narrow therapeutic window remains a limitation.

**Objective**

We hypothesize that combining VSVd51 with a MT destabilizing agent (MDA) with a broader therapeutic window would allow for increased OV spread and replication while maintaining tumour selectivity.

**Results**

We propose to assess the ability of novel MDAs to enhance VSVd51 spread, replication, and cytotoxicity in tumour cell lines to determine if they are well suited to improve OV therapy.

**Conclusions**

The combination of VSVd51 MDAs could improve OV spread and replication in resistant tumour cell lines. Furthermore, there would be an added advantage to delivering the microtubule disrupting agent only to the tumour cells which could potentially limit cytotoxicity in normal tissues.

**19 PTEN represses glioblastoma tumor initiating cell differentiation via the polarity proteins aPKC and Lgl**

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**Background:** Glioblastoma is the most deadly form of brain cancer with a median survival time of approximately one year. A subset of glioblastoma cells, known as glioblastoma tumor initiating cells (GTICs), has an essential role in the malignancy of this disease. Similarly to neural stem cells, GTICs express stem cell markers and are able to differentiate along multiple lineages. Differentiation of GTICs appears to be accompanied by a loss of malignant behaviour and thus may hold therapeutic potential. Atypical protein kinase C (aPKC), acting on its substrate Lgl, has previously been shown to regulate *Drosophila* neuroblast self-renewal over differentiation.

Objective: Investigate the mechanism of Lgl inactivation in glioblastoma and determine the role of Lgl in inhibiting glioblastoma malignancy (invasion, proliferation, differentiation status).

Methods and Results: The phosphorylation status of Lgl was assessed using an antibody which recognizes protein phosphorylated at a serine or threonine within the PKC consensus amino acid sequence. A serine to alanine mutant form of Lgl (Lgl3SA) in which the known atypical PKC phosphorylation sites were mutated was not phosphorylated. Both the knockdown of atypical PKC  $\zeta$  and expression of PTEN resulted in decreased phosphorylation of Lgl. Lineage specific differentiation of the GTICs was assessed by immunofluorescence detecting TUJ1 (neuronal-lineage) and GFAP (astrocytic-lineage). Expression of Lgl3SA as well as PKC  $\zeta$  knockdown and PTEN expression resulted in increases in the proportion of TUJ1+ (neuronal-lineage) cells. To evaluate the importance of this pathway in vivo, we engineered GTICs for doxycycline-inducible expression of constitutively active Lgl3SA. These cells were injected intracerebrally into immunocompromised SCID/Beige mice. Mice were then randomized to chow with or without doxycycline and the effects on GTIC differentiation and invasion were assessed. In vivo induction of constitutively active Lgl3SA significantly increased differentiation of GTICs along the neuronal lineage. In addition, it significantly decreased invasion of GTICs into the contralateral hemisphere.

Conclusions: That PTEN loss, acting via PKC  $\zeta$  and Lgl, has a key role in maintaining GTICs in an undifferentiated, highly malignant state both in cell culture and in vivo.

## 20 Induction of Activating Transcription Factor 3 is associated with cisplatin cytotoxicity in NSCLC

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Platins are one of the most widely employed chemotherapeutic agents in North America and are used for the treatment of a variety of cancers, including NSCLC. The effectiveness of platins is reduced due to acquired or intrinsic resistance to these agents in many cancers. The goal of this study is to identify treatment induced biomarkers of platin activity that may predict response to this important class of chemotherapeutic agents, as well as to identify potential combination therapies that may overcome platin resistance. Using RNAseq transcriptome analysis we compared two parental NSCLC cell lines (Calu6 and H23) to their resistant sub-lines which were derived following exposure to high dose cisplatin. This analysis identified a stress pathway consisting of GADD45a, ATF3 and DDIT3/CHOP that was specifically induced in cisplatin treated parental cell lines but not in the resistance clones. Furthermore, ATF3 was not expressed in untreated parental or resistant clones but was robustly induced only in the parental sensitive cell lines following cisplatin treatment. Cisplatin induced MAPK-JNK activation, a regulator of ATF3 expression, was also attenuated in the resistant cell lines. In ex-vivo NSCLC tumours, ATF3 was induced in 2/4 tumours evaluated but not in their corresponding normal adjacent lung tissue (0/4) following cisplatin treatment, indicating a potential utility as a predictive biomarker of platin response that requires further study.

## 21 GREB1 is a potential mediator of estrogen effects on ovarian cancer progression in a mouse model

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Background: Hormone replacement therapy containing estrogen increases the risk of developing ovarian cancer, and 17 $\beta$ -estradiol (E2) promotes growth and survival of ovarian cancer cell lines.

Objective: We have shown previously that E2 promotes ovarian cancer progression in transgenic and allograft mouse models, and now investigate the mechanism of E2-stimulated tumour progression.

Methods: To identify genes altered by E2 treatment, we used an allograft model in which SCID mice were implanted with E2 pellets and injected with mouse ovarian cancer cells (MAS) derived from the ascites of the tgCAG-LS-TAg transgenic mouse model of ovarian cancer. One E2-upregulated gene of particular interest is Growth regulation by estrogen in breast cancer-1 (Greb1). GREB1 is an estrogen receptor (ESR1) target gene which mediates the proliferative actions of hormones in human breast and prostate cancer cells. In order to investigate the function of GREB1, we used lentiviral vectors to cause knockdown and overexpression in

MAS cells.

Results: Survival of mice engrafted with MAS ovarian cancer cells was shortened by E2 treatment and microarray analysis showed upregulation of 197 genes and downregulation of 55 genes in tumours from E2-treated mice. QPCR confirmed upregulation of Greb1 and other genes of interest in tumours from E2-treated mice and cultured MAS cells as well as two human ovarian cancer cell lines. MAS cell proliferation was decreased by GREB1 knockdown and increased by GREB1 overexpression. When injected into SCID mice, MAS cells with GREB1 knocked down resulted in fewer metastases and mice had prolonged survival relative to mice injected with control MAS cells. GREB1 is highly expressed in human ovarian tumours of 4 histological subtypes relative to normal ovarian epithelial cells (on average, 347-fold higher in tumours). GREB1 levels correlate with ESR1 expression in these tumours, suggesting that GREB1 may be regulated by ESR1 in ovarian cancer, as it is in breast cancer.

Conclusions: This study is the first to examine GREB1 action in mouse models and its expression in ovarian cancer cell lines and tumours. Characterization of the function of E2-target genes will elucidate the mechanisms by which E2 increases the risk of ovarian cancer and help clarify the effects of estrogen antagonists on ESR1-positive ovarian cancers.

## 22 Tailored Oncolytic viral Therapeutics for Pancreatic Cancer

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Pancreatic Carcinoma (PCa) has the highest mortality rate of all the major cancers –94% of patients die within 5 years of their diagnosis and 75% of patients die within the first year, statistics that have not changed in the last 30 years. The unique biology of PCa including its unusually fibrotic nature contributes to what is largely a treatment refractory disease. It is now clear that Cancer Associated Fibroblasts (CAFs), the predominant component of PCa stroma contribute to tumor growth, angiogenesis, metastasis and acquired resistance to therapy. CAFs are epigenetically and biologically distinct from their normal fibroblast counterparts suggesting that they are potential new therapeutic targets for PCa. In this regard, we have previously shown that several oncolytic virus (OV) platforms can replicate specifically in PCa tumour cells and their supporting CAFs but not in normal fibroblasts. The aim of our research is to select/engineer oncolytic viruses that have enhanced capacity to replicate in and kill both PCa tumour cells and the CAFs that support their malignant growth/progression. Our research strategy uses a novel screening approach that links selective virus replication to expression of an artificial microRNA (amiRNA). We have conducted an amiRNA-encoding virus library screen in cultures of pancreatic cancer cells, normal fibroblasts, and CAFs. Cell selected virus populations are being analyzed by deep sequencing and bioinformatics analysis to identify amiRNAs that are enriched on PCa cells and/or CAFs but are not enriched on normal fibroblasts. Our preliminary data shows that amiRNAs expressed from a virus backbone can improve virus growth in certain cell types suggests that potentially amiRNAs can be selected to further enhance growth of OVs in PCa tumour cells and CAFs.

## 23 A randomized, double-blind trial evaluating the palliative benefit of either continuing pamidronate or switching to zoledronate in patients with high risk bone metastases from breast cancer (The Odyssey Study)

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Background:

Questions remain around the optimal use of bone-targeted agents (BTA) in patients with bone metastases (BM) from breast cancer (BC). In Canada pamidronate (PAM) is the most commonly used BTA in BC. We explored whether a switch to a more potent BTA, like zoledronic acid (ZA), in patients who remain at high risk of skeletal related events (SREs) despite PAM use is associated with significant palliative benefit.

Methods:

BC patients with high risk BM (prior SRE, bone progression, bone pain or levels of bone turnover marker serum C-telopeptide (sCTX) >400ng/L) despite >3 months of PAM use were eligible. Patients were randomized in a double-blind manner to either switch to ZA or continue on PAM every 4 weeks for 12 weeks. Primary outcome was the proportion of patients achieving a fall in sCTX at 12 weeks. Secondary outcomes were pain control (BPI and FACT-BP) and toxicity.



**Results**

73 patients completed the study. Median age 61 years (range 37 – 87), prior duration of PAM use 10 months (range 3 – 118). sCTX levels for all patients at baseline, 372 +/- 471, week 12, 209 +/-290. Proportion of patients achieving a fall in sCTX from week 0 to week 12, 26/31 (84%) in ZA arm, 17/30 (57%) in PA arm, p=0.0262. Two patients were unable to complete the study due to deterioration in renal function (both receiving PAM), four due to progressive disease (two receiving ZA, two PAM), two patients chose to discontinue the study before completion (both receiving ZA). Four patients (5%) had SRE's during the study, two receiving ZA, two receiving PAM. Quality of life and pain analysis shows no difference between week 0 and week 12 scores in either arm. Toxicity was predominantly grade 1 and 2, numerically there were more adverse effects in the ZA arm than PAM.

**Conclusion**

Switching patients with high risk BM from PAM to ZA leads to a reduction in sCTX levels but may be associated with more toxicity. Quality of life and pain scores were similar between the two treatments. While the literature suggests that a reduction in sCTX may correlate with reduced rate of SREs, given the lack of symptom improvement a switching strategy cannot be recommended. NCT01907880

## 24 **Overcoming tumour-induced immunosuppression using an oncolytic VSV expressing miR155**

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**Background:** Tumour-induced immunosuppressive mechanisms in the tumour microenvironment (TME) are one of the major reasons for the limited current success of therapeutic cancer vaccines. Various immunosuppressive cells have been identified within the TME including myeloid-derived suppressor cells (MDSC), tumour-associated macrophages (TAM) and regulatory T cells all of which contribute to the induction of tumour induced tolerance and prevent the successful eradication of tumour cells. Therefore, reversing the tumour-induced immune tolerance will be critical to generate effective anti-tumour immunity. Oncolytic viruses (OV) specifically replicate in and lyse tumour cells. In addition to direct cytotoxicity, OV stimulate the immune system and facilitate the generation of anti-tumour immune responses. The generation of this anti-tumour immunity following oncolytic virotherapy has shown to be critical for successful therapy in numerous pre-clinical models. The ability of OV to overcome immunosuppressive and tolerogenic mechanisms has yet to be fully characterised, and like other cancer immunotherapies may to the stumbling block for effective translation in the clinic.

MiRNAs are small endogenous non-coding RNAs implicated in the post-transcriptional control of gene expression and are critical regulators of virtually all immune cell types. Modulating the activity of miRNAs provides opportunities for novel cancer interventions. However, low bioavailability and poor cellular uptake are major challenges for delivering miRNA mimetics specifically to cells within the TME.

**Objective:** Determine whether expressing immunomodulatory miRNAs, such as miR155 from OVs will lead to enhanced anti-tumour immune response compared to OV alone.

**Method:** The mature MiR155 sequence was constructed into the miR30 cassette and cloned into an oncolytic Rhabdovirus, VSV?51 (VSV-155).

**Results:** Mature MiR155-5p is expressed in tumour cells following VSV-155 infection. Initial studies using ovarian cancer patient's ascitic cells (containing both tumour and immune cells) indicate that miR155 is productively expressed from VSV-155 as a 7.3-fold increase in IFN gamma was detected in supernatants from VSV-155 infected cells (miR155 is a positive regulator of IFN gamma secretion) compared to VSV?51. The ability of VSV?51 and VSV-155 to generate an anti-tumour immune responses have been investigated in in vitro priming experiments, these experiments demonstrate that VSV-155 generates an increased anti-tumour T cell responses compared to VSV?51. While, tumour bearing mice treated with VSV-155 demonstrate a reduced tumour burden and an increased anti-tumour T cell response compared to VSV?51 treated mice.

**Conclusions:** VSV ?51 expressing miR155 demonstrates an increase anti-tumour efficacy compared to VSV ?51 by enhancing anti-tumour immune priming.

## 25 **Tailored oncolytic viral therapeutics for the treatment of malignant melanoma by harnessing recombination evolution.**

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**Background:** Malignant melanoma is the most sinister form of skin cancer due to its propensity to become aggressively metastatic

in advanced stages. The incidence of malignant melanoma has been increasing for the past several decades, especially in countries with predominantly fair-skinned individuals. Oncolytic viruses are viruses that have the ability to specifically infect and replicate in cancerous cells by taking advantage of their unique biology. The use of these viruses is a relatively under-explored area of therapeutic development that could be refined for more effective treatments against malignant melanoma and other cancers.

**Objective:** Our main objective is to exploit the natural tendency of viruses to evolve through recombination according to Darwinian principles in an effort to design a specific and oncolytic viral backbone.

**Methods:** This study aims to take advantage of the natural propensity for different strains of vaccinia virus (VacV) to evolve through recombination when co-infecting the same cell. Wild-type VacV strains of Copenhagen, Western Reserve, Lister, and Wyeth were used to co-infect HeLa cells at an MOI of 10. After 24 hours of infection, viruses were isolated and purified, and new viral titres were obtained (passage 1). These were then used to infect fresh HeLa cells. After 3 passages, the resulting viral libraries were purified and deep sequenced. Sequences will then be compared with those of original viruses to determine genetic contributions by parental viruses and the frequency of recombination events. Cores of fresh melanoma surgical specimens from multiple patients will then be treated in tissue culture with the novel library to select for viruses that preferentially propagate within fresh human tumour tissue. With novel virus backbones identified, we will further study these viruses and determine manipulations that could lead to more oncolytic and potentially immunogenic agents.

**Results:** Recombination events are known to be common when co-infecting host cells with VacV at high MOI. This methodology is effective in the generation of a diverse viral library. Thorough data analysis will provide us with a strong understanding of the frequency of recombination events in this context as well as an understanding of the contribution of various genetic elements to VacV phenotype.

**Conclusions:** We have taken advantage of the natural tendency for different strains of VacV to evolve according to Darwinian principles through genetic recombination. This approach has the potential to yield important lessons regarding the fundamental biology of VacV and could be tailored to select for other cancer-specific VacV-based oncolytics.

## 26 **The Role of LIM-domain binding 1 (Ldb1) in Muscle Development**

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**Background:**

The LIM-domain binding 1 (Ldb1) is a co-transcription factor for LIM only proteins. It is known that Ldb1 can negatively regulate the Canonical Wnt signaling pathway. Recent experiments conducted in our laboratory showed expression of Ldb1 in cycling and differentiating myoblasts.

**Objective:**

Given its role in the Wnt/ $\beta$ -catenin signalling pathway, we believe it has a role in regulating myogenesis. Our objective is to determine if the overexpression and knockdown of Ldb1 has any effects on the process of myogenesis and the localization of target proteins

**Methods:**

Using western blotting analysis along with immunofluorescence we will be able to see any differences between the overexpression and the knockdown of Ldb1 in C2C12 myoblasts.

**Results:**

Knockdown of Ldb1 showed a decrease in differentiation while the overexpression showed an increase. The localization of  $\beta$ -catenin seem to remain nuclear in the knockdown model. We assessed the expression of Ldb1 throughout the differentiation time course and the expression of Ldb1 was localize in the cytoplasm. The expression levels did not change during the time course.

**Conclusions:**

Our data suggests that Ldb1 is essential for myogenesis in the C2C12 cell line. Further work needs to be done to verify this finding in primary myoblasts.

27 **Surgery-induced vaccine dysfunction in a murine B16 melanoma model**

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**Background**

Surgery resection is the leading treatment of most solid tumours but surgical stress creates an immunosuppressive environment that promotes tumour recurrence and metastases. A global reduction in T cell numbers and function post-surgery has been documented in preclinical studies and cancer patients however, the effects on tumour associated antigen (TAA)-specific T cells remains unclear. Preclinical studies from our lab have demonstrated that complete protection from tumour challenge, conferred by a TAA encoded vaccine, is rendered ineffective post-surgery in both a prophylactic and a therapeutic murine melanoma model.

**Objective**

The objective is to evaluate the impact of surgical stress on TAA-specific adaptive T cell immunity. Specifically, the number, distribution and function of antigen-specific cytotoxic T lymphocytes (CTLs) in a prophylactic and therapeutic murine B16 melanoma model of surgical stress.

**Methods**

**Prophylactic model:** C57BL/6 mice were immunized (intramuscular) with AdhDCT, a non-replicating adenovirus expressing human dopochrome totaumerase (hDCT), a melanoma TAA. Seven days post-AdhDCT injection, mice were challenged with B16 tumor and remained untreated or underwent an abdominal nephrectomy to induce surgical stress. The function and number of DCT-specific CTLs was quantified with intracellular staining for IFN $\gamma$ , TNF $\alpha$  and Granzyme B by flow cytometry. The total number of T-cells and DCT-specific T cells was assessed using anti-CD3, anti-CD8 and a customized DCT-loaded MHC 1 tetramer. The number of DCT-specific CTLs undergoing apoptosis was determined using Annexin V and 7-amino-actinomycin D viability stain. Lastly, proliferation was assessed by BrdU incorporation following antigen stimulation. **Therapeutic Model:** B16lacZ cells were implanted subcutaneously 7 days prior to AdhDCT vaccination and 14 days prior to surgery. DCT-specific IFN $\gamma$  and TNF $\alpha$  production was assessed by flow cytometry.

**Results**

**Prophylactic model:** Surgical stress significantly attenuates proportion ( $p < 0.001$ ) and absolute numbers ( $p < 0.05$ ) of total IFN $\gamma$ + DCT-specific CTLs by over 2-fold in the prophylactic model. Similar results were detected for TNF $\alpha$  and Granzyme B. DCT-specific tetramer stain established that surgery does not affect the proportion of DCT-specific CTLs. There was no significant difference among apoptotic CTLs in untreated mice and those undergoing surgery but a postoperative attenuation in proliferation was observed. **Therapeutic Model:** Total IFN $\gamma$ + and TNF $\alpha$ + DCT-specific CTLs were attenuated following surgical stress.

**Conclusion**

Our results suggest that surgical stress does not cause antigen- specific T cell apoptosis but does attenuate proliferation and function associated with preoperative vaccination. Understanding the mechanisms of T cell dysfunction following surgery will facilitate the development of targeted immunotherapies to reverse this effect.

28 **Bone-targeted Therapy Use in Metastatic Lung Cancer: A Systematic Review**

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**Background**

Lung cancer is now the most common global malignancy. Patients frequently present with advanced, incurable disease with a median survival of 1 year. Bone is one of the most common sites of metastases occurring in 30-40% of patients. Bone metastases are associated with significant morbidity and reduced quality of life. However, unlike other malignancies such as breast and prostate cancer the use of bone-targeted therapies (e.g. bisphosphonates and denosumab) in this patient population has not been extensively studied.

**Objective**

A systematic review was conducted to explore whether or not there is any evidence from randomized controlled trials (RCTs) to support the routine use of bone-targeted therapy in patients with bone metastases from lung cancer. The endpoints of interest were: incidence of skeletal related events (SREs) (i.e. radiotherapy/surgery to bone, fractures, hypercalcaemia), overall survival, progression-free survival, quality of life and bone pain.

**Methods**

Medline (1946-June 2014), Embase (1947-June 2014) and Cochrane CENTRAL (May 2014) databases were searched for English-language RCTs looking at the use of bone-targeted therapy in patients with metastatic lung cancer. Using pre-defined criteria, two reviewers independently screened citations and full text articles. Data extraction was performed by two reviewers independently. Random effects meta-analyses of clinically important outcomes were planned, provided homogeneous studies were identified.

**Results**

A total of 263 articles were identified from the initial database search. Following abstract screening, 31 citations were retained for review of the full texts. Of these, 15 met the inclusion criteria and were retained for analysis. A total of 4840 patients participated in the included studies, 2580 of those included had a primary diagnosis of lung cancer. Characteristics of studies included: nine studies of bisphosphonate versus placebo, three studies of bisphosphonate versus alternate placebo, two studies of denosumab versus bisphosphonate and one study tested bisphosphonate versus an alternate agent (strontium 89).

**Conclusion**

We have identified a small number of studies reviewing the use of bone-targeted agents in metastatic lung cancer. Many of these trials include patients with multiple malignancies. The trials are heterogenous in the agent tested, whether versus placebo or alternate bone-targeted agent. Further analysis as to the evidence to support the use of bone targeted agents in metastatic lung cancer is pending.

## 29 **Management of urogenital atrophy in breast cancer patients - A systematic review**

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**Background:**

Urogenital atrophy can occur due to both natural menopause and also as a consequence of cancer therapies (e.g. endocrine therapy or chemotherapy-induced ovarian failure). Around 75% of breast cancer survivors experience at least one urogenital symptom (i.e. dryness, dyspareunia, itching, discharge, urinary incontinence, recurrent urinary tract infections and pain). The management of urogenital atrophy has become an increasingly important and challenging issue given the improved longevity of breast cancer patients and increasing recommendations for prolonged use of endocrine therapy for up to 10 years. There is concern about using hormone-replacement therapy (HRT) in patients with poorly controlled urogenital symptoms as these products are associated with increasing serum estradiol, possibly increasing the risk of breast cancer recurrence. Given that the evidence to support or refute the use of HRT in this population remains undetermined, treatment options for urogenital atrophy remains a challenging issue for patients and clinicians. A systematic review will be conducted to assess the optimal management strategies for urogenital atrophy.

**Methods:**

EMBASE (1947-September 2014) and Medline (1946-September 2014) were searched for English-language randomized clinical trials reporting urogenital atrophy treatment options. Using pre-defined criteria, two reviewers independently screened citations and full text articles. Data extraction will be performed to assess the optimal type, dose and safety of urogenital treatment.

**Results:**

A total of 324 citations have been identified. Seven relevant citations fitted the criteria. Four full-text articles and 1 abstract will be included in the meta-analysis. Sample size ranged from 7 to 81. Outcome data will be collated and presented.

**Conclusion:**

Determining optimal management strategies for urogenital atrophy is critical to quality patient care. The purpose of this systematic review is to explore if there is high quality evidence to evaluate topical and systemic treatment options for urogenital atrophy. In turn, this will allow for the identification of possible treatment recommendations as well as gaps in evidence that should be addressed in future clinical trials.

## 30 **A new spontaneously transformed syngeneic model of high-grade serous ovarian cancer with a tumor-initiating cell population**

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**Background and objective:**

High-grade serous ovarian cancer (HGSC) is the most common subtype of ovarian cancer with a survival rate <25% in patients with stage III-IV disease. Understanding HGSC etiology has been hindered by a controversy over the origin(s) of HGSC. New spontaneous models of HGSC are essential to understand HGSC origin and to provide better tools to test novel immune therapies. This study highlights the development of a new spontaneous and syngeneic murine model of HGSC.

**Methods and results:**

The M0505 cell line our lab established in 2005 from mouse ovarian surface epithelial (OSE) cells spontaneously transformed during long-term culture into the spontaneously transformed OSE (STOSE) cell line. STOSE cells form colonies in soft agar and have a greatly increased growth rate compared to their parental M0505 counterpart. Microarray analysis in conjunction with Ingenuity Pathway Analysis revealed differential gene expression in 2958 genes, many of which are associated with the Nf- $\kappa$ B and Wnt/ $\beta$ -Catenin signaling pathways, as well as expression changes in Ccnd1 and Cdkn2a consistent with human HGSC identified by The Cancer Genome Atlas. STOSE cells produced HGSC-like tumors indicated by a pancytokeratin+, WT1+, inhibin- and PAX8+ histotype, in both SCID and syngeneic FVB/N studies. The STOSE cell line also contains a stem-like SCA1+ population that formed tumors faster than SCA1- cells in FVB/N mice.

**Conclusion:**

This study identifies STOSE cells as the first spontaneous murine model of HGSC and provides a novel opportunity to study how a normal stem cell could become tumor-initiating cells, highly relevant to improving our understanding of HGSC.

### 31 **Mevalonate Pathway Inhibitors Enhance EGFR-TKIs Efficacy in Head and Neck Squamous Cell Carcinomas**

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The epidermal growth factor receptor (EGFR) is highly expressed in head and neck squamous cell carcinomas (HNSCC), which is associated with advanced disease. The prognosis for advanced disease using conventional therapies is dismal, thus, novel therapeutic approaches are urgently required. Tarceva, a clinically advanced EGFR tyrosine kinase inhibitor (TKI), can target EGFR by preventing ligand induced receptor activation in HNSCC cells. However, HNSCC patients do not show clinical benefit with Tarceva employed as a single agent. Expanding the therapeutic efficacy of EGFR-TKIs in this setting will likely require the addition of other agent(s) in combination. Our laboratory was the first to demonstrate that targeting the mevalonate pathway with statins induced synergistic cytotoxicity with Tarceva in HNSCC cells likely through their ability to inhibit the activation and downstream signaling of the EGFR in a mechanism distinct from EGFR-TKIs. This work led to phase I clinical trial combining Rosuvastatin with Tarceva that has just been completed. This trial showed the feasibility of this approach, however, significant statin-induced muscle toxicities were observed. Alternative strategies that recapitulate the efficacy without the statin-induced toxicities are warranted. In this study, we will emphasis on evaluating downstream mevalonate pathway inhibitors and identify rationale targets that can dissociate the efficacious effects from the toxicities inherent with high dose statin treatment. We have focused on targeting a downstream enzyme of this pathway, GGTase I, and demonstrated that inhibiting its function with GGTI-298 can also induce synergistic cytotoxicity with erlotinib in HNSCC cells. We believe that targeting this downstream mevalonate pathway enzyme may alleviate the toxicities of statins but retain the efficacy in combination with EGFR inhibitors.

### 32 **Antiemetic recommendations for breast cancer patients receiving highly emetogenic chemotherapy: Systematic review and network meta-analysis**

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**Background.** Despite guideline recommendations for antiemetic use in patients receiving highly emetogenic, anthracycline and cyclophosphamide-based chemotherapy (A&C-CT), clinical experience suggests that control of chemotherapy-induced nausea and

vomiting (CINV) is suboptimal. Forty to sixty percent of patients still experience significant CINV.

**Objective.** Conduct network meta-analyses (NMA) of randomised controlled trials (RCTs) comparing antiemetic regimens.

**Methods.** A systematic search of Medline (1947–June 2014), EMBASE (1980–June 2014), and Cochrane CENTRAL (June 2014) for RCTs comparing antiemetic regimens in breast cancer patients receiving A&C-CT was performed. We adhered to PRISMA guidelines. Primary outcome was overall total control of CINV (no nausea, no vomiting, and no rescue antiemetics for five days post-chemotherapy). Secondary outcomes included complete protection (minimal nausea, no vomiting, and no rescue antiemetics), complete response (no vomiting and no rescue antiemetics), as well as no vomiting, and no nausea in the 5 days following chemotherapy. Established methods for Bayesian network meta-analyses were used. The American Society of Clinical Oncology (ASCO) recommended regimen of 5HT3 receptor antagonist (day 1) with dexamethasone (days 1-4), and an NK1 receptor antagonist (days 1-3) was used as our reference treatment.

**Results.** From 1081 citations, 189 were retained after abstract screening, and 31 after full-text screening. Our largest network contained 16 of the 31 RCTs (n=8,839 patients), and contained 15 different antiemetic regimens. We identified over 15 different endpoints across RCTs. Consequently, we were unable to formulate a connected network of all treatments for several key outcomes. There was heterogeneity between trials in terms of design, chemotherapy administered, and tumour types. Network meta-analyses for three outcomes were performed: 1) no vomiting in the five days following chemotherapy; 2) no nausea in the five days following chemotherapy; 3) post-hoc analysis of composite endpoints, including complete protection and complete response. No summary estimates demonstrated clear benefits of the ASCO-recommended regimen over its competitors. While comparisons were largely inconclusive due to statistical uncertainty, there was suggestion that regimens involving cheaper, generic agents, like olanzapine, may offer comparable efficacy.

**Conclusions.** NMA was challenging due to extensive heterogeneity and inconsistent reporting of CINV outcomes. Future trials should report outcomes in a standard fashion. Findings did not demonstrate clear differences between the ASCO-recommended regimen and competitors. Based on these results, we have applied for funding for an RCT comparing guideline-recommended treatment with a regimen containing the cheaper and possibly more effective agent, olanzapine.

### 33 **Role of the Ste20-Like Kinase in Muscle Development and Repair**

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**Background:**

The Ste20-Like kinase is highly expressed in both embryonic and adult muscle tissue. The expression of a dominant negative SLK in C2C12 myoblasts resulted in decreased myoblast fusion. Additionally, the expression of the dominant negative SLK in adult mice resulted in a reduction in force generation.

**Objective:**

This study aims to further evaluate the role of SLK in muscle development, as well as in muscle repair. In addition, known downstream targets of SLK will be assessed for their capacity to interact with SLK and propagate myoblast fusion and muscle integrity

**Method:**

Using a muscle specific Cre, SLK was genetically deleted from the myogenic lineage. This animal model was then used to study muscle repair after cardiotoxin injection, as well as muscle development in the absence of SLK. C2C12 and primary myoblasts were also used to study SLK's role in myoblast fusion.

**Result:**

As expected, both C2C12 and primary myoblasts exhibited reduced fusion in the absence of SLK. Interestingly, this did not perturb the differentiation of myoblasts. Muscle repair was also delayed significantly in adult knockout mice. The signal from SLK to stimulate myoblast fusion appears to be mediated in part by RhoA-GTPase. Adult mice also displayed reduced force generation, and an increased amount of central nuclei in the absence of SLK, implicating SLK as being essential in muscle integrity and function.

**Conclusions:**

SLK is a critical regulator of myoblast fusion and muscle repair, but does not interfere with the genetic program of myogenic cells. SLK is also responsible for the development and stability of fully functional adult muscles.

34 **Ontario Tumour Bank Initiative at The Ottawa Hospital**H. Sekhon<sup>1</sup>, A. Arnaout<sup>1</sup>, S. Gilbert<sup>1</sup>, C.A. Jodouin<sup>1</sup>, **N. Rayne**<sup>2</sup>, M. Beckstead<sup>2</sup>, J. Bartlett<sup>3</sup>, M. Albert<sup>3</sup>

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3. Ontario Institute for Cancer Research<sup>3</sup>

The Ontario Tumour Bank (OTB) is a province-wide biorepository and data bank focused on the collection of tumour-related human biospecimens. Operating at four academic teaching hospitals across Ontario (Kingston General Hospital, London Health Sciences Centre, St. Joseph's Healthcare Hamilton, and The Ottawa Hospital (TOH)), the OTB is a program of the Ontario Institute for Cancer Research (OICR) which is funded by the Government of Ontario. The OICR is a not-for-profit corporation that supports research on the prevention, early detection, diagnosis, treatment and control of cancer. Collaboration with the OTB provides academic and industry cancer researchers with a diverse selection of high quality tumour-related specimens and data obtained directly by dedicated tumour bank staff, who follow a stringent set of procedures and ethical guidelines. The biospecimens and clinical data are an important resource for scientists engaged in translational research who are developing better diagnostic tools and new drug therapies. Researchers depend on the OTB to provide research biospecimens of high quality, diversity, and integrity.

The OTB coordinates the collection, storage, analysis, annotation, and distribution of tumours and peripheral blood samples. Working alongside local pathologists, medical oncologists, surgeons and other hospital personnel, specially trained OTB staff obtain patient consent, collect tissue and assemble comprehensive clinical information for each donor and their corresponding samples.

TOH-OTB site has been acknowledged in 2 papers in the July 2014 issue of Nature for the contribution to the TCGA (The Cancer Genome Atlas) study. The researchers involved in the studies identified promising therapeutic targets for lung adenocarcinoma and reported molecular characterizations for gastric adenocarcinoma.

TOH-Ontario Tumour Bank team, Dr. Harman Sekhon (PI); Dr. Angel Arnaout and Dr. Sebastien Gilbert (Co-PI's); Carol-Ann Jodouin, EORLA, Clinical Research Manager and OTB Program Administrator; TOH-OTB staff, Nikita Rayne and Matthew Beckstead.

35 **A Novel RNAi Screen to Identify Host Factors that Modulate Oncolytic Virus Replication**Dominic Roy<sup>1,2</sup>, Theresa Falls<sup>1</sup>, Benjamin tenOever<sup>3</sup>, John Bell<sup>1,2</sup>

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Background: Oncolytic viruses (OVs) infect and kill tumor cells while leaving normal cells unharmed. Specificity towards cancer cells can be a natural feature of the virus, or it can be selected for or engineered into the virus. OVs are often genetically attenuated by reducing their ability to antagonize antiviral defenses, therefore increasing tumor specificity. This strategy leads to enhanced replication in tumor cells, which often possess defects in antiviral pathways, while sparing normal cells. However, not all tumors have defects in their antiviral defenses and thus OV replication in these tumors is rather limited. Identifying and modulating host factors that regulate OV replication in OV-resistant cancer cells, but not normal cells, will lead to increased OV replication in these tumors and potentially improved therapeutic outcomes.

Objectives: To increase oncolytic virus replication in cancer cells using an RNAi screening approach in order to identify host factors that modulate oncolytic virus replication in cancer cells.

Methods:

1. A Sindbis virus shRNA library screen was conducted in cultures of various cancer cells as well as in vivo. Cell selected virus populations were analyzed by deep sequencing and bioinformatics analysis was used to identify shRNAs that were enriched.
2. Selected shRNA sequences were tested for enhancement of OV growth in cell culture as well as in vivo.
3. The cellular mRNA targets of the enriched shRNAs will be identified.

Results: Serial passages of the Sindbis virus shRNA library in various cancer cell lines followed by deep sequencing of the selected virus populations led to the identification of several shRNA sequences that were enriched. Follow-up experiments demonstrated that the identified shRNAs increase replication of Sindbis virus, as well as Vesicular Stomatitis Virus (VSV), another OV. shRNA sequences identified in the in vitro screen also enhance virus replication in vivo.

Conclusions: The Sindbis virus RNAi screening approach is a useful tool to identify shRNAs, and potentially their corresponding cellular mRNA targets, which modulate oncolytic virus replication in OV-resistant cancer cells. Viruses expressing the identified shRNAs demonstrate an enhancement in replication. Future studies will be aimed at identifying the cellular mRNA targets of the identified shRNAs, determining if the increase in virus replication is tumor-specific, and if shRNA-expressing viruses demonstrate enhanced anti-tumor activity in vivo.



### 36 **Efficacy and safety of a vaccinia virus oncolytic candidate lacking the soluble interferon gamma receptor gene (B8R).**

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**Background:** In 2011, 29.9 % of deaths in Canada were due to Cancer making this disease the leading cause of death. Current standard treatments like chemotherapy and radiotherapy are non-specific and result in significant damage to normal tissue. Oncolytic viruses (OV) are an emerging class of therapeutic replicating viruses that selectively lyse and kill tumour cells while sparing normal cells. Tumour selectivity of OV's stems from genetic changes within the tumour creating a more favourable environment to infection than normal tissue. These differences allow for deletion of viral genes required for infection of normal cells but dispensable for infecting tumour cells resulting in enhanced safety of the therapeutic. Vaccinia virus (VV) is a large double stranded DNA poxvirus historically used as a vaccine for the eradication of smallpox. JX-594, a VV clinical candidate with a deletion in the thymidine kinase (TK) gene and armed with GM-CSF, an immune stimulatory gene, is in clinical trials and has shown promise in terms of extending patient survival. Despite this success, VV encodes an armament of immunosuppressive genes that may hamper anti-tumour immunity and oncolytic potential. Thus, exploiting the vast potential of VV as an oncolytic platform by altering immunosuppressive genes remains a largely untapped area. The VV B8R gene encodes a soluble receptor for interferon gamma and thus aids in controlling the immune response against the virus. **Objective:** We have deleted B8R from the Copenhagen strain of VV and assessed the effect of this deletion on the oncolytic ability and safety of vaccinia. **Results:** We show that a B8R deleted virus is better able to control tumour outgrowth and metastases in a murine model of breast cancer compared with a TK deleted virus. This effect may be due to better infectivity of the B8R deleted virus. We also show an increase in NK cell activation 3 days following treatment with B8R deleted virus, reduced CD31 staining and increased MHCII staining at 7 days post treatment suggesting B8R deletion results in specific beneficial changes within the tumour microenvironment. In addition, we show that a B8R deleted virus is more cytotoxic than control virus in glioblastoma cells and has a favourable safety profile in both an intranasal toxicity model and an intracranial model of glioblastoma. **Conclusion:** Taken together, deletion of the B8R gene may result in favourable changes in the tumour microenvironment resulting in enhanced anti-tumour effects of a VV oncolytic virus.

### 37 **SIMONE-Simulation in Medical Oncology Education**

#### **Part I: Pilot study**

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4. Skills and Simulation Centre, University of Ottawa

**BACKGROUND:** Advances in medical oncology therapeutics have led to novel "challenging cancer scenarios" (CCS), including an expanding spectrum of unique treatment toxicities and chronic cancer complications from increased longevity. There is no defined curriculum addressing CCS management. Simulation based training with debriefing (SBTD) is an educational method utilizing a virtual medium to mimic clinical scenarios.

**OBJECTIVES:** This pilot study tests the feasibility of SBTD in medical oncology education, and compares SBTD to didactic teaching of CCS management.

**METHODS:** With ethics approval, a curriculum highlighting CCS topics was created. Three clinical scenarios were developed and programmed using the high-fidelity SimMan™ mannequin. Scenarios lasted 10 minutes, and participants' decisions determined the course of the scenario. After receiving the curriculum, participant demographics were collected and they were randomized 1:1 to intervention Arm A or B. Both arms performed three simulation scenarios. After simulation#1, participants took a quiz testing CCS-relevant knowledge. Then, Arm A received an expert-facilitated debriefing; Arm B received a didactic lecture covering CCS management. The next day all participants performed simulation and quiz#2, with simulation and quiz#3 done eight weeks later. Each simulation was videotaped for two independent reviewers to grade clinical performance using the Ottawa Crisis Resource Management Global Rating Scale. Beyond feasibility, outcomes included change in performance and quiz scores, and participants' satisfaction with educational method as assessed by questionnaire after simulation#2-3. Differences between the three simulation scores in both arms were calculated, and assessed using independent t-test.

**RESULTS:** Eleven participants were enrolled from the division of medical oncology and the internal medicine training program. The majority had 0-5 years of clinical medical experience, 67% in Arm A and 60% in Arm B. Most had previous experience with SBT, 67% in Arm A and 100% in Arm B. Overall performance improved steadily in Arm A with median score increasing from 4.5/7 (simulation#1) to 5/7 (simulation#2) to 5.1/7 (simulation#3). Arm B median performance scores improved from 4.4/7 (simulation#1) to 4.8/7 (simulation#2) but decreased to 4.1/7 by simulation#3. In Arm A 83% of learners were either very satisfied

or satisfied with the educational method used, compared to only 40% in Arm B.

**CONCLUSION:** This pilot study demonstrates the feasibility of SBTD in medical oncology education and suggests potential superiority compared to didactic lectures in improving learner's clinical skill. We plan to validate these findings with a larger study. Our ultimate goal is to create a national CCS management curriculum.

### 38 **Accumulation of myeloid derived suppressor cells following cancer surgery impairs natural killer cell activity**

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**Background and Objective:** Activating the immune system has emerged as a promising way to treat cancer. However, immune suppression induced by frontline therapy such as surgery, limits their potency. Myeloid derived suppressor cells (MDSC) are a heterogenous population of immune regulatory cells that have been shown to inhibit immune function. We have previously shown that surgery induced impairment of natural killer (NK) cells facilitates the formation of postoperative metastases. While MDSC are known to suppress NK cells, this has effect has not been evaluated in the postoperative period. To characterize the mechanism of postoperative NK cell impairment, we sought to investigate the molecular basis of surgery-regulated accumulation of MDSC and their functional consequence on NK cell activity.

**Methods:** The suppression of NK cell cytotoxic activity by MDSC from control and surgically stressed mice was compared both in vitro and in vivo. In human cancer patients, MDSC expansion and function were assessed prior to and following surgical resection.

**Results:** MDSC expansion was observed following surgery in mouse melanoma and breast tumor models of surgical stress. Co-cultures of NK cells with MDSC from control and surgical stressed mice resulted in their functional suppression with surgically stressed MDSC, but not with control MDSC. Adoptive transfer of MDSC from surgically stressed donor mice into PolyI:C primed recipient mice resulted in impaired in vivo clearance of NK cell sensitive RMA-S tumor cells. Further, surgically stressed mice depleted of MDSC, demonstrated enhanced ability to clear RMA-S tumor cells. In human cancer surgery patients, MDSC expansion was observed in the early postoperative period.

**Conclusions:** Our results suggest that MDSC are increased in both number and suppressive activity following surgery. Importantly, our study reveals that MDSC represent a hurdle to successful treatment of postoperative metastatic disease. Preoperative targeting of MDSC in cancer surgery patients could reduce recurrences and improve survival in over 65,000 Canadians who undergo surgery to remove their primary tumor every year.

### 39 **Evolution of Alternative Splicing in Ubiquitin Specific Protease 4**

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#### Background

Ubiquitin specific protease 4 (USP4) is a highly networked deubiquitinating enzyme with reported roles in cancer, development, and innate immunity. In mammals it has two dominant isoforms arising from inclusion or exclusion (skipping) of exon 7 (E7).

#### Objective

We studied two plausible mechanisms for the generation of these isoforms: (1) E7 skipping due to the long upstream (I6) intron of mammalian USP4 and (2) E7 skipping due to inefficient 5' splice sites (5'SS) and branchpoint sites (BPS).

#### Methods

Employing a combinatorial in silico and in vitro approach, we first derived a predictive framework by characterizing relative intron lengths and splice site strengths bioinformatically. We then generated a series of minigene constructs to pinpoint the mechanism of exon skipping in mammals.

#### Results

Both transcript variants were generated from a USP4-E7 minigene construct with short flanking introns, an observation consistent with the second mechanism whereby differential splice signal strengths are the basis of E7 skipping. This mechanism was confirmed by optimization of sequence elements through site-directed mutagenesis. Bioinformatic analysis predicted that exon skipping would not occur in two vertebrate species. This prediction was confirmed experimentally.

#### Conclusions

The protein domain encoded by E7 is poorly conserved and may be dispensable; in the absence of strong selection E7 exon

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skipping appears to have arisen from genetic drift in the 5'SS7 element. E7 exon skipping occurs is predicted to occur in many vertebrate species but is not universal.

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40 **17 $\beta$ -estradiol sensitizes ovarian surface epithelium to transformation by suppressing Dab2 expression.**

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Background: Ovarian surface epithelial (OSE) cell proliferation and morphology are tightly regulated by the asymmetrical distribution of polarity proteins. Maintenance of polarity is important because it provides positional cues for surface localization and growth inhibition. In a mouse model of ovarian cancer, we have established that prolonged exposure to high levels of 17 $\beta$ -estradiol (E2) accelerates tumor onset and increases the incidence of morphologically dysplastic/hyperplastic OSE - lesions that we believe can be sites of cancer initiation.

Hypothesis: I hypothesize that E2 can cause OSE hyperplasia by inhibition of a tumor suppressor gene called Disabled-2 (Dab2). Dab2 is critical in mediating the polarized distribution of cell surface proteins and is highly expressed in ovarian tissue but is lost in hyperplastic OSE and the majority of ovarian carcinomas.

Methods: To reproduce the E2-induced OSE hyperplasia seen in vivo, I have established an in vitro model system using primary cultures of mouse OSE (mOSE) and have observed the effects of E2 in this model via growth curve experiments, immunofluorescence staining, quantitative RT-PCR, and western blot analysis.

Results: I have confirmed mOSE become hyperplastic after prolonged E2 exposure and display evidence of loss of contact inhibition and polarity. Dab2 and other polarity-related proteins that have been reported to be differentially expressed in ovarian cancers relative to normal OSE such as E-cadherin, Cytokeratin-19,  $\beta$ -catenin, and Snail are all observed to be inappropriately expressed in mOSE displaying hyperplasia due to prolonged E2 exposure.

Conclusions: E2 can sensitize OSE to tumor initiation by increasing OSE proliferation and suppressing regulators of polarity.

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## Chronic Disease Program

### 41 DISEASE SPECIFIC MUTATIONAL ANALYSIS OF CD127 SIGNALING, EXPRESSION & REGULATION

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Interleukin (IL)-7 is an essential cytokine for CD8 lymphocyte differentiation, proliferation and homeostasis. IL-7 signals through a heterodimeric receptor complex composed of a common,  $\alpha$  chain (CD132) and an IL-7 specific,  $\beta$  chain (CD127). Disease states such as HIV and multiple sclerosis (MS) have shown varying levels of CD127 expression. Dysregulation of IL-7 signaling has been shown in HIV infection, where CD8 T-cell activity is decreased. In addition, genome wide association studies (GWAS) have identified several single nucleotide polymorphisms (SNP) in and around the CD127 gene that may be linked to autoimmune diseases such as multiple sclerosis (MS) and rheumatoid arthritis (RA). Our objective is to develop an in vitro model to determine how mutations within CD127 affect IL-7 signaling, expression, and regulation. In accordance with the objective, mutations have been made within CD127 gene, on a pCMV-CD127 plasmid. These mutations are based on identified SNPs (identified in GWAS) within the CD127 gene. The Jurkat cell line has been chosen for the in vitro model to study IL-7 signaling and expression due to low level of endogenous CD127 expression, the presence of STAT5 and high level of endogenous CD132. Future work in the development of this model will be the use of a lentivector to transduce the CD127 gene into the Jurkat cell line. These stable cell lines containing either wild type and mutant CD127 will be used to examine IL-7 signaling and CD127 expression and regulation. By expanding our basic knowledge of IL-7 signaling and receptor expression and regulation, we can potentially develop therapeutics that can in disease states like HIV or MS return the function of CD127 to normalcy.

### 42 Long Acyl Chain Sphingolipids Govern Visible Microdomains and Cholesterol in both Model and Plasma Membrane

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**Background** - Lateral membrane microdomains, or lipid rafts, are fundamental to a wide range of biological processes in mammalian cells. Generated through spontaneous association of sphingolipids and cholesterol, lipid rafts are thought to be phase-separated from the more fluid environment. Indeed, such phase separation could be seen as micron-sized microdomains in giant unilamellar vesicles (GUVs). Giant plasma membrane vesicles (GPMVs), isolated from live cells with complex lipid and protein compositions, also form micron-sized microdomains. However, similar microdomains have not been observed in the plasma membrane of living cells. A possible explanation lies in the unique nature of live cell plasma membrane – a high degree of lipid asymmetry coupled with high levels of long acyl chain sphingolipids exclusively in the exofacial leaflet.

**Objective** - We aim to characterize how plasma membrane transbilayer asymmetry influences membrane properties, such as microdomain formation, in live cells and model membrane systems.

**Methods** - We use a combination of fluorescence microscopy and a newly developed methyl-beta-cyclodextrin assay to examine lipid behaviour in symmetric and asymmetric membranes.

**Results** - Here, we report that GUVs could mimic the phase behaviours of live cell plasma membrane if long acyl chain sphingomyelin (SM) was placed exclusively in the exofacial leaflet - micron-sized microdomains were no longer formed in these asymmetric GUVs. To better understand the mechanism, we developed a novel methodology to analyze cholesterol in the individual leaflets of bilayer membranes. We found that cholesterol preferred tail-to-tail interdigitation with exofacial long acyl chain SM. This forced the majority of cholesterol into the inner leaflet and, at the same time, depleted cholesterol from the exofacial leaflet. Both conditions are known to abolish visible microdomains. We further analyzed cholesterol in the leaflets of live erythrocytes and found cholesterol highly enriched in the cytoplasmic leaflet, identical to the asymmetric GUVs described above.

**Conclusion** - Thus, we identified a novel cholesterol-sphingolipid interleaflet coupling mechanism that can explain why live cell plasma membrane do not form visible microdomains. This work helps to elucidate how lipid assembly in the plasma membrane can regulate protein function.

43 **The effects of high glucose on insulin resistance and adipogenesis in primary human preadipocytes and differentiated adipocytes**

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**Background:** Obesity is caused by a chronic positive energy imbalance and is associated with cardiovascular disease, and diabetes/insulin resistance. Metabolic factors such as nutrient excess cause impairment of normal insulin signalling, which in adipocytes, may lead to dysfunction.

**Objective:** The aim of this study is to assess the susceptibility of human preadipocytes and differentiated adipocytes to nutrient stress. The effect of high glucose on insulin signalling pathways (such as Akt phosphorylation) and insulin-dependent cellular responses (adipogenesis and glucose uptake) will be characterized in response to high glucose stress.

**Methods:** Preadipocytes were isolated from the stromal vascular fraction of human subcutaneous adipose tissue. Preadipocytes and differentiated adipocytes were pre-incubated in 5 mM glucose (normal glucose) or 25 mM glucose (high glucose) in the presence or absence of 0.6 nM insulin for 48h, followed by acute (5min) insulin (100nM) stimulation. Immunoblot analyses were performed to examine insulin-dependent Akt phosphorylation of Ser473. Insulin-stimulated 3H-deoxy-glucose uptake was also quantified in adipocytes.

**Results:** In adipocytes, levels of Ser473 phosphorylated Akt normalized to Akt, were not significantly changed following stimulation in the high glucose chronic insulin condition, when compared to levels in the normal glucose condition (n=5). Similarly, insulin-stimulated glucose uptake was comparable between all conditions (n=3), suggesting that excess glucose has little effect on insulin action in differentiated adipocytes. In contrast, preadipocyte levels of Ser473 phosphorylated Akt normalized to Akt, were significantly reduced following stimulation in the high glucose chronic insulin condition, when compared to levels in the normal glucose condition (p<0.01; n=3).

**Conclusions:** Taken together, current data suggest that human preadipocytes are susceptible to the inhibitory effects of high glucose/chronic insulin treatment. Future directions will aim at elucidating the mechanism underlying this inhibition by examining upstream regulators of Akt phosphorylation, as well as downstream effectors. We will also investigate if this inhibition leads to impairment of insulin stimulated physiological events, such as adipogenesis. Characterizing adipogenesis in the context of an insulin resistant state may in turn provide therapeutic targets to address nutrient excess-induced adipose tissue dysfunction.

44 **The timing of gestational weight gain & infant adiposity in the Maternal Obesity Management (MOM) Trial**

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**Background:**

Excessive gestational weight gain (eGWG) is a modifiable risk factor for, and independent predictor of, fetal overgrowth. Early eGWG has also been shown to increase the odds of unfavorable neonatal body composition. Thus, the timing of eGWG may affect infant adiposity and subsequent child obesity risk.

**Objective:**

We aimed to assess adherence to the 2009 Institute of Medicine (IOM) GWG guidelines, the timing of GWG, and its relationship with anthropometric markers of infant body composition.

**Methods:**

A subset of women enrolled in the MOM trial, a randomized controlled trial of prenatal lifestyle intervention, were categorized as: 1) appropriate GWG (i.e., within IOM) in the first and second halves of pregnancy (overall appropriate, OA); 2) appropriate GWG in the first half of pregnancy and eGWG in the second half of pregnancy (late excessive, LE); 3) eGWG in the first half of pregnancy and appropriate GWG in the second half of pregnancy (early excessive, EE); and 4) eGWG throughout pregnancy (overall excessive, OE).

**Results:**

Prepregnancy body mass index (BMI) and total GWG differed between OA (n=9), EE (n=4), LE (n=17), and OE (n=19) ( $p < 0.01$ ). LE and OE had greater GWG and were more likely to exceed recommendations ( $p < 0.01$ ); although women with obesity were the most likely to exceed GWG targets. Women gained most of their weight in the later stages of pregnancy, independent of pregravid BMI. No between group differences were observed for infant weight-for-length z-scores, % body fat or fat mass at 3 and 6 months postpartum.

**Conclusions:**

The majority of women had eGWG by term and gained the most weight later in pregnancy. Neonatal body composition did not differ with timing of GWG. Future studies should examine if appropriate rate of GWG relates to infant body composition. Particular attention should be given to GWG management in late pregnancy.

#### 45 **Hexokinase II-mediated Warburg effect is required for chemoresistance in ovarian cancer cells.**

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**Background**

In human ovarian cancer (OVCA), recurrence and resistance to cisplatin (CDDP) is a major hurdle to the successful treatment. Most cancer cells are highly dependent on aerobic glycolysis for additional energy, commonly called Warburg effect. Hexokinase II (HKII) is an enzyme that catalyzes the first committed step in glycolysis, converting glucose to glucose 6 phosphate, and high HKII expression has been reported to be highly correlated with poor survival in ovarian cancer patients. Whether HKII could be considered as protective molecule against apoptosis beyond its metabolic function is still elusive.

**Hypothesis and Objectives**

Objective of this research is to examine the role of HKII in accelerated glucose metabolism rate in chemoresistant ovarian cancer cells, and how HKII-mediated increased aerobic glycolysis leads to anti-apoptosis and CDDP resistance. Furthermore, how HKII content and translocation is regulated by aberrantly expressed Akt and p53 in ovarian cancer will be examined. We hypothesized here that HKII is a key molecule involved in chemoresistance of ovarian cancer.

**Methods**

Chemosensitive (A2780s) and chemoresistant cell lines (A2780cp, SKOV3, Hey, and ES2), were examined in time-course and CDDP concentration-response studies. HKII content and mRNA abundance, and glucose uptake level were determined by Western blot, RT-qPCR and Amplex red assay, respectively. Apoptosis and HKII localization was assessed by Hoechst staining confocal microscopy.

**Results**

CDDP decreased HKII protein content and mRNA level in chemosensitive cells whereas no significant change of HKII was observed in chemoresistant cells in culture duration and concentration-dependent manner. In addition, high glucose uptake level was observed in chemoresistant cells, especially in Akt overexpressed Hey cells. Inhibition of glucose uptake in OVCA cells by 2-DG suppressed glycolysis and sensitized the cells to cisplatin. Confocal microscopic studies suggested that CDDP treatment of chemosensitive cells (A2780s) resulted in HKII translocation from cytosol and outer membrane of mitochondria to the nucleus whereas HKII remained in the cytosol in chemoresistant cells (A2780cp). Knockdown of p53 in chemosensitive cells by siRNA showed increased HKII content, suggesting that p53 is required for HKII regulation.

**Conclusion**

Collectively, these results suggested that HKII is a key molecule engaged in accelerated glycolysis rate, subsequently leading to anti-apoptosis. Therefore, HKII could be a candidate therapeutic target to overcome CDDP resistant ovarian cancer.

#### 46 **Proteomic Characterization of Pig Sperm Anterior Head Plasma Membrane Reveals Roles of Acrosomal Proteins in ZP3 Binding**

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**Background:** Capacitation is a process whereby mammalian sperm gain full ability to bind to the zona pellucida (ZP), and to fertilize the egg. Reorganization of sperm plasma membrane components is one known event during capacitation. The anterior sperm head plasma membrane (APM) is the ZP-binding site and it is likely that capacitation-related changes occur mostly at this site.

**Objective:** Our objective was to characterize and compare APM proteins of sperm before and after capacitation with the anticipation to gain an insightful understanding of the mechanisms underlying the capacitation and sperm-ZP interaction processes.

**Methods:** We have performed quantitative mass spectrometry (MS) based proteomic analyses of APM vesicles isolated from non-capacitated and capacitated pig sperm by nitrogen cavitation at 650 psi. The presence of high molecular weight (HMW) protein complexes in APM vesicle extracts was detected by blue native gel electrophoresis (BN-PAGE) and the ZP affinity of these complexes was assessed by far western blotting with biotinylated pig ZP3. The presence of selected APM proteins on the sperm surface after capacitation was examined by immunofluorescence and flow cytometry.

**Results:** The MS results revealed that ZP-binding proteins were the most abundant group of proteins, with a number of them showing increased levels in capacitated sperm. BN-PAGE and far-western blotting revealed presence of HMW protein complexes in APM vesicles of both non-capacitated and capacitated sperm, but the complexes (~750-1300 kDa) from capacitated sperm possessed much higher binding capacity to pig ZP3 glycoprotein. Proteomic analyses indicated that a number of proteins known for their acrosome localization, including zonadhesin, proacrosin/acrosin and ACRBP, were components of capacitated APM HMW complexes, with zonadhesin being the most enriched protein. Our immunofluorescence results further demonstrated that a fraction of these acrosomal proteins was transported to the surface of live acrosome-intact sperm during capacitation. Co-immunoprecipitation indicated that zonadhesin, proacrosin/acrosin and ACRBP interacted with each other and they may traffic as a complex from the acrosome to the sperm surface. Finally, the significance of zonadhesin in the binding of APM HMW complexes to pig ZP3 was demonstrated; the binding ability was decreased following treatment of the complexes with anti-zonadhesin antibody.

**Conclusion:** Our findings suggested that proteins having inherent ZP affinity and previously existing in the acrosome may traffic together as HMW complexes to the sperm APM during capacitation and play roles in the initial sperm-ZP binding.

47 **Withdrawn**

48 **Ovarian Ring finger protein (RNF6) regulates follicular growth via a modulation of androgen receptor (AR) activity**

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**Background:** Follicular cell proliferation and survival are tightly regulated by androgen and the oocyte-derived factor Growth Differentiation Factor 9 (GDF9). Androgen receptor (AR) is the key transcription factor mediating androgen signaling, which plays important regulatory roles in follicular development. In addition to AR expression, its degradation also plays an important role in regulating the intracellular steady state level of AR and the responsiveness of the cells to androgen. Ring finger protein 6 (RNF6), a member of the small nuclear Ring finger protein family, induces AR ubiquitination although its role in AR degradation is still unclear. The relative expression of RNF6 and its role in the regulation of ovarian folliculogenesis by androgen and GDF9 is not known.

**Objective:** The objective is to examine the expression and role of RNF6 in cell fate determination during the regulation of ovarian follicular development by androgen and GDF9.

**Methods:** To examine the expression of AR and RNF6 in the rat theca cells, GC and oocyte at different stages of follicular development, ovarian sections in 21 days old rats were assessed by immunofluorescence. To examine if GC RNF6 expression is regulated by GDF9 and DHT, GCs from preantral follicles from DES-treated rats were cultured in the presence of GDF9 (0-100 ng/ml) and/or DHT (0-10 µM) for 24 hours and RNF6 content was examined by Western blot. In addition, GC proliferation (S-G2 phase) and apoptosis (sub-G1) were examined using flow cytometry.

**Results:** Immunohistochemistry studies indicated that AR immunostaining in rat ovary was strongly observed in the granulosa cells of all follicular stages examined. But AR expression was not observed in the oocyte and theca cells. In addition, RNF6 was strongly expressed in granulosa cells at all follicular stage in rat ovary. Different from AR expression pattern, RNF6 expression was observed

in the oocyte and theca cells although much lower than granulosa cells. Depending on the concentration of DHT, RNF6 expression in cultured GC gradually decreased but gradually increased with the DHT concentration in the presence of GDF9.

Conclusion: This study was demonstrated, for the first time, the expression of RNF6 during ovarian follicular growth and that this response is regulated by the actions and interaction of androgen and GDF9. Our findings significantly contribute to the current understanding of the cellular basis of androgen action in ovarian follicular growth.

#### 49 **Hyperandrogenism induces M1 macrophages polarization in the rat ovary: implications in Polycystic Ovarian Syndrome (PCOS).**

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2- Ottawa Fertility Centre

Background: Polycystic ovary syndrome (PCOS) is a reproductive and endocrine disorder in women, characterized by chronic anovulation, hyperandrogenism, and polycystic ovaries. Female rats chronically treated with 5 $\alpha$ -dihydrotestosterone (DHT) exhibit symptoms similar to women with PCOS, including increased levels of blood and ovarian chemerin, a chemoattractant for macrophages expressing its receptor, CKLMR1. We hypothesize that DHT up-regulates chemerin, which drives the inflammatory macrophages recruitment to the ovary causing follicular apoptosis/growth arrest.

Objective: To determine the short-term effects of hyperandrogenism in ovarian macrophage and whether the increased number of CKLMR1-expressing M1 macrophages is related with the apoptosis/arrest of specific ovarian follicles.

Methods: 21 days old female Sprague Dawley rats were androgenized with DHT-silicone capsules subcutaneously implanted for 3 and 7 days. Empty capsules were used as control. Body, spleen and ovary weight were recorded. Ovaries were processed for paraffin. Serial sections were performed, followed by TUNEL assay and immunofluorescence for CD68 (pan-macrophage), CD163 (resident macrophage) and CKLMR1. The data were statistically analyzed using One-way ANOVA or Three-way ANOVA, followed by Bonferroni's post-test.

Results: Ovarian weigh of the 7 days DHT treated group were significantly reduced compared to their controls. Frequency of apoptotic antral (AF) and pre-ovulatory (POF) follicles was increased in 3 and 7 days of DHT treatment. DHT increased the M1(CD68+CD163-) numbers, while prevented the physiological up-regulation of M2(CD68+CD163+) in an ovarian follicle stage-specific manner. M1 were significantly increased in AF and POF of rats treated with DHT for 7 days; while the abundance of POF-specific M2 was reduced in 3 and 7 days of DHT treatment. In both control and DHT-treated ovaries, cells expressing CKLMR1 did not differ in number. CKLMR1 was expressed in a subset of CD68+ cells and endothelial cells.

Conclusion/future directions: DHT treatment induces anatomical and morphological changes in the ovaries and the apoptosis of mature ovarian follicles is coincident with increased incidence of M1 and reduced M2. In the future, identification of ovarian and systemic cytokines will contribute to understand the shift of M2 towards M1 immunophenotype of macrophages in hyperandrogenic females. A functional assay of co-culture of ovarian follicles and in vitro differentiated M1 and/or M2 macrophages will also be essential to determine the mechanism of follicular apoptosis induced by macrophages. The validation of results in human samples will be conducted in collaboration with Ottawa Fertility Centre. This project examine if and how the immune system, particularly macrophages, is involved in the PCOS pathophysiology.

#### 50 **Role of Chemerin on the Dysregulation of Mitochondrial Dynamics in Polycystic Ovarian Syndrome**

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2. Cellular and Molecular Medicine, University of Ottawa

##### BACKGROUND

Polycystic ovarian syndrome (PCOS) is characterized by early antral follicle growth arrest, chronic anovulation, and suppressed granulosa cell proliferation. FSH is an anti-apoptotic gonadotropin that promotes follicular growth and steroidogenesis, and its dysregulation via the adipokine Chemerin appears to be involved in the pathogenesis of PCOS. Whether follicular growth arrest is a consequence of chemerin-induced granulosa cell apoptosis is unknown.

Mitochondria are highly dynamic organelles, constantly dividing (fission) and elongating (fusion) to form a network which maintains mitochondrial function and regulates apoptotic cell death. Dysregulation in the proteins involved in mitochondrial fission (Fis1, Drp1) and fusion (Opa1, Mfn1/2) has been linked to the pathogenesis of human diseases. However, it remains unclear as to how mitochondrial dynamics regulate ovarian follicular growth and whether dysregulation of mitochondrial fission play a role in PCOS. The role of FSH and chemerin in this process also remains uncertain.

##### OBJECTIVES/HYPOTHESIS

My objectives are to determine whether FSH regulates mitochondrial proteins and if chemerin inhibits these FSH-regulated



processes, to examine whether chemerin induces apoptosis in early antral granulosa cells, and to explore whether mitochondrial dynamics are associated with follicular arrest in a dihydrotestosterone (DHT)-treated PCOS model. My hypothesis is that FSH induces follicular growth by suppressing granulosa mitochondrial fission and apoptosis, and that chemerin inhibits these responses and contributes to PCOS.

#### METHODS

Granulosa cells from early antral rat follicles will be cultured with chemerin and/or FSH, and apoptosis will be assessed. These investigations will involve loss-in-function studies to ascertain the importance of mitochondrial fission/fusion proteins in chemerin-induced apoptosis and follicular growth arrest using techniques such as TUNEL, Western blot, ELISA and immunofluorescent confocal microscopy. A DHT-treated rat PCOS model will be used to determine the link between dysregulated mitochondrial dynamics and PCOS.

#### RESULTS

Chemerin induced apoptosis in granulosa cells from early antral follicles during a 48h period but did not attenuate FSH-induced cAMP production. Although FSH significantly decreased phospho-Ser616 Drp1 (stimulatory site) and increased phospho-Ser637 Drp1 (inhibitory site) content, chemerin did not attenuate these responses. Preliminary data show that FSH downregulates Opa1 and Fis1 content.

#### CONCLUSIONS/FUTURE DIRECTIONS

The mechanism of action of chemerin does not appear to involve in the regulation of cAMP and Drp1. Precisely how it is involved in the pathophysiology of PCOS remains to be determined. Further examination of chemerin and FSH on other mitochondrial proteins, mitochondrial morphology, and mitochondrial dynamics in a DHT-induced model will be completed in future studies to demonstrate the potential role of mitochondrial fission/fusion in PCOS.

## 51 PCOS phenotypes regulation in a DHT-treated rat model and the role of Chemerin

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#### Background

Polycystic Ovarian Syndrome (PCOS) is a heterogeneous syndrome affecting 10 % of women in reproductive age and account for 75% of anovulatory fertility. The etiology of the PCOS is complex and is not clearly known. Serum, adipose tissue and ovarian follicle levels of the adipokine Chemerin are elevated in PCOS patients. We have previously demonstrated the ability of a post-natal DHT-treated rat model to recapitulate the phenotypes of human PCOS, including elevated ovarian chemerin levels, obesity, insulin resistance, disrupted estrous cycle, and dysregulated follicular growth and steroidogenesis. Whether and how the PCOS phenotype is mediated by chemerin is not known. Our hypothesis here is that the PCOS phenotypes are differentially and temporarily regulated by DHT and that chemerin is involved in these regulations.

#### Objective

In order to validate our hypothesis we have extended this model of PCOS to include shorter duration of DHT treatment to determine if (i) the PCOS phenotypes could be demonstrated with shorter DHT treatment durations, and (ii) the chemerin levels are associated with the PCOS phenotypes.

#### Methods

Female SD rats received a DHT or control subcutaneous implant for 1, 2, and 3 months (n = 8). Estrus cyclicity (daily vaginal smear in the last 2 weeks before sacrifice) and insulin sensitivity (IV insulin injection and blood glucose level test in the week before sacrifice) were assessed. On the day of sacrifice, body and spleen weight and serum Chemerin levels (Western blot, n=7) were compared. Two ways ANOVA and Bonferroni post-hoc tests were performed.

#### Results

(i) Early changes in spleen weight (DHT effect, at 1 month), ovarian size and cyclicity (DHT and DHTxTIME interaction effect at 1, 2, and 3 months) were noted; (ii) Neither insulin sensitivity differences (time and DHT effect at 2 months) nor body weight differences (time, DHT and time xDHT interaction at 2 and 3 months) were observed until the 2nd month; (iii) Chemerin levels increased significantly at 1, 2 and 3 months (Time and DHT effect)

#### Conclusions

Androgens in PCOS have an impact at the ovarian and at the systemic level, and these responses may be regulated temporarily and may involve the synthesis and action of chemerin at ovarian and extraovarian sites. These data also suggest that the immune system may be involved in the PCOS pathogenesis.

#### Perspectives

Granulosa cell expression in response to chemerin, particularly in the context of PCOS will be explored.

52 **Effect of glucose on modulating the secretion of anti-adipogenic factors by macrophages**Vian Peshdary<sup>1,2</sup>, AnneMarie Gagnon<sup>1,2</sup>, and Alexander Sorisky<sup>1,2</sup>

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2. Biochemistry, University of Ottawa

**Background** – Obesity is an excess accumulation of adipose tissue that results from a chronic positive energy balance, via the recruitment and differentiation of preadipocytes into adipocytes (adipogenesis) and/or hypertrophy of existing adipocytes. Insufficient adipogenic capacity leads to dysfunctional adipocyte hypertrophy that raises the risk of insulin resistance and type 2 diabetes. Obesity is also associated with increased inflammatory (M1 state) macrophage infiltration within adipose tissue. Our laboratory and others have shown that macrophage-secreted factors prevent adipogenesis. High glucose concentrations activate macrophages but little is known about their effect on the anti-adipogenic effect of macrophages.

**Objective** – The objective of this project is to determine whether exposure of macrophages to high glucose levels increases their anti-adipogenic effect on human adipogenesis.

**Methods** – Human monocyte derived macrophages (MDM) were derived from peripheral blood mononuclear cells (PBMCs) isolated from blood donated by 6 (3 male, 3 female) healthy volunteers aged 25±5.6 years (mean±SD). MDMs were exposed to 5 mM (NG) or 25 mM (HG) glucose for 24 hrs. The conditioned medium was collected and its effect on differentiation of human subcutaneous abdominal preadipocytes was evaluated. Human subcutaneous abdominal adipose tissue samples were obtained from 6 female patients undergoing elective abdominal surgery aged 56±6.0 years, and with body mass index (kg/m<sup>2</sup>) of 30±7.9 (mean±SD). These studies were approved by the Ottawa Health Science Network Research Ethics Board.

**Results** – Macrophage-conditioned medium (MacCM) generated in the presence of HG inhibited triacylglycerol (TG) accumulation and protein expression of peroxisome proliferator-activated receptor  $\alpha$  (PPAR $\alpha$ ) by 32±5%\* and 46±11%\*\* (mean±SE) respectively (n=6, \*\*p<0.01 and \*p<0.05) compared to control. There were no changes in responses to NG-MacCM (n=6, p= n.s for these markers).

**Conclusions** – These preliminary results suggest that high glucose concentrations may influence adipose tissue function by modulating anti-adipogenic factors produced by macrophages.

53 **The oncolytic virus MG1 preferentially targets and kills latently HIV-infected OM10.1 cells**Nischal Ranganath<sup>1,2</sup>, Dr. Sandra C. Côté<sup>1,2</sup>, Dr. Jonathan B. Angel<sup>1,2</sup>

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**BACKGROUND:** Despite effective viral suppression by HAART, latent HIV reservoirs represent a major barrier to eradication. We propose a novel strategy to target the latent HIV reservoir using a class of oncolytic viruses (OV) including the recombinant Maraba Virus (MG1), which have been engineered to target cancer cells by exploiting defects in type I interferon (IFN)-signaling. Similar alterations in IFN-mediated antiviral responses are also seen in HIV-infected cells. We therefore hypothesize that MG1 can selectively target and kill latently HIV-infected cells.

**OBJECTIVES:** We aim to investigate the ability of MG1 to preferentially target and kill HIV infected cells, including latently HIV infected cell lines.

**METHODS:** Latently HIV-infected promyelocytic cell line OM10.1 and parental HIV-uninfected cell line HL60 were infected with increasing MOIs (0.000 01 to 0.1) of recombinant GFP-expressing MG1 virus. At frequent time points upto 28 hours, productive OV infection was quantified by assessing GFP by flow cytometry. The effect of MG1 infection on cell viability was assessed by MTT and Alamar Blue assays at each time point.

**RESULTS:** Latently HIV-infected OM10.1 cells were significantly more susceptible to MG1 infection and viral cytopathic effects than HL60 controls in a dose- and time-dependent manner. After 24 hours, 82.1 ± 2.3% OM10.1 were infected compared to 43.4 ± 4.4% of HL60 at an MOI of 0.1 (n=5). In parallel, MG1 infection (MOI of 0.1) of latently HIV-infected OM10.1 cells resulted in 71.6 ± 4.1% loss in viability compared to 25.6 ± 6.6% loss in viability in HL60 controls at 28 hours post-infection (n=5).

**CONCLUSION:** Latently HIV-1 infected OM10.1 cells are more susceptible to infection and killing by MG1 when compared to HIV-uninfected HL60 cells, suggesting that MG1 can target and kill latently HIV-infected cells. Further experiments will be conducted to delineate the underlying Type I IFN defects in the cell lines, as well as the consequences of HIV reactivation on MG1 infectivity and HIV production. Selective OV targeting of HIV-infected cells will also be explored in additional in vitro cell line and primary cell models of HIV latency. The use of OV represents a novel approach to selective targeting and elimination of the HIV reservoir, which if successful can significantly advance the management of HIV infection.

54 **Glycine-Dependent Cell Volume Control in Mouse Oocytes is activated due to Release of Inhibition Mediated by the Intact Antral Follicle**

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**Background**

The ability of pre-implantation oocytes and embryos to regulate cell volume is vital for normal growth and development to produce a healthy blastocyst for implantation. After the LH surge at ovulation, oocytes and embryos will accumulate glycine as an organic osmolyte via the GLYT1 transporter. This mechanism of GLYT1-mediated volume regulation is unique to pre-implantation embryos. How GLYT1 is activated to initiate cell volume regulation is currently unknown. We previously found that GLYT1 activation occurs independently of protein synthesis following removal of the oocyte from the antral follicle and GLYT1 can be maintained in a quiescent state in cultured antral follicles with functional gap junctions.

**Objective:**

Determine how GLYT1 is activated in oocytes following removal from the antral follicle.

**Hypotheses:**

- 1) GLYT1 activation following removal of the oocyte from the follicle is not dependent on an increase in the amount of GLYT1 localized at the oocyte surface.
- 2) An inhibitory factor from granulosa cells acts directly or through signaling mechanisms via gap junctions to suppress the GLYT1 transporter and activation occurs indirectly after removal of this inhibitory factor after ovulation.

**Methods:**

I will perform immunofluorescence and western blot analyses to determine the amount of GLYT1 protein localized at the oocyte surface in freshly isolated oocytes and in oocytes cultured 4hr in vitro to allow GLYT1 to fully activate.

To test hypothesis 2, antral follicles will be cultured with or without various inhibitors of signalling pathways and GLYT1 activity measured using a [3H]-glycine uptake experiment.

**Results:**

Immunofluorescence and western blot analysis results did not show a significant increase in the amount of GLYT1 localized at the oocyte surface during GLYT1 activation. However, preliminary results of antral follicles cultured with or without inhibitors suggest a possible role of PKG signaling and/or tyrosine kinase signaling is required to maintain GLYT1 quiescence in the antral follicle. This data suggests that GLYT1 activation occurs due to the removal of an inhibitory factor from mural/cumulus granulosa cells resulting in activation of the transporter. Understanding the mechanisms involved in this method of volume regulation is important in providing optimal conditions for oocyte/embryo culture to avoid failure of infertility treatments, and damage that could result in dysregulation in fetal development, and disease in the offspring. Knowledge of the healthy intra-follicular environment will be vital to further improving in vitro maturation (IVM) techniques to prevent hormonal stimulation in women undergoing fertility treatments.

55 **Antimicrobial Host Defence Peptides, LL-37, Peptide-X and Peptide-Y, as a Potential Vaginal Contraceptive**

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Background: LL-37, a cationic antimicrobial peptide, exerts its microbicidal effects through the disruption of microbial cytoplasmic membranes following its interaction with microbial surface anionic phospholipids. Since sperm contain a substantial amount of anionic sulfogalactosylglycerolipid (SGG) on their surface, treatment of sperm with LL-37 may cause sperm membrane disruption in an analogous manner to that occurring on microbial membranes. Related cationic 12-mer peptide-X and 20-mer peptide-Y (shorter than LL-37) may have effects on sperm similar to LL-37.

Objective: Determine the contraceptive effects of LL-37, peptide-X and peptide-Y

Methods: Peptide concentrations used for sperm treatment were based on physiological amounts of sperm SGG (up to 10.8  $\mu\text{M}$ ). The SGG-dependent binding of peptides on mouse sperm was predicted by computational docking and validated by immune-detection on sperm. Sperm motility was assessed by video-microscopy and the sperm acrosomal status examined by Coomassie blue staining or exposure of CD46, an inner acrosomal membrane. Sperm plasma membrane disruption was assessed by intracellular incorporation of Sytox Green, and electron microscopy. Mouse sperm were determined for their ability to fertilize eggs in vitro. Ultimately, contraceptive effects of LL-37 were assessed by pregnancy outcomes following transcervical injection of sperm +/- LL-37 into naturally cycling female mice. Effects of LL-37 on the reproductive tract of these females were also evaluated histologically.

Results: Computational docking indicated that Arg23 interacted with SGG. Indeed LL-37 bound to mouse and human sperm with partial dependence on sperm surface SGG. Sperm lost their motility and became prematurely acrosome reacted upon treatment with LL-37. The initial action of LL-37 appeared to be through permeabilization/disruption of sperm surface membranes evidenced by intracellular incorporation of Sytox Green and by electron microscopy revealing ultrastructural damage on the sperm surface. LL-37-treated mouse sperm were incapable of fertilizing eggs in vitro. No pregnancy was observed in all 26 females transcervically injected with sperm + LL-37. However, these females showed no abnormality of their reproductive tract and were capable of resuming their fecundity in subsequent mating with fertile males. Similar to LL-37, peptide-X and peptide-Y induced disruption of sperm plasma membranes and premature acrosome reaction in a dose dependent manner.

Conclusions: Our results reveal selective inhibitory effects of LL-37 on sperm fertilizing ability without an apparent damage to the female reproductive tract. It is possible that peptide-X and peptide-Y, both shorter than LL-37, may have

## 56 **sCD127 release depends on MMP2 and MMP9 in human CD8+ T cells and thymocytes**

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Background: Interleukin-7 (IL-7) regulates the development, homeostasis and cytotoxic (CTL) activity of CD8+ T-cells. IL-7 downregulates the expression of the membrane bound IL-7 receptor  $\alpha$  chain (mCD127) and induces the release of a soluble form (sCD127). sCD127 alters IL-7 activity and plasma concentrations are increased in the course of HIV infection. Despite the potential biological importance of sCD127, the mechanisms of its production and release have been only partially described.

Methods: Human thymocytes and blood-isolated CD8+ T cells were treated with IL-7 (10ng/ml) and TcR-stimulating antibodies (anti-CD3/CD28 1 $\mu\text{g}/\text{ml}$  each). Culture supernatants were collected every 24 hours over 96 hours and sCD127 concentration was measured by ELISA. To evaluate the contribution of shedding of mCD127, surface protein biotinylation assays were performed and analyzed through Western blots. To further characterize the signaling pathways leading to the shedding of sCD127, pharmacological inhibitors for JAK, STAT5 and PI3K were also used. Specific MMP2/9 and MMP3 inhibitors were used to evaluate the implication of proteolytic cleavage in sCD127 release.

Results: IL-7 in combination TcR stimulation significantly enhanced sCD127 release by blood isolated CD8+ T-cells however no modulation was detectable in human thymocytes. Biotinylation assays revealed that shedding of mCD127 contributes to the release of sCD127. In combination with pharmacological inhibitors, biotinylation assays also revealed that JAK/STAT5 and PI3K pathways were both involved in the shedding of sCD127 by CD8+ T-cells. Proteolytic cleavage by MMP2 and 9, but not MMP-3 contributes to IL-7/TcR-induced sCD127 release by CD8+ T-cells. In human thymocytes both MMP2 and MMP9 were shown to be involved in the basal release of sCD127.

Conclusions: Our results demonstrated that sCD127 release can be mediated by different mechanisms. Firstly, IL-7/TcR stimulation can induce direct shedding of mCD127 from the cell surface, which is mediated by the JAK/STAT5 and PI3K pathways. Secondly, a shedding-independent mechanism of sCD127 production relies on MMP2 and 9 activities. Furthermore, the role of mRNA splicing in the IL-7/TcR-induced release of sCD127 is under investigation.

57 **IL-7 Induces Mediators of Cell Survival and Proliferation in Th17 Cells****Alana Stilla**<sup>1,2</sup>, Sandra C. Côté<sup>1,2</sup>, Jonathan B. Angel<sup>1,2,3</sup>

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**BACKGROUND:**

In the gut-associated lymphatic tissues, Th17 cells maintain homeostasis and initiate immune responses against pathogenic organisms. Blood- and gut-derived Th17 cells become reduced in number and functionally impaired in HIV-infected individuals for reasons that are poorly understood. IL-7 is a cytokine that is known to promote cell survival through binding and activation of its specific receptor. While IL-7 is involved in promoting Th17 cell function and survival, the effects of HIV on IL-7 signalling in Th17 cells have not yet been investigated.

**HYPOTHESIS:** HIV inhibits Th17 cell survival by dysregulating IL-7 signalling

**METHODS:**

First, the expression of the IL-7 receptor on Th17 cells was determined. CD4+CXCR3-CCR6+ memory T cells (436 cells) were isolated from the blood of healthy donors using the EasySep™ Human Th17 Cell Enrichment Kit from StemCell. Upon stimulation with 50ng/mL PMA and 1000ng/mL ionomycin, a proportion of these cells express IL-17A and represent "true" Th17 cells. To determine whether Th17 cells express the IL-7 receptor (CD127), 436 cells were stimulated with PMA/ionomycin and the expression of both CD127 and intracellular IL-17A were measured by flow cytometry. IL-7 responsiveness of 436 cells was then characterized. 436 cells were stimulated with IL-7 and the expression of phospho-STAT5 and anti-apoptotic molecule, Bcl-2, were measured by flow cytometry. To determine whether HIV interferes with IL-7 responsiveness in Th17 cells, the above experiments will be repeated using 436 cells obtained from the blood of untreated, HIV-infected individuals.

**RESULTS:**

As expected, approximately 15% of PMA/ionomycin-stimulated 436 cells express IL-17A, those being true Th17 cells. Thirty percent of cells expressing IL-17A also express CD127.

Very few (4%) unstimulated 436 cells express phospho-STAT5 and following stimulation with IL-7 (0.1ng/mL), pSTAT5 becomes expressed in an average of 60% of 436 cells. A mean of 51% of unstimulated 436 cells express Bcl-2, which increased to 76% following stimulation with IL-7 (1 ng/ml).

**CONCLUSIONS:**

The above data indicate that 436 cells, the source of Th17 cells, are responsive to IL-7, suggesting an important role for IL-7 in promoting Th17 cell survival.

**FUTURE CONSIDERATIONS:**

The depletion of Th17 cells that occur as a result of HIV infection may be a consequence of impaired IL-7 signalling. Gaining insight into the potential effects of HIV on IL-7 signalling in Th17 cells could lead to the development of treatments more effective at restoring Th17 cells and reducing associated pathology.

58 **Peri-ovulatory putrescine supplementation to reduce miscarriage and birth defects in older women****Yong Tao**, Supervised by Johné Liu

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Advanced maternal age (35 and older) carries significantly increased risks of miscarriage and birth defects, mostly due to poor quality of the eggs. According to Statistics Canada, 52% of all Canadian births in 2011 were by women age 30 and older, up from 24% in 1981 (<http://www.statcan.gc.ca/pub/91-209-x/2013001/article/11784-eng.htm>). Although increasingly sophisticated IVF procedures have helped millions older women to achieve parenthood, the risk of miscarriage and birth defects similarly increases in older women undergoing IVF procedures. Currently, there is no nutritional or medical intervention to reduce the risk of aging-related miscarriage and birth defects.

Mammalian ovaries exhibit a luteinizing hormone-mediated transient rise of ornithine decarboxylase (ODC) activity and its product putrescine, coinciding with oocyte maturation. We have recently demonstrated that ODC deficiency, either chemically induced in young mice or occurring naturally from aging, is correlated with increased egg aneuploidies. Most remarkably, putrescine supplementation significantly reduces egg aneuploidy in older mice (Liu and Tao. 2012. *Aging* 4:1-3; Tao and Liu. 2013. *Aging Cell* 12:42-49). Since then, we have demonstrated that older mice exhibited significantly reduced levels of ovarian putrescine, which was restored by peri-ovulatory oral putrescine supplementation. Putrescine supplementation significantly reduced the number of miscarried fetuses when examined at midgestation, and increased the number of live pups at birth. Genetic analyses of miscarried fetuses revealed an additional role for putrescine supplementation independently of preventing egg aneuploidies. Specifically,

while older mice produced blastocyst embryos with greatly reduced cell number when compared to those by young mice, oral putrescine supplementation significantly restored blastocyst cell number. Significantly, reduction of blastocyst cell number is the single most important indicator of egg and embryo quality in mice and in human IVF practice (most IVF clinics culture human embryos in vitro until blastocyst before implanting into the uterus of the patients). Furthermore, exogenous putrescine exhibited rapid absorption and excretion, and showed no toxicity to mothers and fetuses even when applied beyond conception (Tao et al., submitted).

Putrescine is a naturally occurring metabolite that has been used as a dietary supplementation for neonatal calves and pigs in conjunction with soybean proteins as milk replacement, indicating safety of putrescine supplementation in animal physiology. The acute oral toxicity dose of putrescine (2000 mg/kg in rats) is comparable to that of table salt (3000 mg/kg). Our approach is unique in that it is natural, it targets the reproductive process and it entails a very short period of intervention (at most, days). In addition,

## 59 **Studies on the Role and Regulation of Drp1 in the Control of Autophagy and Chemosensitivity in Ovarian Cancer Cells**

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### Background

Cisplatin (CDDP) chemotherapy is a key treatment for ovarian cancer, but resistance to chemotherapeutic agents remains a major hurdle of successful treatment and is associated with poor prognosis. The mechanism of chemoresistance in ovarian cancer is not fully understood. Mitochondrial fission is associated with the induction of apoptosis and autophagy (mitophagy) in cancer cells, and can be augmented via post-translational modifications of dynamin related protein 1 (Drp1). It has been previously reported that Ca<sup>2+</sup>/calmodulin-dependent protein kinase I (CaMKI) phosphorylates and activates Drp1 which in turn induces mitochondrial fission. It is known that CaMK1 mediates intracellular Ca<sup>2+</sup> action and is activated by phosphorylation via CaMK kinase with increase cytosolic Ca<sup>2+</sup>. However, whether CaMKI regulates Drp1 and chemosensitivity in a Ca<sup>2+</sup>-dependent manner in ovarian cancer cells is not known and needs to be elucidated.

### Overall Objective

The overall objective of my research program is to understand the interactions between Ca<sup>2+</sup> signaling, mitochondrial fission and autophagy in ovarian cancer cell and to determine if their dysregulation is associated with chemoresistance.

### Specific Objectives and Experimental Approaches

To simulate in vivo tumor growth environment closely reflecting the actual physiological conditions, we have implemented a 3D cell culture system based on hanging drop spheroid culture of chemo-sensitive A2780s and -resistant A2780cp cells. We performed time course and dose response studies to optimize the treatment with CDDP to 3D spheroids. Apoptosis, determined by TUNEL assay, was significantly increased in A2780s cells but not in A2780cp cells after 72 h CDDP treatment. This suggested that cells cultured in 3D spheroid show increased chemoresistance compared to cells cultures in 2D. In addition, CDDP also significantly increased apoptosis in A2780s cells in concentration-dependent manner whereas A2780cp cells showed no response.

### Conclusions and Future Investigations

A 3D culture system of ovarian cancer cells and CDDP treatment have been optimized and established. Using this 3D spheroid cell culture system, we will investigate the protein contents of CaMKI, phospho-CaMKI, and phospho-DRP1 to CDDP treatment. Moreover, we will investigate the relationship between the mitochondrial fission, autophagy and chemosensitivity through gain- and loss- of function strategies [Supported by a grant from CIHR (BKT), Kizawa Memorial Hospital Doctor Scholarship Studying Abroad (HT) and a CIHR-QTNPR Postdoctoral Fellowship (PDAL)].

## 60 **Cobalt protoporphyrin injection recruits M2-like fibrocytes to the pancreas and inhibits development of type 1 diabetes**

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**Introduction:** Type 1 diabetes (T1D) is an autoimmune disease in which the insulin-producing  $\beta$ -cells are destroyed by infiltrating inflammatory cells. It is not well explored whether a counter-regulatory repair mechanism is activated in the pancreas.

**Hypothesis:** Induction of heme oxygenase-1 (HO-1), a cytoprotective enzyme, by injection of cobalt protoporphyrin (CoPP), inhibits

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development of T1D in diabetes-prone BB (BBdp) rats in association with activation of repair mechanisms.

Methods: 30 d rats received IP injections of CoPP (6.5 mg/kg, n=33) or saline (n=31) twice/wk for three weeks. Subsets of rats (n=14-16/group) were killed at 51 d (pre-diabetic) and pancreata were snap frozen or fixed. Immunohistochemistry, confocal microscopy, qRT-PCR, and microarrays were used to evaluate early changes in the pancreas. Remaining rats (n=15-17 per group) were monitored for T1D.

Results: T1D incidence was markedly inhibited in HO-1-induced rats (29% vs 73%). We observed a striking infiltrate in the interstitial space of the exocrine pancreas at 51 d. CoPP injections increased pancreas Hmox1 expression by 32 fold along with a 10 fold increase in CD68+, HO-1+ cells around islets and in the peri-lobular region. A majority of these cells expressed the mesenchymal marker vimentin which co-localized with CD34; >80% of vimentin+ cells were HO-1+. Some of the infiltrating cells expressed desmin, MHC class II, CD163, S100 and exhibited fibrocyte morphology. Fibrocytes are collagen-producing, spindle-like cells expressing CD34 or CD45 that are involved in tissue repair and remodeling. More than half of the cells also expressed KLF4, a transcription factor involved in epithelial to mesenchymal transition and possible marker of anti-inflammatory M2 macrophages. Cell proliferation and cell death were increased in the peri-lobular region. Collagen V positivity, a feature of fibrocytes, was identified in CD34+vimentin+ cells. Microarray analysis revealed that HO-1 induction also resulted in upregulation of regenerating factors (Regs) and host defence proteins (a-defensins) in pancreas accompanying an increase in acinar and duct-associated extra islet insulin+ clusters characteristic of islet neogenesis. qRT-PCR analysis of whole pancreas revealed upregulation of Cd204, Cd206, Il10 and Tgfb1 which are markers of M2 macrophages. Additional immunohistochemical and confocal analyses revealed that some interstitial infiltrate cells expressed M2 macrophage markers: CD163, KLF4, CD204 and/or CD206.

Conclusion: We propose that HO-1 induction prevented T1D by recruiting an interstitial infiltrate consisting of M2 macrophages, fibrocytes and M2-like fibrocytes that contribute to anti-inflammatory, pro-repair and islet renewal processes.

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## Clinical Epidemiology Program

### 61 **Want people to download your new app? Analyzing the success of promotional strategies in driving adoption of ImmunizeCA; a new Pan-Canadian Immunization App.**

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#### Background

There is a paucity of literature on what drives downloads of mobile health apps. In partnership with the Canadian Public Health Association (CPHA), we launched a smartphone app to help individuals track their immunizations. ImmunizeCA was released for free on Apple, Android and Blackberry(BB) platforms in March 2014. The app has been promoted through a variety of channels.

#### Objective

To measure uptake of ImmunizeCA and to assess the effectiveness of various promotional strategies in disseminating a mobile health app. Tactics examined included: press release and app store placement at launch, government mailouts with child benefit packages, targeted news articles and social media.

#### Methods

MediaMiser SNAP software was used for media monitoring. We filtered results using inclusion criteria and manual review by research staff. Downloads were collected through iTunes Connect, Google Play and Blackberry World. Google Analytics collected usage metrics and website traffic.

#### Results

In the first 6 months following release(March 20- September 20), ImmunizeCA had 52,356 downloads and 2,254 updates from ImmunizeON, for a total of 54,610 users. Of these, 39,547(72.4%) were iOS, 11,762(21.5%) Android and 940(1.7%) BB. There have been 834,537 screen views in 155,722 sessions. The app has had 775 media mentions (494 tweets, 68 online news articles, 152 Facebook posts, and 42 print articles) and 51,485 website visits.

The highest proportions of total downloads occurred in March(launch) and July(government mailouts), with 20,835(38.1%) and 11,945(21.9%) respectively. March saw 359(46.0%) media mentions; 256 tweets, 34 online news articles, 16 print news articles, and 53 Facebook posts. July had only 30(3.8%) media mentions; 23 tweets, 1 online news article, and 6 Facebook posts.

March downloads were 85.0% iOS, 14.5% Android and 0.5% BB. In July, uptake was 61.6% iOS, 35.5% Android and 2.9% BB. March downloads accounted for 44.8%, 25.5%, and 1.27% of total iOS, Android, and BB downloads. July accounted for 18.6%, 36.0% and 35.4% of total iOS Android and BB downloads, respectively.

#### Conclusions

The two major drivers of downloads were launch communications and government mailouts. Social media strategies did not appear to have a detectable independent effect on uptake. The higher proportion of iOS downloads at launch may be secondary to placement in the iOSAppStore "Best New Apps" section. In contrast, mailouts in July drove uptake more uniformly across platforms than any other strategy. Based on our experience, when releasing new health apps we recommend seeking endorsement from app stores and using direct to consumer marketing communications strategies.

### 62 **Rates of dialysis usage and one-year mortality after implementation of automated eGFR reporting.**

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**Background:** Chronic Kidney Disease (CKD) is a major public health concern, estimated to affect 12.5% of the Canadian population. It is a disease associated with high rates of morbidity and mortality as well as increased health costs, all of which can be partially mitigated by early detection of CKD and referral to nephrology. Over the last decade many laboratories have moved towards routine reporting of an estimated GFR (eGFR) using the MDRD or CKD-EPI study equation in pursuit of increasing the identification of patients with CKD. Widespread implementation of automated eGFR reporting has altered the practice and management of CKD, however the effect of population-level automated eGFR reporting on dialysis utilization and early dialysis mortality are unknown.

**Objective:** The purpose of this study was to examine rates of dialysis utilization and early dialysis mortality post-implementation of



automated eGFR reporting.

Methods: All adult incident dialysis patients from four Canadian provinces that implemented province-wide, automated laboratory reporting of eGFR were included in the study (N= 23, 788). Data were obtained from the Canadian Organ Replacement Registry (CORR) from Jan 1, 2001 to Dec 31, 2010. Crude and age, sex-standardized dialysis usage rates were calculated pre-and post-eGFR reporting using Canadian Census data. One-year dialysis mortality was examined using an interrupted time series and adjusted multilevel logistic regression models to determine changes pre- and post-reporting. Analyses were stratified by age groups and presence of pre-dialysis care.

Results: The pre- and post-eGFR reporting crude rates for dialysis utilization were 222.69 and 216.46 per million person years at risk respectively. In an unadjusted interrupted time series, there was a borderline improvement in one-year mortality post-eGFR reporting. In a multi-level model adjusting for case-mix, facility and the survival trend over time, there was a negative slope for mortality after implementation of automated eGFR reporting (adjusted odds ratio 0.98 (95%CI 0.97-1.00) per 90 day period, p=0.01). Among age groups, the reduction in one-year mortality was observed in patients aged 73 and younger. No changes in mortality between pre- and post-eGFR reporting were observed among patients with pre-dialysis care.

Conclusions: Laboratory-based eGFR reporting was associated with no substantial change in the rates of dialysis usage and marginal improvement in one-year mortality, primarily among those < 74 years of age.

### 63 **The Contribution of Child Behaviour Problems to the Health of Caregivers**

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Background: Parenting is a daunting and demanding task, especially for those who must care for a child with health problems. Considering that caregivers of children with health problems have been demonstrated to show generally poorer physical and psychological health, efforts have been made to understand the factors that influence caregiver health. It has been suggested that child behavioural problems account for a large proportion of the variance in caregiver health. Behavioural problems have been categorised into two groups: Externalizing behavioural problems which are overt and internalizing behavioural problems which are covert. Currently, the literature linking child behaviour problems and caregiver health is varied, and the relation between the two kinds of behaviour problems in terms of their effect on caregiver health is unclear.

Objective: We conducted a systematic review in order to describe and compare the effect of internalizing and externalizing behaviour problems on caregiver health.

Methods: Studies were included if: 1) They measured a psychological or physical outcome on the caregiver, 2) The caregivers were caring for a child (2 to 18 years old) with a behaviour problem, and 3) Child behaviour problems were measured at the same time or prior to caregiver outcomes. Studies were screened and inclusion was determined by two independent investigators. All pairwise comparisons were made between children with internalizing behaviour problems, children with externalizing behaviour problems, and healthy children.

Results: A total of 47 studies were included in the meta-analysis. Three caregiver outcomes were investigated frequently enough to allow for meta-analyses: depression, psychiatric symptoms, and stress. Results suggest significant associations between child externalizing behaviour problems and caregiver depression 0.46 (95% CI 0.36 to 0.55), psychiatric symptoms 0.51 (95% CI 0.37 to 0.66), and parental stress 1.29 (95% CI 0.85 to 1.73). Significant associations were also noted between child internalizing behaviour problems and caregiver depression 0.51 (95% CI 0.09 to 0.94), as well as with psychiatric symptoms 0.56 (95% CI 0.19 to 0.94). No evidence suggests differential effects of internalizing and externalizing behaviour problems on caregiver health.

Conclusions: Consistent and significant effects were found associating child behaviour problems with higher levels of caregiver depression, stress, and psychiatric symptoms. Further studies are needed in order to assess the impact of externalizing behaviour problems compared to internalizing behaviour problems. Additional studies are needed to elucidate the relation between behaviour problems and physical health. The current project sheds light on the impact of behaviour problems on caregiver health.

64 **Effect of folic acid supplementation in pregnancy on preeclampsia – Folic Acid Clinical Trial (FACT)**

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**Introduction**

FACT is an international, multi-centre, double-blind, placebo-controlled, Phase III trial of 3,656 women, sponsored by the Ottawa Hospital Research Institute (OHRI) and funded by the Canadian Institutes of Health Research (CIHR).

Observational studies suggest that folic acid supplementation during pregnancy reduces the risk of preeclampsia (PE). No randomized controlled trial has been conducted to demonstrate the effect of folic acid supplementation on PE.

**Objectives**

FACT aims to determine efficacy of a PE prevention strategy using high dose folic acid supplementation from early pregnancy until delivery in women at high risk of developing PE.

**Methods**

**Subjects**

Pregnant women between 80/7 -166/7 weeks gestation, aged >18 years, taking =1.1mg of folic acid supplementation with at least 1 of the following risk factors for PE: Pre-existing hypertension; Pre-pregnancy diabetes; Twin pregnancy; History of PE; BMI >35kg/m<sup>2</sup>

**Primary Outcome**

PE, defined as =d90 mmHg on 2 occasions =4 hrs apart and proteinuria developed in pregnancy > 200/7 weeks gestation.

Or

HELLP (Haemolysis, Elevated Liver Enzymes, Low Platelets):

Haemolysis; Serum LDH =600U/L; Serum AST =70U/L; Platelets <100 x10<sup>9</sup>/L

Or

Superimposed PE, defined as history of pre-existing hypertension with new proteinuria. Proteinuria is defined as:

Urinary protein =300mg/24 hr, or =2+ protein dipstick, or Random protein-creatinine ratio =30mg protein/mmol

**Results**

As of September 30th, 1235 participants (864 Canadian, 152 Australian, 86 Argentinean, 19 Jamaican and 114 UK participants) have been randomized.

**Conclusions**

Results will establish if high dose folic acid supplementation is an effective preventative strategy in women at high risk of developing PE.

65 **Clinical studies of hematopoietic stem cell expansion to accelerate engraftment after umbilical cord blood transplantation: a systematic review**

**Pauline Damien**<sup>1,2</sup>, Lauralyn McIntyre<sup>2,3</sup>, Dean Fergusson<sup>2,3</sup>, Risa Shorr<sup>4</sup>, Alan Tinmouth<sup>1,2</sup> and David Allan<sup>1,2</sup>

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4. Medical Library Services, The Ottawa Hospital

**Background**

Since the first human umbilical cord blood (UCB) transplant in 1988, UCB has emerged as a major source of hematopoietic stem cells (HSCs) for transplantation, allowing rapid access. Moreover, UCB requires less stringent HLA-matching compared with bone marrow or peripheral blood grafts, that may be especially useful for patients without matched donors. A significant barrier to greater use of UCB is the limiting dose of HSCs that can be collected. Lower numbers of CD34+ cells/kg recipient weight compared with other HSCs sources contribute to delayed or failure engraftment.

**Objective**

A key strategy to overcome the limiting doses of HSCs is stem cell expansion. Although a number of studies have been published regarding UCB stem cells expansion, a comprehensive review of the various strategies has not been previously performed but may help identify promising strategies. This information may assist transplant centres and cord blood banks to plan for the future in terms of expertise and management of precious resources.

**Methods**

Regarding the search strategy, we focused on human clinical studies that included patients (children and adults) undergoing UCB transplant. For inclusion, UCB unit had to be ex vivo treated with the goal of expanding HSCs. Studies that did not specifically aim to expand CD34+ cells were excluded (ie. expanding mesenchymal cells). The same is true about studies with expanded but non-infused HSCs. Clinical outcomes to be extracted include HSCs proliferation, hematopoietic engraftment rates in transplant recipients (neutrophils and platelets following) and post-transplant survival. Our systematic search was performed in MEDLINE, EMBASE, Cochrane as well as clinical trials website and on the Portal of the World Health Organization's International Clinical Trials Registry Platform regarding a research strategy that includes following filters (i) UCB-derived HSCs, (ii) transplantation conditioning/stem cells manipulation and (iii) human clinical trials.

**Results**

To date, the research results (3247 MEDLINE+EMBASE, 175 Cochrane) are under screening. After duplicates removing and title/abstract screening relevant articles will be manually reviewed. Descriptive statistics and analysis of subgroups (ie. adults vs. children studies) will be performed taking into account risk of bias (clinical trial method and technical use of the UCB).

**Conclusions**

A better understanding of the ways to expand UCB HSCs could increase the transplantation process and subsequently avoid engraftment failure. The second part to consider is an economic aspect. Beyond haematological disorders, this advances should be extrapolated to non-hematopoietic cells also containing in UCB that have a promising feature in regenerative therapy.

## 66 **The Effect of Insulin Sensitizers on Treatment and Liver-Related Outcomes in HCV-Infected Patients: A Systematic-Review and Meta-Analysis.**

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**Background and Aims:** Insulin resistance (IR) associated with hepatitis C negatively impacts treatment and liver-related outcomes. With the development of HCV protease inhibitors, sustained virological response (SVR) rates have improved significantly. However due to cost, access and other comorbidities, IFN/Rib remains the mainstay of HCV antiviral therapy in many circumstances. We reviewed and summarized current studies examining the role of insulin sensitizers in improving hepatitis C antiviral therapy response and reducing liver-related complications in hepatitis C patients.

**Methods:** Medline and Embase databases were searched to identify relevant studies. Studies that evaluated the benefit of insulin sensitizers on sustained virologic response to hepatitis C therapy or liver-related outcomes were included. A random-effects model was used to calculate a pooled risk estimate and estimate differences in treatment-related outcomes.

**Results:** There were 4 randomized controlled trials identified that measured the effect of insulin sensitizers on treatment response rates and 2 observational studies that evaluated long term liver-related outcomes. A pooled analysis of sustained virological response (SVR) trials did not indicate an improvement (OR 1.44, 95% CI 0.73-2.85) but exclusion of studies with a high risk of bias did demonstrate improved treatment response rates (OR 1.98, 95% CI 1.26, 3.09). A subgroup analyses of metformin only studies demonstrated increased SVR rates (OR 1.82, 95% CI 1.06, 3.10). For the observational evidence, a trend towards reduced hepatocellular carcinoma (HCC) event rate was noted in two studies (1 case-control, 1 cohort study) [case-control OR 0.39, 95% CI 0.13-1.27, cohort-study OR 0.20, 95% CI 0.04-0.92]. There were no studies that investigated the impact of insulin sensitizers on preventing the progression of liver fibrosis.

**Conclusion:** Although insulin resistance has been consistently associated with reduced SVR, increased fibrosis and increased risk for HCC and liver transplant, there are very few studies that have examined the benefits of insulin sensitizers in improving these outcomes. Insulin sensitizing agents are safe, well-tolerated, accessible and inexpensive, and may represent an attractive adjuvant to current interferon-based hepatitis C antiviral therapies. There is a need for larger, high quality studies to evaluate these potential benefits and understand the mechanisms by which IR may impact outcomes in this population.

## 126 **Selection of Resistance Settings for a Variable Resistance Orthotic Knee Joint**

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**Background:**

Knee-ankle-foot orthoses (KAFO) are leg braces designed for individuals with knee extensor weakness. A hydraulic variable resistance orthotic knee joint is being designed, based on the Ottawalk-Speed knee joint (developed at the Ottawa Hospital Rehabilitation Centre) and a variable flow hydraulic valve adapted from a commercial prosthetic ankle. The new joint is being

designed to improve locomotion during level ground walking, stair descent, stand-to-sit, as well as minimize the dimensions so the orthosis can be worn under clothing.

**Objective:**

The purpose of this study was to determine resistance settings for the hydraulic valve to be used in the orthotic knee joint. This new design uses a variable resistance valve to control hydraulic fluid flow from a linear hydraulic cylinder. By varying the flow, different resistive moments can be applied to the knee joint to appropriately support the user.

**Methods:**

A jig was designed to hold the Ottawalk-speed knee joint by the shank upright. A weight basket was attached to the end of the thigh upright. A custom brass orifice part was threaded onto the joint's hydraulic cylinder and a fluid reservoir was attached to the orifice exit. The orifice exit hole was re-drilled after each test to increase the diameter. Nine orifice sizes were tested, between 0.25-2.00 mm in diameter. The hydraulic cylinder was filled with hydraulic oil and the weight basket was dropped from 20° to 95° knee flexion. Reflective markers were attached to the thigh and shank uprights and an 8-camera motion capture system was used to collect 3D movement data at 100 Hz. For each orifice, four weights were dropped in the weight basket (12 trials per orifice).

**Analysis and Results:**

Marker data were reconstructed using Vicon Nexus software and analyzed using Visual 3D. Angular position and velocity were compared to knee angle and angular velocity from the literature (able-bodied, transfemoral amputee, stance-control KAFO for level ground walking and stair descent). Orifices settings were selected to support knee flexion for 50-100 kg individuals throughout stance but allow free knee motion during swing. Resistance settings were selected to allow the user to "ride" the knee joint when lowering the body's centre of gravity for stair descent and stand-to-sit.

**Conclusions:**

A viable hole pattern was generated for the hydraulic valve that will be used in the variable resistance orthotic knee joint. These setting will provide appropriate knee flexion resistance across a variety of walking tasks and daily activities.

67 **Toileting: a neglected topic in adult rehabilitation populations. A clinical review**

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**Background:** The ability to toilet independently is critical to dignity and self-esteem. Rehabilitation patients' reduced balance and arm mobility can impair their ability to safely and independently toilet themselves — i.e., defecate, urinate and clean the perianal/urethral regions. Discharge home after a rehabilitation stay may depend on the ability to perform these functions independently.

**Objective:** The purpose of this review was to synthesize the available information on toileting in rehabilitation populations and identify gaps in research concerning this neglected area.

**Methods:** The following databases were searched for articles on toileting in adult rehabilitation populations: Cochrane Library, Ageline, CPIO, CINAHL, Medline and PubMed.

**Results:** Studies on toileting were found in populations with chronic low-back pain, hip fracture, rheumatoid arthritis, amputation, multiple sclerosis, stroke, traumatic brain injury, Parkinson's disease, spinal cord injury and dementia. The review was divided into 1) causes of toileting impairment (physical vs. cognitive disability) (17 studies), 2) measurement of toileting performance (degree of cleanliness, need for assistive devices) (8 studies), 3) consequences of impairment (7 studies) and 4) interventions to enhance toileting independence (5 studies). Physical disabilities predominated over cognitive ones. No dedicated measures for toileting were found, but the Functional Independence Measure, a universally accepted measure of burden of care, has a toileting component. Both patients and health care professionals are embarrassed to discuss toileting, and affected patients feel distress and loss of dignity.

**Conclusions:** Although toileting impairment is a barrier to achieving independence, the rehabilitation literature on this topic is sparse. Currently available technologies may be effective toileting adjuncts.

68 **A Microsoft-Excel-based tool for running and critically appraising network meta-analyses for evaluation of health technologies: an overview and application of NetMetaXL**

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**Background**

Many questions regarding evaluation of health technologies for a medical condition are associated with the availability of multiple active treatment choices. Because of the complex structure of the evidence base in such situations, traditional approaches to meta-analysis (which deal with only two treatments) often fall short in providing a meaningful answer for clinicians, decision-makers, and patients. A technique called network meta-analysis has helped to greatly address these issues, however such analyses are statistically complex and difficult for researchers to implement. WinBUGS, a freely available Bayesian software package, has been the most widely used software to conduct network meta-analyses. However, its learning curve can be daunting, especially for new users. Furthermore, critical appraisal of network meta-analyses conducted in WinBUGS can be challenging given its limited data manipulation capabilities and the fact that generation of graphical output from network meta-analyses often relies on different software than the analyses themselves.

**Objective**

We set out to develop a tool that would (1) afford more researchers the ability to conduct network meta-analyses by developing a user friendly software tool; and (2) ease the challenges authors face in terms of generating complex graphical summaries for manuscripts by automating these tasks to produce publication-ready figures.

**Methods**

We developed a freely available Microsoft-Excel-based tool called NetMetaXL, programmed in Visual Basic for Applications, which provides an interface for conducting a Bayesian network meta-analysis using WinBUGS from within Microsoft Excel. This tool allows the user to easily prepare and enter data, set model assumptions, and run the network meta-analysis, with results being automatically displayed in an Excel spreadsheet. It also contains macros that use NetMetaXL's interface to generate evidence network diagrams, forest plots, league tables of pairwise comparisons, probability plots (rankograms), and inconsistency plots within Microsoft Excel. All figures generated are publication quality, thereby increasing the efficiency of knowledge transfer and manuscript preparation.

**Results**

We demonstrate the application of NetMetaXL using data from a recent network meta-analysis which compares combined resynchronization and implantable defibrillator therapy in left ventricular dysfunction. We replicate results from the previous publication while demonstrating result summaries generated by the software.

**Conclusions**

Use of NetMetaXL successfully demonstrated its ability to make running network meta-analyses more accessible to novice meta-analysts by allowing analyses to be conducted entirely within Microsoft Excel. NetMetaXL also allows for more efficient and transparent critical appraisal of network meta-analyses and enhanced standardization of reporting which can improve reporting of studies using this methodology.

69 **Predicting cancer incidence in the Ontario population based on health behaviours**

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6. Department of Family Medicine, University of Ottawa

**Background:**

Health behaviours are important and modifiable risk factors for the development of malignancies, however they are often not included in cancer risk prediction tools. Furthermore, most risk prediction tools for cancer are developed for individuals, rather than assessing risk at a population level. This study is intended to determine the ability to accurately predict incident cancer among individuals in the Ontario population on the basis of easily measured and widely available data on behavioural factors.

**Objective:**

The primary objective is to utilize self-reported health behaviours to predict the incidence of malignancy, from any site, among the Ontario population. The secondary objective aims to validate the derived risk algorithm in a separate population to ensure predictive accuracy and discrimination between those at low and high risk of malignancy.

**Methods:**

This prospective cohort study will involve Ontario household respondents aged >20 years old to the Canadian Community Health Survey (CCHS) from 2003, 2005 and 2007. Individuals without Ontario universal health insurance and those with a prior diagnosis of cancer will be excluded from the analyses.

The specific predictor variables of interest from the CCHS are intake of fruits and vegetables, activity level, smoking status and alcohol consumption. Other potentially important covariates such as individual or household income, education level and ethnicity will also be determined from the CCHS. The primary outcome, cancer from any site, will be defined as a first diagnosis of cancer in the Ontario Cancer Registry following CCHS survey administration.

Cox proportional hazard models will be used to generate a multivariable model including all pre-defined health behaviours to establish hazard of incident cancer. Model performance will be assessed using calibration (difference between observed and predicted values in the overall population and across pre-identified subgroups) and discrimination (based on C-statistic). Statistical analyses will be completed in SAS, version 9.3.

The final risk algorithm will be validated by applying it within the CCHS 2001 cohort, and assessing calibration and discrimination in this population.

**Relevance:**

The intent of creating this risk algorithm is to assess the contribution of lifestyle factors, both individually and in combination, on incident cancer, and subsequently to evaluate the potential for cancer risk reduction within the population through alteration of health behaviours.

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**Health and healthcare impacts of the Ottawa Model for Smoking Cessation**

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**Background:** Smoking-related illnesses contribute to over 10% of all healthcare expenditures in Canada. On a given day, more than 20% of Canadian hospital beds are being occupied by current smokers.

**Objective:** To determine the effects of a hospital-initiated smoking cessation intervention on healthcare utilization and mortality as compared to usual care.

**Methods:** This was a multi-centre, two group, retrospective, before and after cohort study involving 14 Ontario hospitals that were implementing the Ottawa Model for Smoking Cessation (OMSC) intervention. Participants from each hospital were: current smokers (i.e. daily smoker in the past 7 days); over the age of 17; and, eligible for the Ontario Health Insurance Plan (OHIP) throughout the study period. The usual care group (n=641) received no formal smoking cessation program while admitted to hospital. The intervention group (n=726) received the OMSC intervention. Baseline participant data from each group was linked to Ontario healthcare administrative databases. Our main analyses compared the cumulative incidence of re-hospitalization, emergency department (ED) visits, physician visits and death in smokers that received the OMSC intervention compared to smokers that did not. We used competing risks regression and assessed outcomes at 30 days, 1 year and 2 years following index hospitalization.

**Results:** Smokers in the intervention group were more likely to be smoke-free at 6 months. Over the two-year follow up period, smokers who received the OMSC intervention experienced: a lower incidence of all-cause re-hospitalization (Hazard ratio (HR) = 0.79 [0.68-0.91], p<.0001); a lower incidence of smoking-related re-hospitalization (HR = 0.79 [0.66-0.92], p>.0001); a lower incidence of all-cause emergency department visits (HR = 0.91 [0.84-0.99], p=0.04); and a lower risk of death (HR = 0.61 [0.43-0.86]), p=.0007).

**Conclusions:** Implementation of the OMSC hospital-based intervention is associated with significant reductions in overall health care utilization and mortality at 30-day, 1-year, and 2-year follow-up. These impacts are achieved with a modest per patient intervention cost of \$85.

127 **Subjective and objective measures of cognition in MS: A preliminary analysis of correlations and test-retest reliability**

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2. University of Ottawa,

3. The Ottawa Hospital

Background: The Multiple Sclerosis Neuropsychological Questionnaire (MSNQ) is a screening tool for cognitive impairment in MS. The relationship between MSNQ (self- and informant- report) and both cognition and depression were examined. Significant correlations were expected between MSNQ-informant and objective cognition, as well as between MSNQ-self and self-report ratings of depression. Moderate test-retest reliability was expected for the MSNQ.

Objectives: The relationship between subjective cognition and mood, and objective cognition was measured over time. Test-retest reliability was examined.

Methods: 27 individuals with MS (21 RRMS, 4 SPMS, 2 PPMS) recruited from the Ottawa Hospital MS Clinic, completed the Brief International Cognitive Assessment for MS (BICAMS) at baseline and two weeks follow-up. The BICAMS includes the Symbol Digit Modalities Test [SDMT], the California Verbal Learning Test – Second Edition learning trials [CVLT-II] and the Brief Visuospatial Memory Test – Revised learning trials [BVRT-R]. Other measures include: Auditory Consonant Trigrams (9" & 18"; ACT), Paced Auditory Serial Addition Test (PASAT), Controlled Oral Word Association Test (FAS & Animals), Daily Fatigue Impact Scale (D-FIS). Participants completed self-report measures of depression: Beck Depression Inventory Fast Screen for Medical Patients (BDI-FS); Patient Health Questionnaire – 9 (PHQ-9); and Center for Epidemiological Studies in Depression Questionnaire (CES-D). Participants and an informant completed the MSNQ.

Results: At time 1 the only significant correlation was between MSNQ-I and BDI-FS ( $r = 0.42, p < .05$ ). At time 2 MSNQ-S correlated with PHQ-9 ( $r = 0.39, p < .05$ ) and MSNQ-I correlated with SDMT ( $r = -.43, p < .05$ ), ACT-9 ( $r = -0.44, p < .05$ ) and ACT-18 ( $r = -0.43, p < .05$ ). Both MSNQ-S ( $r = 0.83, p < .001$ ) and MSNQ-I ( $r = 0.81, p < .001$ ) showed high test-retest reliability.

Conclusions: Expected relationships between self-report cognition and mood, as well as informant-report cognition and objective cognition, were observed at time 2. The lack of similar relationships at time 1 is unexpected. Post-hoc regression analyses were conducted to determine if D-FIS (i.e. fatigue on the day of testing) impacted target relationships. Although not statistically significant, D-FIS approached significance ( $p = 0.066$ ) as a potential moderator when examining informant ratings of cognition and mood (Adjusted  $R^2 = 0.094$ ). Informants' ability to estimate cognition may have been confounded by MS participants' level of fatigue on the day of testing.

71 **COLONIC POLYPS IN PATIENTS WITH CYSTIC FIBROSIS: A PROSPECTIVE PILOT STUDY**

Marc R. Fournier, Avijit Chatterjee, Shawn Aaron presented by **Piriya Paramanathan**

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Background - Objective

Cystic fibrosis (CF) is the commonest lethal genetic disease to affect Caucasian populations. It is an autosomal recessive disease characterized by a defect in the CF transmembrane conductance regulator (CFTR), a chloride-transporting channel. The CFTR gene lies in the chromosome band (7q31) adjacent to the MUC3, MUC11, and MUC12 genes (ref). These genes are believed to be involved in the pathogenesis of colon cancer. In a study reviewing over 30,000 patients with CF, there was clear evidence of an excess of digestive tract cancers and no evidence of an increased risk of any other cancers (ref). In a 10-year study examining the risk of colon cancer, CF patients developed more colon cancer than expected with a standardized incidence ratio of 7.4 compared to the Surveillance, Epidemiology and End Results database (SEER) (ref, Maisonneuve). Currently, there is no data on polyp prevalence in patients with Cystic Fibrosis. We sought to assess the feasibility of conducting a screening colonoscopy to detect the polyp rate in a young population with cystic fibrosis.

Methods

Enrolment, Eligibility, and Data Collection

All patients were recruited prospectively at The Ottawa Hospital Cystic Fibrosis clinic from August 2010 through July 2011. Baseline demographical and Cystic Fibrosis Disease characteristics were collected from a CF patient database. Included in this database are information such as genotype, transplantation history, and immunosuppressive drugs if applicable. All patients enrolled were aged 18 years or greater, along with: 1) a confirmed diagnosis of cystic fibrosis (sweat chloride value higher than 60 mmol/litre or 2 disease-causing mutations), 2) clinically stable cystic fibrosis, (ie. no recent acute exacerbations of airway disease within the last 4 weeks). Key exclusions included: 1) Patients with a recent and acute exacerbation of pulmonary symptoms within the previous 4 weeks, 2) Pregnancy 3) baseline FEV1 < 25% predicted, and 4) inability to provide informed consent.

Results: analysis partially completed (fully will be completed prior to research day).

**Conclusion:**

Given the young age of study participants undergoing colonoscopy, we believe this result to be significant. The key limitation of our pilot study is our sample size. It is possible that our sample of subjects undergoing colonoscopy and the polyp detection rate may not reflect the true incidence in subjects with cystic fibrosis. However, we feel that our results do demonstrate evidence supporting a larger scale prospective study to evaluate this question.

## 72 **Real-time signal quality analysis of ambulatory ECG for improving positive predictive value of myocardial ischemia detection**

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**BACKGROUND:** Around the world, approximately 100 million people undergo non-cardiac surgery each year. Of these more than 1 million will experience a major cardiac complication as a result of their surgery. Prophylactic  $\beta$ -blockade has been shown to reduce the incidence of these cardiac complications in at-risk patients; however a large scale study of the treatment has demonstrated that it also increases mortality rates – this is likely due to administration of  $\beta$ -blockers to less at-risk patients. The Perioperative Ischemia Reduction Studies (PROSE) seeks to evaluate tailored administration of  $\beta$ -blockers only to patients experiencing myocardial ischemia (a lack of oxygen in the heart tissue that often precedes complication). Myocardial ischemia is detectable on a patient's electrocardiogram (ECG) up to two hours before lasting cell damage occurs, thereby providing an intervention window to stop the ischemic cascade. Unfortunately, the environment surrounding postoperative patients following non-cardiac surgery subjects the ECG to high levels of noise. As such, myocardial ischemia detection techniques still return significant numbers of false alarms despite supplementary engineered "white box" solutions, rendering monitoring clinically impractical.

**OBJECTIVE:** To build upon a previously developed signal quality index (SQI) and investigate potential alarm modification strategies on the basis of signal quality, such that positive predictive value (PPV) is improved while maintaining alarm sensitivity.

**METHODS:** Motion artifact was added to two 24 hour ECG records containing ischemic episodes to simulate patient movement. These two contaminated ECG records were then provided to a commercially available bedside monitor, which logged estimates of ST segment deviation on a personal computer; ST estimates are used to trigger ischemia alarms. Signal quality of the contaminated ECG records was analyzed using a previously developed signal quality index (SQI), and the SQI values were used to modify ischemia alarms. Resulting modified alarms were then compared to known ischemic episodes in the ECG records. **RESULTS:** Modifying alarms on the basis of signal quality improved PPV from 0.41 to 0.82 while maintaining sensitivity.

**CONCLUSION:** Proof of concept testing using two offline ECG records showed that modifying ischemia alarms on the basis of signal quality effectively increases PPV while maintaining alarm sensitivity. This suggests that using signal quality to modify alarms makes a significant contribution toward clinically practical ischemia monitoring.

## 73 **Efficacy of Mesenchymal Stromal Cell Therapy for Acute Lung Injury in Preclinical Animal Models: A Systematic Review**

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10. Department of Cell and Molecular Medicine, University of Ottawa, Ottawa, Ontario, Canada

**Background:**

Acute Respiratory Distress Syndrome (ARDS) is a devastating clinical condition characterized by hypoxemia, increased pulmonary alveolar and capillary membrane permeability, inflammation and progressive respiratory failure. Recently mesenchymal stromal cells (MSC) have emerged as a potential novel treatment due to their ability to modulate inflammation, enhance bacterial clearance, and repair tissues in pre-clinical models of ARDS.



**Objective:**

To systematically review the effects of MSC therapy on death in preclinical animal models of acute lung injury (ALI).

**Methods:**

A systematic search of MEDLINE, EMBASE, BIOSIS and Web of Science (June 2013), a grey literature search and a manual search of bibliographies was conducted to identify preclinical control comparison studies using in vivo models of ALI. Data was collected for the primary outcome mortality, as well as for general characteristics allowing for subgroup meta-analyses. Results were pooled and expressed as odds ratios (OR) and 95% confidence intervals (CI). Studies that administered human MSCs were examined for MSC characterization criteria as defined by Dominici et al (2006). Risk of bias was assessed using the Cochrane approach, and funnel plots were generated to examine publication bias.

**Results:**

3810 citations were reviewed; a total of 54 studies met our inclusion criteria. Twenty-one experiments (within 18 studies) reported mortality. Meta-analysis revealed that treatment with MSCs as compared to controls significantly decreased the odds of death in animals with ALI overall (OR 0.24 (95% CI 0.17-0.34)), and at pre-specified time points: =2 days OR 0.30 (95% CI 0.21-0.44), 2 to =4 days OR 0.32 (95% CI 0.18-0.54) and >4 days OR 0.18 (95% CI 0.09-0.35). Subgroup analyses according to animal, ALI and MSC characteristics also generally suggested reductions in death with MSC treatment. Of the 18 experiments that utilized human MSCs, none met all the MSC characterization criteria. None of the 54 studies were considered to have a low risk of bias across all 7 domains and funnel plot analysis showed evidence of asymmetry, suggesting publication bias.

**Conclusions:**

Results from our meta-analysis convincingly demonstrate that treatment with MSCs reduce the odds of mortality in animal models of ALI. Overall reporting was sub-optimal, including reporting on MSC characterization for studies that used human MSCs. Visual funnel plot analysis suggested the possibility that negative MSC ALI comparative efficacy studies are not published.

1. Dominici et al. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. *Cytotherapy* 2006; 8(4): 315-7.

## 74 **Wearable ultrasonic sensor for continuous monitoring of plantar soft tissue for diabetic patients**

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**Background**

One of the complications of diabetes is peripheral neuropathy, where insensitive nerves can lead to serious foot ulceration. Studies have shown that the mechanical properties of plantar soft tissues change with diabetes. Ultrasound methods could be used to measure plantar tissue thickness and mechanical properties, as a quantitative assessment tool. A wearable and flexible ultrasonic sensor, made of a piezoelectric polymer film, was developed and verified for continuous, real-time measurement of soft tissue layer thicknesses.

**Objective**

The objective of this study was to develop an ultrasonic sensor, that can be embedded into footwear, for continuous real-time monitoring of plantar soft tissue mechanical properties during physical activities such as walking and standing.

**Methods**

Wearable ultrasonic sensors were constructed using a piezoelectric PVDF film. A smaller sensor was 16.5 mm diameter, 400 µm thick, and 0.2 g weight. Experiments were performed using water and pork meat to investigate measurement accuracy. The water layer thickness between the sensor and a reflector was changed in steps of 0.3048 mm for water and 0.1524 mm for pork, using a precision mechanical translation stage. The developed wearable sensor and a commercial ultrasonic sensor were used in the experiments for comparison purpose. Following lab tests, an in-vivo test was conducted with a human subject. The developed sensor was attached to a person's left heel and a force was applied on the heel using the person's own weight to measure the plantar soft tissue thickness change.

**Results/Discussion**

Good agreement was found between thicknesses obtained by the developed wearable sensor and commercial sensor. The average distance change in each step was 0.3071 mm (SD=0.0037 mm, 1.2 %) for the developed sensor and 0.3038 mm (SD=0.0046 mm, 1.51 %) for the commercial sensor. Results for pork meat were 0.1529 mm (SD=0.0033 mm, 2.19 %) for the developed sensor and 0.1514 mm (SD=0.0013 mm, 0.87 %) for the commercial sensor. Planter tissue thickness at the heel decreased from 14.3 mm to 8.5 mm with the pressure change from 0 to 122 kPa.

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**75 Vaccination Attitudes and Mobile-Readiness: A Survey of Expectant and New Mothers.**

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**Background**

Sub-optimal vaccination coverage and recent outbreaks of vaccine-preventable diseases serve as a reminder that vaccine hesitancy remains a concern. We developed a smartphone application(app) to enable and empower individuals to track their immunizations via their mobile device. Although not the initial goal, the app may address several reasons for not vaccinating.

**Objectives**

- 1) To ascertain attitudes and beliefs about vaccinations and their relationship with mobile-readiness amongst a sample of women within three months of childbirth.
- 2) Determine if baseline attitudes toward pediatric vaccination vary based on mobile phone usage.

**Methods**

We recruited women within three months of childbirth from a hospital in Ottawa, Canada. We used surveys to collect demographic information, examine attitudes, behaviour, and information sources regarding immunization and self-reported mobile phone usage.

**Results**

A total of 54 women participated. The majority of participants had positive attitudes towards vaccination(96%) and intended to vaccinate their children(98%). Participants were interested in information on pediatric vaccination(94%), and found information from public health the most reliable and accessible(78%) compared to the Internet(52%), mobile devices(35%) and alternative medicine providers(10%). Participants trusted information from their doctor or nurse and public health(83%) more than information from other sources. Participants reported using apps on their smartphone regularly. Information apps were the most highly used(83%), followed by apps providing organizational tools(59%). Almost half(46%) of participants use their smartphone to manage bank transactions regularly. We found no evidence of an association between mobile device usage and attitudes and behaviours about vaccination.

**Conclusions**

This is the first evaluation of mobile-readiness for a smartphone app to track immunizations. We found that only a third of respondents identified information on immunization from mobile devices to be reliable/accessible though they reported using apps regularly for other purposes. There exists an opportunity to provide reliable information on vaccination through mobile devices to better inform the public.

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## Neuroscience Program

### 76 Probing the Structural Determinants of the Central Region of Third Intracellular Loop of Human Dopamine D1-class Receptor Subtypes

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The D1-class dopaminergic receptors (D1, D5) are transmembrane (TM) G protein-coupled receptors (GPCRs) modulating major physiological effects in the brain and in the periphery. The first and the second intracellular loops are highly conserved between D1R and D5R while the third intracellular loop is the most divergent. The alpha-helical N- and C- terminal ends of the third intracellular loop (IL3) of GPCRs play an important role in receptor activation and G proteins coupling. However, whether the central region of the IL3 linking the cytoplasmic alpha-helical extension of TM5 and TM6 is required for activation and G protein coupling of D1 and D5 remains to be fully appreciated. To address this issue, we performed deletions of the conserved N-terminal or the divergent C-terminal moieties of the central region of IL3 of human D1-class receptors (D1?N and D5?N; D1?C and D5?C). Agonist affinities were significantly increased and decreased at D1?N and D5?N, respectively. Meanwhile, D1?C and D5?C exhibited a drastic increase in agonist affinity. We also noted elimination of constitutive activity of D1?N and D5?N, and a stark reduction in agonist-induced cAMP formation by these mutants. In contrast, constitutive and agonist-dependent activity of D1?C and D5?C were substantially augmented. Taken together, our results imply a major role of the central region of IL3 in subtype-specific activation of D1-class dopaminergic signaling. We propose that this may be done via distinct motifs found in the N- and C-terminal moieties of the central region of IL3. Work is in progress to assess the role of these moieties in agonist-induced receptor desensitization. This work is supported by a Graduate Scholarship from King Saud University (to AB) and OMHF grant (to MT).

### 77 Generation of a mouse model for recessive Parkinson disease: The parkin/MnSOD double mutant mouse

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**Background:** Recessively inherited loss-of-function mutations in the Parkin gene are associated with familial cases of early-onset Parkinson disease (PD) (Kitada et al., 1998). Early studies investigated Parkin's function as an E3 ubiquitin ligase, and loss-of-function of Parkin was speculated to induce accumulation of substrates resulting in neurodegeneration of midbrain neurons (Shimura et al., 2000). Other groups established a second theory regarding Parkin's function; namely, that Parkin is essential for "mitophagy". They speculated that lack of Parkin causes accumulation of damaged mitochondria with low mitochondrial membrane potential and impaired oxidative phosphorylation generating power (Narendra et al., 2010). However, new evidence has recently surfaced regarding Parkin's protective effects against oxidative stress and mitochondrial dysfunction (Henchcliffe & Beal, 2008). Furthermore, Manganese superoxide dismutase located within mitochondria, and acting as the first line of defense against the generation of superoxide, and in turn H<sub>2</sub>O<sub>2</sub>, has been reported as a susceptibility factor for PD.

**Hypothesis:** Haploinsufficiency at the MnSOD locus will increase the level of ROS in mitochondria from parkin-null mice (upstream event) resulting in calcium dysregulation and other mitochondrial dysfunction, and therefore lower the threshold for neuronal death (downstream event) in dopamine-producing cells. Here, we present the generation of a parkin/MnSOD double mutant mouse model for recessive PD. We will examine ROS levels including of superoxide and H<sub>2</sub>O<sub>2</sub>, additionally monitoring mitochondrial and cytosolic calcium levels, in the double mutant mice, while comparing them to parkin knock-out animals and wild-type control. We anticipate that higher ROS levels in the parkin/MnSOD double mutant mice will result in primary mitochondrial dysfunction, including calcium dyshomeostasis.

**Summary:** The molecular analysis of parkin/MnSOD double mutant mice will help us elucidate the cascade of events from the generation of ROS and calcium dysregulation to mitochondrial dysfunction.

78 **Characterizing alterations in the protein components in synaptosome fractions from neural tissues in a mouse model of SMA**

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**Background:** Neuromuscular junctions are a primary site of pathology in the course of Spinal Muscular Atrophy (SMA). However, there is also evidence that synapses within central nervous system (CNS) might be affected in this disease. For example, it is shown that Smn is highly expressed within mouse CNS during development and SMN deficiency leads to cell death and pathological foci in the mouse telencephalon during this period. There are also reports of impairments of spinal circuits in SMA mouse models. Using the Smn2B/- mouse model of SMA, we are investigating the synaptic alterations in the CNS before the onset of the disease. Specifically, we are preparing synaptosomes from cortices and spinal cords to assess proteomic changes upon Smn depletion.??

**Results:** Smn2B/- mice were sacrificed at P11 and P16, and their cortices and spinal cords were dissected immediately. Enriched synaptosomal preparations were prepared by ultra-centrifugation of crude lysates on the top of non-continuous Percoll gradients. In the first phase of this study, a biased approach was used to investigate the alterations of specific synaptic markers in Smn2B/- mice. Interestingly, minimal alterations in protein amounts of synaptic markers were observed in synaptosomal fractions of P11 Smn2B/- mice compared to Smn2B/+ littermates. In the next phase of the study, we will use an unbiased proteomic approach to determine the phospho/proteomic profile of synaptosomal fractions in synaptosomes from both pre-phenotypic and phenotypic mice. The results will be analyzed to identify which signalling pathways are affected before the onset of disease in Smn2B/- mice. ??

**Conclusions:** By studying aberrant phospho/proteomic profiles in Smn2B/- mice, we will be able to identify new pathways as possible targets for treating spinal muscular atrophy.??

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79 **Behavioural deficits in the SYNERGY mouse: a bi-genic model of Parkinsonism and dementia with Lewy bodies**

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**Background:** Parkinson's disease (PD) and dementia with Lewy bodies (DLB) are related neurodegenerative diseases characterized by neuronal loss and  $\alpha$ -synuclein accumulation. The SYNERGY mouse (Synucleinopathy related to Gaucher's and Lewy body dementia) is a novel model of parkinsonism and DLB with three genetic manipulations strongly linked to PD and DLB: 1) amplification of the human SNCA ( $\alpha$ -synuclein) gene, 2) A53T point mutations in all SNCA alleles, and 3) Gba1D409V (glucocerebrosidase) knock-in mutations.

**Objective:** Perform a comprehensive behavioural characterization of the SYNERGY mouse at 1½, 3, and 6 months of age (moa) and analyze pathological  $\alpha$ -synuclein accumulation at each time point. We hypothesize that SYNERGY mice will show age-dependent motor, olfactory, and cognitive behavioral deficits that correlate with  $\alpha$ -synuclein load in specific brain regions.

**Methods:** Behavioural characterization is carried out in three separate cohorts for each age group of SYNERGY mice: 1) Motor/sensorimotor, 2) Olfactory, 3) Learning/Memory. For each group, SYNERGY mouse performance is compared to SYNERGY littermate controls that are wildtype for Gba1 and wildtype mice on the same mixed background. Following behavioural testing, brain tissue is collected for immunohistochemical and biochemical analysis of  $\alpha$ -synuclein accumulation.

**Results:** At 1½ moa SYNERGY mice have a mild motor deficit compared to controls; underperforming in the rotarod task while demonstrating normal motor function in all other tests. This motor deficit appears to be progressive: by 3moa SYNERGY mice have more pronounced and significant motor deficits in all tests. They also show sensorimotor deficits with normal olfaction and cognitive performance. Based on characterization of the parental strains, we predict SYNERGY mice will develop cognitive deficits by 6moa.

**Conclusions:** This preliminary behavioural data suggest that the SYNERGY mice have an age-dependent decline in motor function. Further behavioural and histopathological characterization is ongoing. These data suggest that the SYNERGY mouse has the potential to be the most relevant model of parkinsonism and DLB and a useful tool in studying disease mechanisms and drug development.

80 **Monitoring Viral Encephalitis in LRRK2 Mutant Mice: Implications for Parkinson Disease**

**Irene E. Harsmen**<sup>1,2</sup>, Mansoureh Hakimi<sup>1,3</sup>, Jay Majithia<sup>3</sup>, Julianna J. Tomlinson<sup>1</sup>, Earl Brown<sup>1,3</sup>, Michael G. Schlossmacher<sup>1,3</sup>

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**Background**

Leucine-rich repeat kinase-2 (LRRK2) has been implicated in modulating the risk of Parkinson's, Crohn's disease and leprosy. One of its functions is thought to lie within the innate immune system at the interface of host susceptibility, environmental triggers and tissue inflammation (Hakimi et al., 2011).

**Objective**

To better study its role in complex diseases, we employed a recently described infection model using a neurotropic, respiratory-enteric-oral (REO) virus, serotype-3-Dearing (Gauvin et al., 2013). We hypothesized that LRRK2 modulates disease severity.

**Methods**

After nasal administration of REO-virus to suckling *lrrk2* knock-out mice and wild-type littermates (Tong et al., 2009), we used holocranomicroscopy (ie, whole skull mounts) to monitor infection from rhinitis to terminal encephalitis. To probe for genotypic differences, we quantified anti-REO antibody positivity by Aperio-ImageScope software examining serial, 4 micrometer-thin sections. We juxtaposed these data with available viral titres of select organs.

**Results**

The rate of neuronal infection at the time of euthanasia (day 11) was higher in knock-out mice for three regions examined. Mean percentage counts for anti-REO antibody-positive neurons in knock-out (versus wild-type) mice of thalamus, midbrain, and cerebellum measured 4.72(3.12), 8.20(2.00) and 0.37(0.20), respectively; corresponding p-values for the differences (0.17-6.20%) were calculated at 0.0871,  $1.75 \times 10^{-8}$  and 0.1895, respectively. These neuropathological results were associated with preliminary findings of higher viral titres in the liver, lungs and brain of knock-out (versus wild-type) mice three days post inoculation.

**Conclusions**

REO-T3-Dearing virus-infected *lrrk2* knock-out mice show significantly greater rates of neuronal infection in the midbrain by day 11 when compared to wild-type animals. We conclude that LRRK2 modulates host susceptibility and/or response after nasal exposure to a neurotropic pathogen.

81 **Functional role of second intracellular loop for D1-class dopaminergic receptors**

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Dopamine (DA) regulates several physiological processes in brain and periphery through interaction with specific class A G protein-coupled receptors (GPCRs). DA receptors are divided into Gs-coupled D1-class (D1R and D5R) and Gi-coupled D2-class (D2Rshort/long, D3R and D4R). The structural basis underlying the distinct signaling properties of the highly homologous D1R and D5R are not fully understood. Crystal structures of antagonist-bound  $\beta_1$ -adrenergic receptor and inverse agonist-bound  $\beta_2$ -adrenergic receptor suggest that the second intracellular loop (IL2) adopt distinct conformations. We hypothesize that conserved residues of IL2 among class A GPCRs play a critical role in D1R and D5R signaling properties. To test our hypothesis, we created D1-PD2 and D5-PD2 mutants, in which all serine and threonine residues of IL2 were mutated to alanine and valine, respectively. We have assessed the ligand binding and G protein-coupling properties in transfected HEK293 cells. We show that the global mutation of serine and threonine residues differentially modulates agonist affinity, agonist-independent (constitutive) and -dependent activity of D1R and D5R. D5-PD2 displays a drastic loss in agonist affinity and constitutive activity whereas those remain unchanged for D1-PD2 when compared with their respective wild type counterparts. Moreover, D1-PD2 mediates a higher maximal activation of adenylyl cyclase (AC) whereas D5-PD2 couples less efficiently to AC stimulation. Interestingly, when compared to wild type receptors, D1-PD2 and D5-PD2 mutants do not undergo DA-induced desensitization but internalize to a similar extent following a brief exposure to DA. Overall our studies suggest that IL2 of D1-class receptors differently regulate agonist binding, constitutive activity and DA-induced AC stimulation, and are critical in mediating DA-induced D1R and D5R desensitization. Work is underway in our lab to delineate the role of each serine and threonine residues within IL2.

82 **Genes that regulate antero-posterior motor neuron spacing in *C. elegans*****Aysha C. Rankin**<sup>1,2</sup>, Matthew Tanner<sup>1,2</sup>, Antonio Colavita<sup>1</sup>

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2. Cellular and Molecular Medicine, University of Ottawa

**Background**

Planar cell polarity (PCP) is the driving force behind a diverse complement of cellular processes that occur during embryonic development. There are many genes in the planar cell polarity pathway that contribute to the migration and polarization of the embryonically-derived dorsal D (DD) motor neurons in the nematode *Caenorhabditis elegans*. Mutations in certain planar cell polarity genes, in particular Prickle (*prkl-1*) and Van Gogh (*vang-1*), can cause defects in the spacing of the DD neurons along AP axis. It is hypothesized that these positional defects can be traced back to problems with the normal intercalation process of the neurons which occurs early on during embryonic development. With the goal of identifying more genes or allelic variations responsible for this departure from the wild-type phenotype, an investigation was conducted by making use of a forward genetic screen.

**Objective**

The discovery-driven investigation into the genetic mutations responsible for dorsal D motor neuron positional defects that result in their abnormal spacing along the AP axis, and the subsequent characterization of function, and localization of the defective gene to a planar cell polarity pathway.

**Methods**

A forward genetic screen was conducted using the mutagen ethylmethanesulphonate. Visualization of the DD motor neurons for screening and subsequent characterization of the mutant phenotypes was enabled by the fluorescent DD reporter, *ynls37* [*flp-13p::GFP*].

**Results**

To-date six genetic screens have been performed, and approximately 7300 haploid genomes examined. Upwards of 110 genetic screen mutants displaying the positional defect phenotype of interest were characterized, the strongest of which are being referred to as *npd-1* through -4. The genetic identities and functions of *npd-1* through -4 are currently being elucidated.

**Conclusions**

This research aims to gain more insight into genes in the planar cell polarity pathway. The discovery of new genes in the planar cell polarity pathway that contribute to proper migration and polarization of the DD neurons would be significant to the field of *C. elegans* research because as of yet only *prkl-1* and *vang-1* have been linked to the 1234-motor neuron positional defect mutation. The uncovering of other genes that function in a manner similar to *prkl-1* or *vang-1*, may also be implicated in other Wnt-directed signaling functions. Additionally, orthologues of alternative planar cell polarity genes could be identified in higher order species and could have implications for other nervous system developmental planar cell polarity pathologies like neural tube closure defects in vertebrates, or certain cancers in post-embryonic cells.

83 **THE ROLE OF ALS8-LINKED VAMP-ASSOCIATED PROTEIN B (VAPB) IN CAENORHABDITIS ELEGANS MOTOR NEURONS****Wendy Zhang** 1,2, Jiravat Visanvimol 1, Antonio Colavita 1,2, John K. Ngsee 1,2

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2. Cellular and Molecular Medicine, University of Ottawa, Ottawa, ON, Canada

**Background:**

Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disease that affects both upper and lower motor neurons, resulting in a loss of motor neurons of the spinal cord, brainstem and motor cortex. A familial form, ALS8, is linked to a single missense mutation in the VAPB gene, resulting in a substitution of Proline at residue 56 by Serine (P56S). Although the formation of dilated ER membranes induced by VAPB-P56S is suggested to compromise various intracellular processes, ALS is a motor neuron disease that specifically causes the degeneration and death of motor neurons.

**Objective:**

Currently, neither the *Drosophila* nor the mouse model shows evidence of motor neuronal death. Therefore, this study aims to generate a *C. elegans* animal model for mutant VAPB to determine the underlying mechanism of selective, age-dependent vulnerability of motor neurons.

**Methods:**

- 1) One transgenic strain where the expression of VAPB-WT and VAPB-P56S was placed under the control of the *unc-4* promoter, which is active in "dorsal A" (DA) motor neurons that control backward locomotion, was created.
- 2) Because VAPB-P56S is believed to be a loss of function mutation causing ALS8 in humans, a second transgenic worm was created to knockdown the VAP homologue in *C. elegans* (*vpr-1*) in DA neurons.

These transgenic worms were further crossed to a GFP reporter line to highlight the cell bodies and axons. Locomotor defect, axonal guidance phenotype and age-dependent death were measured.

Results:

The VAPB-WT and VAPB-P56S transgenic worms show a backward locomotory defect and the axons of DA motor neurons are misguided. There is also a significant increase in DA motor neuron loss from Day 3 to Day 11. This loss is accelerated by a brief exposure to Paraquat, a known oxidative stress inducer. Further, *vpr-1* knockdown worms exhibit a significant loss of DA neurons by Day 6 of adulthood. The most susceptible DA neurons in both models are DA6 and DA7.

Conclusion:

Worms expressing the transgene in the DA neurons have a locomotor defect, misguided axons and age-dependent loss of DA neurons. The *vpr-1* knockdown worms also show a similar loss. The progressive neuronal loss in both transgenic worm models recapitulates the age-dependent onset of disease symptoms in human ALS8.

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## Regenerative Medicine Program

84 **Withdrawn**

85 **Maintenance of muscle stem cells by CpG methylation at Myf5**

**Gregory C. Addicks**<sup>1,2</sup> and Michael A. Rudnicki<sup>1,2</sup>

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2. Cellular and Molecular Medicine Program, University of Ottawa

**Background:** There are two classes of muscle satellite cells which allow restoration of muscle after injury and growth and maintenance of muscle through out life. The first are satellite stem cells which can be transplanted and are able to repopulate the muscle stem cell niche and extensively replicate and contribute to myogenesis and which importantly, have never activated the myogenic gene Myf5. The second are committed muscle satellite cells which have expressed Myf5 and which have a decreased ability to contribute to myogenesis after transplant.

**Objective:** Myf5 expression is under the control of Pax7 and loss of Pax7 through knockout or knockdown results in the loss of Myf5 expression. Pax7 however is expressed in both satellite stem cells which do not express Myf5 and committed satellite cells which do express Myf5. It is not clear why Pax7 does not activate Myf5 expression in satellite stem cells. This work aims to address this question.

**Methods and Results:** We have found that at the embryonic stem cell stage of development Myf5 gene DNA is methylated at CpG sites and that this methylation is lost during development. The Myf5 gene is no longer methylated in myoblasts which are derived from muscle satellite cells, nor is DNA methylation restored after differentiation when the Myf5 gene is turned off. DNA methylation has been suggested as an epigenetic mechanism to suppress gene expression and our work suggests that DNA methylation at Myf5 acts to prevent premature expression of Myf5 during development. To address at what stage of development methylation at Myf5 DNA is lost, we assessed whether DNA methylation is found at Myf5 in satellite stem cells. Satellite stem cells and myogenic satellite cells were isolated using FACS and MeDIP was used to assess DNA methylation in these two cell populations. Intriguingly, it was found that satellite stem cells are CpG methylated at Myf5, but this methylation is lost when Myf5 expression is activated in myogenic satellite cells.

**Conclusion:** This work suggests a mechanism by which muscle stem cell status is maintained by DNA methylation at Myf5 and when this DNA methylation is lost, likely through recruitment of DNA demethylating Tet proteins (via a specific but unknown mechanism), muscle stem cell status is lost.

86 **DNA Damage Repair Protein XRCC1 is Essential for driving Myogenic Differentiation**

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**Background:**

Changes in DNA structure are hallmarks of the maturation process that converts a stem cell into an adult form differentiated cell type. During myogenesis, satellite cells are converted into myotubes with a great deal of alteration to the genetic expression profile of these cells.

**Objective:**

Using primary myoblasts isolated from various mouse models, we study the role of X-ray Repair Cross Complementing protein 1 (XRCC1) in altering gene expression during early differentiation.

**Methods:**

Generated transgenic mouse models for conditional temporal knockout of XRCC1 in skeletal muscle were used for histology, immunofluorescence staining, as well as isolation of primary myoblasts used for cell culture experimental treatments.

**Results:**

We identify a component of the base excision repair pathway, XRCC1 as indispensable for cell differentiation. Caspase triggered XRCC1 repair foci form rapidly within differentiating myonuclei, and then dissipate as the maturation program proceeds. Skeletal myoblast deletion of XRCC1 does not impact cell growth, yet leads to perinatal lethality, with sustained DNA damage and stunted myofiber development.

**Conclusions:**

Our results demonstrate that base excision repair protein XRCC1 manages a temporally responsive DNA repair process to advance the muscle differentiation gene expression program.



87 **Characterization of Pax7+Myf5+ and Pax7+Myf5- satellite cell phenotypes during differentiation.**Caroline Brun<sup>1,2</sup>, Michael A. Rudnicki<sup>1,2</sup>

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**Background:** Skeletal muscle formation is characterized by two temporally distinct waves of differentiation. In mouse, primary myogenesis occurs between E11-14 by fusion of embryonic myoblasts forming primary fibers that constitutes a scaffold for the secondary fibers formed around E14-16 during the secondary myogenesis. During peri- and postnatal periods, myofibers acquire their contractile and metabolic properties. According to their myosin heavy chain (MyHC) expression profile, primary fibers are mainly slow twitch while secondary fibers are fast twitch. A third population of myogenic cells, termed satellite cells (SCs), emerge at the end of fetal development (E17,5). SCs proliferate to provide additional myonuclei to fibers, allowing muscle growth until the end of postnatal development when they enter a phase of quiescence. SCs are a heterogeneous population based on Myf5 expression, the earliest Myogenic Regulatory Factor that marks myogenic commitment. Our group has described the existence of at least two populations of SCs. The Pax7+Myf5+ SCs represent a subpopulation of committed myogenic progenitors whereas Pax7+Myf5- cells define a subpopulation of non-committed stem cells. After a muscle injury, SCs can be activated, undergo a number of divisions producing fusion competent cells that can either fuse with damaged fibers or form new ones. However, the mechanisms regulating SCs activation, division and differentiation into specific myosin heavy chain expressing myofibers remain unknown.

**Objectives:** Our goal is to characterize Pax7+Myf5- and Pax7+Myf5+ satellite cell-derived myoblasts during their differentiation in vitro and their ability to differentiate into specific fiber types.

**Methods:** Using Myf5-Cre:ROSA-YFP mice, we isolated the Pax7+Myf5- and Pax7+Myf5+ cells and expanded them in culture to compare the molecular profiles and phenotypes between both cell types during proliferation and differentiation in vitro.

**Results:** We detected no morphological change between the Pax7+Myf5- and Pax7+Myf5+ primary myoblasts. Both myoblasts can differentiate into MyHC-positive myotubes. However, Pax7+Myf5- cells seem to give rise mostly to fast myotubes whereas Pax7+Myf5+ myoblasts give rise to both fast and slow myotubes. By qPCR and western blot, we confirmed that Pax7+Myf5- primary myoblasts expressed mostly fast MyHC and we showed that these cells express lower levels of myogenic markers than the Pax7+Myf5+ myoblasts.

**Conclusions:** These preliminary results suggest that Pax7+Myf5- satellite stem cells could differentiate without Myf5 expression during regeneration. Further research will dissect genetic and biochemical mechanisms to explain this phenotype.

88 **Use of endothelial colony forming cells for vascular regeneration in bioengineered lungs.**Ketul Chaudhary<sup>1,2</sup>, Duncan Stewart<sup>1,2</sup>

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**BACKGROUND:** Often lung transplantation is the only option for patients suffering from end-stage lung diseases, but there is a critical shortage of donor lungs. Hence, alternative sources of donor lungs are desperately needed. Recent studies have demonstrated the feasibility of generating bioengineered lungs using acellular lung scaffolds. However, the longevity of these constructs was limited mainly by poor vascular competence of the re-cellularized vasculature. We aim to optimize revascularization of acellular lung scaffolds using ECFCs as a critical first step in engineering truly functional grafts.

**OBJECTIVES:** 1) Compare decellularization methods to achieve optimal decellularization with maintained vascular perfusion. 2) Regenerate vascular endothelium using ECFCs.

**METHODS:** Acellular rat lung scaffolds were generated by static incubation and perfusions methods of decellularization. For static incubation, lungs were submerged into Triton-X 100 (0.1%) and sodium deoxycholate (2%) based decellularization buffer. For perfusion decellularization, isolated rat lungs were perfused using CHAPS buffer (8mM CHAPS, 1M NaCl, 25mM EDTA in 1X PBS) or SDS-Triton-X buffer (0.1% SDS followed by 1% Triton-X). Decellularization of lungs was evaluated by tissue histology, total DNA measurement and immuno-blotting. For revascularization, ECFCs were generated from human peripheral blood or rat bone marrow derived mononuclear cells and characterized for ECFC-markers. Further, we also studied initial engraftment of human ECFCs in the rat lung scaffolds.

**RESULTS:** Static incubation method of decellularization generated acellular lung scaffolds with no evidence of intact cells; however, presence of intracellular protein (GAPDH) was evident. Vascular perfusion could not be established in these scaffolds. On the other side, perfusion decellularization using CHAPS buffer produced acellular lung scaffolds with maintained extracellular structure. Moreover, perfusion decellularization with CHAPS buffer consistently demonstrated complete absence of cells, below detectable DNA remnants and intracellular protein, with more optimal vascular perfusion. ECFCs were successfully derived from human peripheral blood and rat bone-marrow mononuclear cells. ECFCs were positive for endothelial specific markers such as Ac-LDL uptake, lectin binding and expressed CD31, von Willbrand Factor (vWF) and VEGFR2. Moreover, these cells did not express pan-leukocyte marker CD45. Initial engraftment study of human ECFCs in rat lung scaffolds showed engraftment of human ECFCs in

vascular compartment of the lung scaffolds.

**CONCLUSION:** We successfully prepared acellular lung scaffolds and identified ECFC, which can be utilized to generate truly functional grafts.

## 89 **Development of an Image Based High Content Screen of Satellite Cells on Flexor Digitorum Brevis Muscle Fibers**

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### Background

Skeletal muscle regeneration is mediated by a small population of self-renewing satellite stem cells. This self-renewal is facilitated by both symmetric and asymmetric expansion of Pax7+Myf5- satellite stem cells. During asymmetric expansion, the stem cell pool is maintained while Pax7+Myf5+ committed progenitors are simultaneously produced to continue through the myogenic lineage, facilitating skeletal muscle regeneration. We have developed an image based high content screen of satellite cells using flexor digitorum brevis (FDB) fibers to facilitate identification of chemical targets that alter asymmetric cell division. Fiber culture is required for this approach due to the maintenance of the fiber niche, which preserves cellular identity. Although the current method of fiber culture is well established and accepted, this method is extremely low throughput.

### Objective

Our objective is to develop a high throughput platform to screen libraries of compounds that ameliorate asymmetric cell division. Through our findings, we will elucidate and define misregulated pathways in asymmetric self-renewal.

### Methods

FDB fibers are isolated from Myf5-Cre Rosa-YFP mice and cultured on a 96-well format. Projection images of fibers and satellite cells are obtained by a Thermo Cellomics Arrayscan VTI HCS Fluorescent Microscope Imager. Satellite cells are recognized by a cellomics script, and further data processing is performed by a script developed in collaboration with Dr. Sharmin Nilufar from Dr. Ted Perkin's bioinformatics laboratory.

### Results

We have previously identified growth factors that stimulate asymmetric self-renewal. Using this platform, we have been able to detect and validate the effects of these growth factors.

### Conclusion

By combining Cellomics high-content analysis and bioinformatics, we are able to identify and discriminate stem cell statuses (Pax7+ vs. Pax7-) and division types (Symmetric vs. Asymmetric). This is a versatile platform, which may be used to screen compounds, validate findings, and establish dose-response curves.

## 90 **Pharmacological Screen of Pro-Myelinating Therapeutics for Multiple Sclerosis in the Inhibitory Lesion Microenvironment**

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3. Department of Medicine, University of Ottawa, Ottawa

### Background:

In multiple sclerosis (MS), oligodendrocyte precursor cells (OPCs) migrate to lesion sites to repair damaged myelin. Throughout MS disease progression, the ability to repair such damage diminishes considerably. This is thought to be a consequence of lesion-associated inhibitory factors (LAIFs) that perturb OPC maturation into myelinating oligodendrocytes (OLs). Recent work has identified compounds that hold promise as pro-myelinating drugs; however, it is unclear if these compounds will be effective in the inhibitory environment of the lesion.

### Objective:

The current study aims to assess the efficacy of newly identified pro-myelinating compounds on OL differentiation in the presence of MS LAIFs.

### Methods:

Our first step is to independently validate the impact of pro-myelinating compounds and LAIFs on OL maturation. OPCs were either seeded in the presence of an inhibitory substrate (Chondroitin sulfate proteoglycans) or administered our first candidate pro-myelinating compound (XAV939). Quantification of OL differentiation ensued including morphological readouts and proteomic expression of common maturation markers.

**Results:**

Previously established inhibitory properties of chondroitin sulfate proteoglycans have been validated, and expanded upon in our primary OL culture model. This is evidenced by a reduction in both process extension and the area of myelin membrane produced. However, the pro-myelinating effect of XAV939, was not perpetuated in our culture system. We were unable to detect any effect of this compound on our maturation readouts.

**Conclusions:**

Future work will involve validation of an alternative pro-myelinating compound, followed by combinatorial experiments in which OLs will be simultaneously cultured in the presence of LAIFs and pro-myelinating compounds. Results of this study will not only highlight which pro-myelinating compounds are most clinically relevant to pursue, but also provide a better understanding of how the lesion microenvironment contributes to MS pathophysiology.

**91 The Human Pluripotent Stem Cell Facility**

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**Human Pluripotent Stem Cell Facility**

Pluripotent stem cells (including iPSCs and ESC) offer a unique opportunity to dissect early human development, generate models of disease, and develop cellular or drug therapeutics that target a disease or target specific patients with a disease (i.e., personalized medicine). Thus, hPSCs are important tools in Regenerative/ Translational/ Personalized Medicine. The goal of The Human Pluripotent Stem Cell Facility is to provide resources that otherwise might not be available to every researcher as well as expertise on how to work with these cells.

**Objectives and Methods:**

The Facility has the ability to provide training and care for iPS and ES cells. The list below outlines some of the available techniques and services.

**hPSC lines:** The Human Pluripotent Stem Cell Facility has a large stock of NIH approved human ESC lines as well as in-house derived human iPSC lines. Cell lines have been distributed to a number of local labs.

**Training:** The Facility is able to train researchers in reprogramming, culture of hPSCs, and validation of pluripotency assays. Furthermore, expertise within the Stanford Lab enables training of researchers in a variety of instruments that can be used in hPSC research.

**Reprogramming:** A variety of reprogramming methods can be used to generate iPSCs including retroviral, lentiviral, Sendai virus, episomal, and mRNA. The Human Pluripotent Stem Cell Facility is able to train researchers in reprogramming.

**Validation:** Validation assays can be performed as a service, in collaboration, or the researchers can be trained in the validation assays.

**Genome Editing (The CRISPR/ Cas9 and TALEN):** The Stanford Lab/ Human Pluripotent Stem Cell Facility has recently developed this expertise in-house and could begin training researchers in these technologies in the near future.

Already, 9 labs in Ottawa or Montreal have made use of our training, technical services, and/ or reagents and protocols and we look forward to discussing additional projects with the Ottawa scientific community.

**92 Dystrophin is required for muscle stem cell asymmetric division**

**Nicolas A. Dumont**<sup>1,2</sup>, Julia von Maltzahn<sup>1,2</sup>, Yu Xin Wang<sup>1,2</sup>, C. Florian Bentzinger<sup>1,2</sup>, and Michael A. Rudnicki<sup>1,2</sup>

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**Background:** Dystrophin is expressed in differentiated myofibers in skeletal muscle where it is required for mechanical integrity. Loss-of-function mutations result in Duchenne Muscular Dystrophy, a devastating genetic muscular disorder of childhood manifested by progressive debilitating muscle weakness and wasting, and ultimately death in the second or third decade of life.

**Objective:** The goal of this study is to determine how dystrophin affects satellite cell behavior.

**Methods and Results:** Here we show, using microarray, qPCR and immunostaining, that dystrophin is also highly expressed in activated satellite cells. Using proximity ligation assay we also demonstrate that the Ser/Thr kinase Par1b, an important regulator of cell polarity, is directly interacting with dystrophin and with its membrane anchoring complex dystroglycan. Par1b establishes polarity by phosphorylating the Par3 complex leading to its asymmetric distribution. In the absence of dystrophin, expression of Par1b protein is lost, and Par3 complex is no longer polarized and is instead dispersed throughout the cell. Importantly, dystrophin-deficient satellite cells display a marked 10-fold reduction in the proportion of asymmetric divisions resulting in a

dramatic reduction in the flux between stem cell and progenitor compartments. Consistently, a significant decrease in asymmetric divisions was observed with siRNA targeting dystrophin, Par1b, and Par3. In vivo, deletion of dystroglycan specifically in satellite cells strongly impaired satellite cell commitment and muscle regeneration.

Conclusion: Therefore, we conclude that dystrophin has an essential role in the regulation of satellite cell polarity. Our findings have important implications for understanding muscle stem cell dysfunction in Duchenne Muscular Dystrophy.

### 93 **Effects of Epidermal Growth Factor Signaling in Muscle Satellite Cells**

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Background :The epidermal growth factor (EGF) and its receptor (EGFR) play an important role in the regulation of cell growth, proliferation, survival and motility. Here, we investigated the role of EGFR signalling in muscle satellite cells.

Methods:EDL fibers from the Myf5-Cre Rosa26YFP mice were isolated and cultured using two different concentrations of EGF (100 ng/ml or 1 ug/ml) for 42 hours. As a positive control for symmetric division, fibers were also cultured with Wnt7a (50 ng/ml) for the same time period.

Results:There was no significant difference in the number of YFP+ cells/fiber or YFP- cells/fiber at either EGF concentration compared to untreated mice. However, there was a 1.8 fold increase ( $22 \pm 2.1$  to  $39 \pm 4.7$ ;  $p < .005$ ) in asymmetric division compared to control mice with 100 ng/ml of EGF. To confirm these results, EGFR levels were knocked down using a siRNA specific to EGFR on EDL fibers. There was a 1.2 fold increase ( $78\% \pm 2.3$  to  $95\% \pm 0.71$ ;  $p < .0005$ ) in YFP- symmetric expansion 42 hours later. In addition, two EGFR tyrosine kinase (TK) phosphorylation inhibitors were also tested and both similarly increased YFP- symmetric expansion by ~1.2 fold.

Conclusions:Thus, blocking EGFR expression using either a siRNA or TK inhibitors increases YFP- symmetric expansion, further confirming the role of EGF signalling on asymmetric division. The molecular mechanism(s) involved in EGFR signalling in muscle satellite cells is currently being pursued.

### 94 **Molecular regulation of satellite cell fate switching**

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Background:

The prevalence of obesity and its related disorders presents a growing obstacle for the medical community worldwide. Where white adipose is the main reservoir for excess energy, brown adipose has the ability to convert excess energy to heat, and in turn poses a promising therapy for obesity. Brown adipocytes are derived from myogenic progenitors (satellite cells) and it has been shown that the microRNA-133 acts as a switch in satellite cells to promote the myogenic over brown adipogenic fate.

Antagonizing microRNA-133 during muscle regeneration leads to de novo brown adipocyte generation, promotes energy expenditure and impedes diet-induced obesity.

Objective:

To study the molecular mechanisms governing the lineage switch in satellite stem cells and their potential as therapeutic targets.

Results:

Using a luciferase based assay to screen small molecule repressors of miR-133, we uncovered the interaction of p53 with the lineage switch in satellite cells. We found Pifithrin-a (an inhibitor of p53 protein) to be a potent inhibitor of miR-133 expression in mouse and human myoblasts and to markedly stimulate brown adipose determination in C2C12 myoblasts and satellite cells. We further characterized the effects of satellite cell-specific p53 genetic depletion on induction of brown adipocytes and regulation of body weight. We discovered that specific deletion of p53 in adult satellite cells results in precocious brown adipose formation within regenerating skeletal muscles, and these mice also demonstrated reduced gain of body weight. These results suggest cyclic Pifithrin-a and other p53 inhibitors hold the potential as anti-obesity compounds.

Conclusion:

The p53 axis poses a novel pathway regulating the skeletal muscle/brown adipose lineage switch within satellite cells where pharmacological inhibition holds potential as a treatment for obesity.

95 **Gene Selection for the Reconstruction of the Hematopoietic Stem Cell Differentiation Tree****Mohamed A. Ghadie**<sup>1,2</sup>, Nathalie Japkowicz<sup>2</sup>, Theodore J. Perkins<sup>1,2,3</sup>

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Background: Stem cells differentiate through an organized hierarchy of intermediate cell types to terminally differentiated cell types. Although this process is largely guided by master transcriptional regulators, the discrete cell types are often identified based on the expression or non-expression of certain marker genes, which are often cell-surface proteins that are fairly easy to assay biochemically but are not necessarily causative of the cell type. This raises important questions about how gene expression across the whole genome controls or reflects cell state, and in particular, differentiation hierarchies. Traditional approaches such as principal components analysis, K-means clustering and hierarchical clustering can group cell types based on gene expression, but they do so without knowledge of the differentiation hierarchy and therefore cannot reproduce the same tree.

Objective: Given the differentiation hierarchy and gene expression data at each node, construct a weighted Euclidean distance metric on a minimal number of genes such that the minimum spanning tree with respect to that metric is precisely the given differentiation hierarchy.

Methods: We provide a set of linear constraints that are provably sufficient for the desired construction and a linear programming approach to identify sparse sets of weights, effectively identifying genes that are most relevant for discriminating different parts of the tree. However, one set of weights might not uniquely satisfy the constraints. Thus, in the style of random-forest training, we construct 70 metrics based on random subsets of the genes and calculate a score for each gene based on how often it receives a nonzero weight in those metrics.

Results: We apply our method to microarray gene expression data describing 38 cell types in the hematopoiesis hierarchy, constructing a sparse weighted Euclidean metric that uses just 175 genes. These 175 genes are different than the marker genes that were used to identify the 38 cell types hence offering a novel alternative way of discriminating different branches of the tree. A DAVID functional annotation analysis shows that the 175 genes reflect major processes and pathways active in different parts of the tree, and 23 of those genes are transcription factors and/or have GO annotations implicating a role in transcriptional regulation. The 175 genes also received high scores over the 70 random metrics, indicating their significance from an empirical point of view as well.

Conclusions: Our work is a novel contribution towards understanding the relationships between gene expression, cell state and differentiation hierarchies.

96 **DYSTROPHIC MUSCLE RECOVERY: SYNERGY OF WNT7A AND FIBRONECTIN****Uxía Gurriarán-Rodríguez**<sup>1,2</sup> Michael Rudnicki<sup>1,2</sup>

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2. University of Ottawa

Background: Satellite Cells (SCs), a class of stem cells residing on the periphery of the myofiber beneath the basal lamina, are responsible for the growth and repair of skeletal muscle. SCs reside in specialized niches supporting many aspects of stem cell identity. Interactions between stem cells and their environment through cell-cell and cell-ECM adhesion are crucial for regulating stem cells. Recently our group discovered that Syndecan-4 (Sdc-4) and Frizzled-7 (Fzd7) form a co-receptor complex in SCs and the binding of the ECM glycoprotein Fibronectin (FN) to Sdc4 stimulates the ability of Wnt7a to induce the symmetric expansion of satellite stem cells. Likewise, direct injection of Wnt7a significantly ameliorated dystrophic changes in the mdx mouse model of DMD. These results provide a glimpse of the pharmacological potential of Wnt7a combined with FN.

Objective: Study the effect of Wnt7a on mdx SCs division and its synergic effect with FN on the activation of the receptor complex Sdc4-Fzd7 to enhance the muscle recovery effect of Wnt7a in mdx mice.

Methods:

- 1- *Ex vivo* fiber culture of Myf5-Cre:R26R-YFP mdx mice and posterior immunofluorescence analysis to analyze SCs division.
- 2- *In vitro* migration assay on C2C12 cells and mdx primary myoblast by wound healing assay.

Results:

Wnt7a stimulation exerts an increase on symmetric satellite stem cell population on fibers from mdx mice. In contrast, there is not an increase on the asymmetric division neither on the total commitment population. Second, FN stimulation in addition with Wnt7a increases the migration on C2C12 cells and primary myoblast from mdx mice, comparing with Wnt7a treatment alone.

Conclusions: The fact that Wnt7a increases satellite stem cell population supports the previous idea that Wnt7a has a muscle recovery effect on mdx mice, due to is necessary and increase in satellite stem cell population expansion for a proper recovery effect. In addition FN, synergizes and enhances Wnt7a migration effect. All these data together confirms that Wnt7a exerts its

recovery effect on mdx increasing satellite stem cell population, as well that this effect could be increased by co-stimulation with fibronectin, highlighting the role that that SCs niche plays on mdx muscle recovery. In conclusion, we have shown that Wnt7a increases satellite stem cell population and its effect synergizes with FN in mdx mice. Further investigation is needed to find out the molecular mechanisms that regulated this process.

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97 **Withdrawn**

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98 **Defining the higher-order chromatin organization of satellite cells.**

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**Background.** The maintenance and repair of adult muscle tissue is directed by satellite cells (SCs). Quiescent SCs are activated by exercise or injury and enter the cell cycle to produce myogenic progenitor cells that undergo multiple rounds of division before entering terminal differentiation and fusing to multinucleated myofibers. Moreover, SCs exist as a heterogeneous population based on transcription factors Pax7 and Myf5 expression, a feature that divides the SC pool into a subpopulation of self-renewing stem cells (Pax7+/Myf5-) and committed progenitors (Pax7/Myf5+). Induction of Myf5 expression requires Pax7 recruitment of a histone methyltransferase complex to enhancer elements upstream of the Myf5 promoter. Furthermore, CARM1 methylates Pax7 in order to regulate its transcriptional activity following asymmetric cell division of a Pax7+/Myf5- satellite stem cell. In addition to these molecular differences between Myf5- satellite stem cells and Myf5+ satellite progenitor cells, new epigenetic mechanisms are still to be revealed.

Increasing evidence suggests that long-range interactions between genomic regions contribute to the regulation of gene expression. In higher eukaryotes individual chromosomes occupy discrete chromosome territories in the 3D space of the nucleus, resulting in potentially functional contacts between chromatin interactions at other loci, specifically between distal enhancers and gene promoters located over long distances within the same chromosome or at different chromosomal location.

**Objectives.** Our aim is to investigate the potential differences of the genome-wide interaction network of the Myf5 gene in Pax7+/Myf5-, Pax7/Myf5+ SCs and primary myoblasts (PMs) in order to determine the specific 3D organization of chromatin for satellite cells.

**Methods.** By using a circular chromosome conformation capture coupled to deep sequencing (4C-seq), we are studying genome-wide chromatin contacts between functional regulatory elements of the Myf5 locus in Pax7+/Myf5-, Pax7/Myf5+ SCs and PMs, obtained by FACS from a Myf5-Cre:ROSA-YFP mice.

**Results.** Preliminary data in PMs shows that our 4C-seq approach provides sufficient informative reads to identify both inter- and intra- chromosomal interactions of the transcriptional start site of the Myf5 gene used as viewpoint. Bioinformatics analysis is currently in progress in order to correlate genomic contacts with our previously generated ChIP-Seq data to identify potential new specific enhancers for Myf5- and Myf5+ SCs.

**Conclusions.** Our initial strategy, suggest that 4C-Seq analysis is feasible to perform in satellite stem cells and satellite myogenic cells with deep resolution in order to interrogate how the 3D organization of chromatin affects transcription and gene expression that determines satellite cells self-renewal and terminal differentiation.

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99 **Identification and Characterization of Mus ISWI Target Genes During Granule Neuron Progenitor Differentiation**

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Epigenetic regulation of chromatin is mediated by ATP-dependent chromatin remodeling enzymes and this serves as a platform to investigate the mechanisms underlying neuronal differentiation. We utilized chromatin immunoprecipitation and high-throughput sequencing (ChIP-seq) to examine the global binding patterns of the mammalian ISWI homologs, Snf2h and Snf2l, in the progenitor (GNP) and differentiated (GN) state of isolated Mus musculus cerebellar granule neurons.

We observed greater than 81 thousand ISWI binding sites within the GNPs with 3.5 times as many Snf2h sites compared to Snf2l sites. In GNs, there is an overall 1.9-fold decrease in the total number of ISWI binding sites and 3.7 times as many Snf2l sites compared to Snf2h sites. Furthermore there was a ten-fold decrease in the proportion of overlapping binding sites in the GNPs compared to the GNs. Snf2h and Snf2l binding at accepted neuronal target genes (FoxG1, Engrailed 1 and Rbfox3), the alpha, beta and gamma protocadherin gene clusters and the ISWI genes themselves are also presented.

While the gene loci of Foxg1 and Engrailed 1 are exclusively regulated by Snf2h throughout differentiation, the gene loci of Rbfox3, Snf2h, Snf2l and the protocadherin gene clusters display joint regulation by both Snf2h and Snf2l. Taken together, we conclude that neuronal differentiation is associated with a shift from primarily Snf2h occupancy to Snf2l occupancy at a genome-wide level.

## 100 EGFRvIII Requires OSMR as a Co-Receptor to Drive Glioblastoma Tumorigenesis

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EGFRvIII/STAT3 signaling plays a significant oncogenic role in glioblastoma pathogenesis, but the underlying mechanisms remain incompletely understood. Here, we identify the cytokine receptor OSMR as a direct target gene of the transcription factor STAT3 in mouse astrocytes and human brain tumor stem cells (BTSCs). Strikingly, we find that OSMR functions as an essential co-receptor for EGFRvIII. Depletion of OSMR impairs EGFRvIII phosphorylation and corresponding STAT3 activation. Conversely, pharmacological inhibition of EGFRvIII phosphorylation inhibits the EGFRvIII/OSMR interaction and activation of STAT3. EGFRvIII/OSMR signaling in tumors operates independently of EGF/OSM, whereas EGFR/OSMR signaling in non-tumor cells is synergistically activated by the ligands EGF and OSM. Finally, knockdown of OSMR strongly suppresses cell proliferation and tumor growth of murine glioblastoma cells or human BTSC xenografts. Our findings identify OSMR as a critical regulator of glioblastoma tumor growth that orchestrates a feed forward signaling mechanism with EGFRvIII and STAT3 to drive oncogenesis

## 101 Investigation of microRNA miR-145-5p as a novel MS therapeutic target through its regulation of critical myelination regulator MYRF

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**Background:** Progressive multiple sclerosis (MS) is a debilitating disease in which demyelinated lesions form in the central nervous system (CNS). In healthy individuals, demyelination leads to recruitment of oligodendrocyte progenitor cells (OPCs). These differentiate into mature oligodendrocytes (OLs) that remyelinate denuded axons. However in progressive MS, recruited OPCs fail to differentiate and remyelinate, leading to neurodegeneration. One characteristic of MS lesions is abnormally high expression of microRNA miR-145-5p. In OPCs, miR-145-5p is also expressed at relatively high levels. However, it is strongly downregulated as OPCs begin to differentiate into OLs. This downregulation likely plays a key role as OPCs transition to maturing OLs, suggesting that high levels of miR-145-5p may contribute to OPCs' inability to differentiate in MS lesions. Importantly, miR-145-5p is predicted to target myelin gene regulatory factor (MYRF), a transcription factor necessary for OL differentiation and myelination which activates expression of critical myelin genes such as myelin associated glycoprotein (MAG).

**Objective:** In this study, we aimed to determine if miR-145-5p does in fact directly target MYRF, and how altering normal expression of miR-145-5p affects OL maturation. This will aid in better understanding the MS lesion microenvironment.

**Methods:** Lentiviral vectors were used to create a stable cell line that inducibly overexpresses miR-145-5p from immortalized OPCs (Oli-Neus). Cells were characterized while proliferating (P), and on differentiation days 3 and 6 (DD3 and DD6). MYRF expression was analysed by qPCR. MAG expression was visualized by immunofluorescence, and quantified by qPCR and Western blot. Direct targeting of MYRF by miR-145-5p was characterized by dual luciferase assay in HEK 293 T cells. Cell morphology was visualized by immunofluorescence. Expression of Olig2 and glial fibrillary acidic protein (GFAP) were quantified by Western blot.

**Results:** Differentiating cells overexpressing miR-145-5p showed significant downregulation of MYRF. Further, a severe reduction in MAG expression was observed. Dual luciferase assays confirmed direct targeting of MYRF by miR-145-5p at two distinct binding sites. Differentiating cells also displayed aberrant morphology, more closely resembling astrocytes than OLs. However, they showed no loss in expression of OL marker Olig2, nor gain in expression of astrocyte marker GFAP.

**Conclusions:** Taken together, these data show that reduced expression of MYRF and its downstream target MAG are due to direct targeting of MYRF by miR-145-5p. Further, while the overexpression of miR-145-5p results in severe alterations in morphology, cells maintain their OL identity. This research may be important in developing remyelination therapies for progressive MS.

102 **Withdrawn**103 **Retinal Interneuron Function and Survival is Dependent on Cell-Extrinsic Atrx Activity**

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2. Department of Biochemistry, Microbiology, and Immunology, University of Ottawa
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5. Department of Ophthalmology, University of Ottawa

**Background:** Retinal degenerative diseases are the leading cause of blindness in the developed world. Therapeutic strategies that aim to replace or bypass the lost photoreceptor cells require the integrity and proper connectivity of the remaining retinal neurons. The survival and functional circuitry of retinal interneurons downstream of the photoreceptors is essential for visual signal processing and transmission. We have generated a mouse model in which retinal amacrine and horizontal cells, the inhibitory interneurons critical for modulation and integration of synaptic activity in the retina, are selectively lost. We are using this model system to delineate the mechanisms that govern retinal interneuron homeostasis and communication in normal and disease states.

**Objective:** To determine the neuronal circuitry and genetic regulation underlying the loss of retinal cells in mutant mice with defects in the chromatin remodeling protein Atrx

**Methods:** We use transgenic mice and conditional knockout approaches to remove Atrx from retinal cell populations in vivo. Phenotypic analysis of the Atrx-deficient tissues is performed using immunohistochemistry and fluorescence microscopy. Retinal function is examined by electroretinography. Gene expression changes are assessed with DNA microarrays and quantitative RT-PCR.

**Results:** Amacrine and horizontal cell disorganization and loss occurs when Atrx is deleted in multipotent progenitor cells early in retinal development, but not when the gene is inactivated in lineage-restricted, post-mitotic amacrine and horizontal precursor cells. Selective genetic ablation of Atrx postnatally in retinal bipolar cells recapitulates the effects of early pan-retinal gene deletion, indicating that Atrx activity in these neurons is responsible for the function and survival of the synaptically connected retinal inhibitory interneurons. Further analysis reveals misexpression of bipolar cell marker genes and bipolar subtype-specific proteins, and alterations in neuronal morphology that may underlie defects in retinal synaptic communication. Transgenic mice harbouring a mutation mimicking ATR-X syndrome patients exhibit phenotypic features similar to the conditional knockout mice, suggesting common mechanisms of visual dysfunction.

**Conclusions:** The loss of amacrine and horizontal cells from Atrx-deleted retinas appears to occur through a cell non-autonomous mechanism. Analysis of Atrx knockout mice and a mouse model of ATR-X syndrome implicate a role for bipolar cells in retinal inhibitory interneuron survival and function. Atrx-mediated chromatin remodeling may be important for the regulation of specific genes that are involved in retinal neuron synaptic activity, connectivity, and homeostasis. These findings provide insight into the basis for visual abnormalities observed in ATR-X syndrome and suggest novel therapeutic avenues for retinal degenerative diseases.

104 **Neural Progenitor Cells Isolated From a Bronchopulmonary Dysplasia (BPD) Model Show Signs of Injury**

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3. Children's Hospital of Eastern Ontario Research Institute

**Background:** BPD, the chronic lung disease of prematurity resulting from oxygen and mechanical ventilation, remains the main complication in this patient population. Adverse neurodevelopmental outcomes affect many BDP patients. The mechanism of associated brain damage is poorly understood.

We hypothesized that neural progenitor cell function is perturbed in experimental BPD.

**Objectives:** 1) To isolate neural progenitor cells from the subventricular zone and hippocampus of mouse pups and culture them as neurospheres. 2) To examine whether or not neural progenitor cells are injured in BPD by comparing the morphology and proliferative ability of neural progenitor cells isolated from normoxia-exposed mouse pups to age-matched hyperoxia-exposed mice.



Methods: Newborn mice were exposed to room air or 80% oxygen (BPD model) for 10 days. The subventricular zone and hippocampus were isolated. Neural progenitor cells were harvested by enzymatic digestion and cultured as neurospheres. Differences in spontaneous differentiation and proliferation capacity were assessed.

Results: Neural progenitor cells isolated from the BPD model appeared to have a higher rate of spontaneous differentiation than those isolated from the normoxia mice. Additionally, by the fourth passage, the hyperoxia neural progenitor cells were less capable of proliferating, as indicated by the lower cell numbers.

Conclusion: The greater ability to spontaneously differentiate and the decreased proliferative capacity of the hyperoxia-exposed neurospheres suggests impaired neural progenitor cell function in this BPD model. A better understanding of the mechanisms underlying the cognitive defects caused by BPD, will help lead to effective treatments to protect the brain of preterm infants.

## 105 **Disruption in the autophagic pathway underlies sensory neuron pathology in dystonia musculorum mice**

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Background: It was recently shown that mutation in the human dystonin gene is the causative factor in a newly identified lethal form of hereditary sensory and autonomic neuropathy in humans (HSAN-VI). The dystonin protein is a giant cytoskeletal linker that has roles in intracellular trafficking, and cytoskeletal dynamics. The murine disease dystonia musculorum (dt) is a result of mutations in the mouse dystonin gene, which leads to defects in these processes within sensory neurons, and subsequent neurodegeneration.

Objective: Since dystonin has a critical role in microtubule-based movement, we aimed to assess autophagy in dt sensory neurons. Methods: Using primary sensory neurons from pre-phenotype and phenotype dt mice, electron microscopy (EM) and immunoblot analysis were performed.

Results: Autophagosome marker LC3-II was significantly higher in dt sensory neurons compared to wild type. In accordance with this, EM confirmed an increase in authentic autophagosomes. Protein turnover was also impaired, as determined by higher levels of the autophagic substrate p62, and poly-ubiquitinated proteins. When the dystonin- $\alpha 2$  isoform expression was restored via exogenous expression under the prion protein promoter, many of these defects were ameliorated. It is likely that the defects in autophagy are mediated in part by a downregulation of the autophagosome motor protein dynein-intermediate chain 1.

Conclusions: These data indicate that a dysregulation in autophagy underlies dt pathogenesis, and this may be due to insufficient autophagosome trafficking by the dynein/dynactin motor complex.

## 106 **POLYCOMB-LIKE 2 (PCL2) IS REQUIRED FOR HEMATOPOIETIC DEVELOPMENT**

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Polycomb genes are epigenetic repressors critical in cell fate decisions. We identified Polycomb-like 2 (PCL2) as a critical regulator of embryonic stem cell (ESC) self-renewal via its role in pluripotency feed-forward networks (Cell Stem Cell 6:153-166; Cell Cycle 10: 45-51). Knockdown of Pcl2 in ESCs causes defects in differentiation and increased ESC self-renewal. Pcl2 is expressed highly but is later largely restricted to adult hematopoietic tissues. We have generated Pcl2 knockout mice using gene targeted ES cells to study the role of Pcl2 in vivo. Mutant mice that lack Pcl2 die at e15.5 and exhibit growth defects, hemorrhage and anemia. Pcl2<sup>-/-</sup> mice have significantly fewer enucleated erythrocytes, suggesting Pcl2 is necessary for definitive erythropoiesis. Moreover, when plated in clonogenic colony forming-unit assays, less BFU-E and more CFU-GEMM colonies are present in the fetal liver of Pcl2<sup>-/-</sup> mice compared with their wildtype littermates. These data indicate that cells lacking in Pcl2 are in a more primitive/progenitor state. In a Friend Virus erythroleukemia model, Pcl2 represses the development of stress erythroid progenitors. RNA-seq analyses in mouse erythroid progenitors revealed a role for Pcl2 in multiple pathways including regulation of cell cycle. Pcl2 is also required for HSC self-renewal as Pcl2<sup>-/-</sup> fetal liver cells fail to reconstitute the hematopoietic system of recipient mice in secondary transplants. Taken together, Pcl2 is required for erythroid development and self-renewal of hematopoietic stem cells.

Funding provided by OGS, CIHR and CCSRI

107 **Role of PAX-FKHR chimeric proteins in alveolar Rhabdomyosarcoma (aRMS) tumorigenesis**Charles Meunier<sup>1,2</sup>, Michael A. Rudnicki<sup>1,2</sup>

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**Background:**

Despite recent improvements in multimodality treatment, the alveolar form of rhabdomyosarcoma (aRMS) is associated with poor clinical outcome and the survival rate for children after metastasis remains well below 20%. For most cases (>80%), aRMS present characteristic chromosomal translocations leading to the expression of chimeric proteins PAX7-FKHR or PAX3-FKHR, the latter correlating with a relative 40% decrease in survival rate. Recent studies have further emphasized that aRMS likely arise from either skeletal muscle satellite cells or late-stage myogenic progenitors. Additionally, aRMS have been shown to express markers of myogenic lineage such as the structural proteins myosin heavy chain, skeletal  $\alpha$ -actin, and desmin, as well as the transcription factors MyoD, myogenin and more recently, Snail1/2.

**Objective and Method:**

In the context of tumorigenesis, the specific clinical features, histology and molecular characteristics observed in fusion-positive aRMS could suggest that expression of PAX3/7-FKHR can influence myogenic differentiation, redirecting transformed muscle progenitor cells into a "satellite stem cell-like" identity, an alternative program which we hope can be overcome in aRMS tumor repopulating cells. We will proceed with the genome-wide characterisation of PAX3/7-FKHR target genes by standard ChIP-seq and RNA-seq approaches, using both normal primary cell populations and aRMS cells obtained from mice and human, and using retroviral vectors to express a TAP-tagged version of the proteins of interest (PAX3-FKHR or PAX7-FKHR).

**Conclusion:**

Ultimately, the present study will provide important new information regarding the identity of aRMS tumour initiating cells and will identify key regulatory networks essential to the impeded differentiation observed in aRMS.

108 **Withdrawn**109 **Proteomics@ohri: Protein mass spectrometry services at OHRI**Lawrence Puente<sup>1</sup>

1. Regenerative Medicine Program, Ottawa Hospital Research Institute

The OHRI's Proteomics Core Facility provides an advanced LC-MS/MS technology platform for the identification and analysis of proteins and peptides, available as a core service to the Ottawa Hospital, University of Ottawa, and external research community.

110 **Caspase-dependent pathways governing cardiac hypertrophy**Charis Putinski<sup>1,2</sup>, Mohammad Abdul-Ghani<sup>1,2</sup>, Rebecca Stiles<sup>1,2</sup>, Steve Brunette<sup>1</sup>, Sarah A. Dick<sup>1,2</sup>, Pasan Fernando<sup>2,3,4</sup>, and Lynn A. Megeney<sup>1,2,5</sup>

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**Background:** Cardiac hypertrophy occurs when the heart size increases to maintain cardiac output at times of stress. Interestingly, this pathological process is characterized by cell behaviours which are typically associated with apoptosis, including cytoskeletal reorganization and disassembly, and altered nuclear morphology. This leads to the hypothesis that a caspase signalling mechanism may direct or propagate the hypertrophy response in cardiomyocytes. It is proposed that cardiac hypertrophy is mediated by the activation of caspase-dependent pathways which activate hypertrophic transcription/gene expression programs and induce cytoskeletal remodelling which originate in part by caspase-mediated cleavage.

**Objectives:** We aim to determine: if an increase in caspase activity occurs during the early stages of cardiomyocyte hypertrophy; if caspase activation is required for the development of cardiomyocyte hypertrophy; if caspase activation is sufficient to induce cardiac hypertrophy; caspase-dependent signalling pathways that promote the hypertrophic phenotype.

Methods: Primary rat cardiomyocytes were treated with hypertrophic agonists, caspase inhibitors followed by hypertrophy agonist treatment or a small molecule activator of caspase 3 (PAC-1). Immunofluorescence analysis allowed the effects on cardiomyocytes to be observed. Infusion of hypertrophy agonists by use osmotic mini-pumps allowed for analysis within the intact myocardium. In vitro cleavage assays were completed with effector caspase 3/7 and proposed substrates HDAC3 and gelsolin followed by mass spectrometry analysis.

Results: Cardiomyocytes treated with hypertrophy agonists displayed rapid and transient activation of the intrinsic cell death pathway. Disruption of the intrinsic pathway at multiple junctures led to a significant reduction of cardiomyocyte hypertrophy, with a corresponding reduction in expression of known hypertrophy markers and transcription factor activity. Similarly, in vivo attenuation of caspase activity blunted cardiomyocyte hypertrophy. PAC-1 treatment resulted in a robust hypertrophy response in the absence of any hypertrophy agonist stimulation. Finally, HDAC3 and gelsolin caspase cleavage sites have been determined.

Conclusions: These results suggest that caspase-dependent signalling is necessary and sufficient to promote cardiomyocyte hypertrophy. This demonstrates that cell death signal pathways behave as active remodelling agents in cardiomyocytes independent of inducing an apoptosis response. Future studies will focus on delineating the caspase-dependent pathways and cleavage targets that promote hypertrophy. This work will contribute to establishing a role for caspases in cardiac hypertrophy and to overall enhance the understanding of the physiological adaptation. Importantly, this may allow for identification of therapeutic targets, including caspase substrates and components of the intrinsic pathway, with the aim of limiting cardiomyocyte size and forgoing the maladaptive transition to heart failure.

## 111 Screening for small molecules inhibiting adenovirus replication

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4. Cancer Therapeutics Program, Ottawa Hospital Research Institute

Background: Human adenovirus (Ad) causes minor illnesses in most patients, but can lead to severe disease and death in pediatric and geriatric populations, and in immunocompromised individuals. Currently, there is no approved antiviral therapy for the treatment of severe Ad-induced disease.

Inside the virion, the virus-encoded protein VII tightly condenses the Ad genome into a structure that is refractory to transcription and replication. Within the first few hours of infection, Ad DNA dissociates from protein VII and associates with cellular proteins containing the histone variant H3.3. Assembly of the viral genome into a repeating nucleosome-like structure is important for efficient expression of viral genes. Thus, one approach to treating Ad-induced disease may be to inhibit host proteins involved in transitioning the viral DNA to the transcriptionally active state, allowing time for non-cytolytic elimination of the DNA from infected cells.

Objective: To identify small-molecule inhibitors of cellular proteins involved in epigenetic regulation of Ad DNA chromatinization in early infection.

Methods: We have produced an Ad construct (Ad-late/RFP), which allows us to follow virus DNA replication by monitoring RFP expression. We are using a human A549 derived cell line that stably expresses GFP as an internal control for compounds that affect Ad-Late/RFP replication through generalized effects on cell health or biology. The cells are infected with Ad-late/RFP and treated with test compounds for 24 hours. RFP and GFP intensities are then measured using the Cellomics High Content Screening Platform. Compounds that do not affect GFP expression, but significantly reduce RFP expression, are further validated.

Results: RFP from the Ad-Late/RFP was only produced in cells permissive for viral replication. The level of RFP expression correlated to the level of viral DNA replication, indicating that the construct provides the necessary stringency for use in the screen. Although initially expected to increase Ad gene expression, we have identified the pan-histone deacetylase inhibitor SAHA as a compound that delays expression by 15 and 72 hours in transformed and non-transformed cells, respectively. Preliminary results indicate that this effect may be more specific to the inhibition of class I HDACs.

Conclusion: We have designed and validated a system for screening small molecules inhibiting Ad replication. Our assays show that SAHA can significantly delay the onset of Ad gene expression and replication. We are currently working on elucidating the mechanism of this inhibitory effect, designing in vivo studies, and setting up a larger screen to identify other novel inhibitors.

112 **Angiopoietin-2 promotes inflammation but protects against vascular leak in a mouse model of LPS-induced acute lung injury**

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Background: Constitutive activation of the endothelial cell-selective Tie2 receptor is necessary for pulmonary vascular quiescence, which is mediated in part by the actions of its principal agonist, angiopoietin-1 (Angpt1). Elevated levels of circulating Angpt2, a partial agonist/antagonist of Tie2, have been reported in patients with Acute Lung Injury (ALI); however, it remains unclear as to whether Angpt2 plays a causal or protective role in ALI.

Objective: To evaluate the biological consequences of elevated circulating Angpt2 levels in an experimental model of ALI.

Methods: Elevated circulating levels of Angpt2 were achieved in transgenic mice (Angpt2OVR) using a liver-specific promoter. ALI was induced via intratracheal instillation of lipopolysaccharide (LPS), and mice were euthanized at 3 h, 6 h, 24 h, 48 h, and 72 h.

Results: Bronchoalveolar lavage (BAL) neutrophil and inflammatory cytokine levels were significantly higher ( $P < 0.05$ ) in Angpt2OVR versus littermate controls at 48 h and 6 h post LPS, respectively. In contrast, vascular leak was attenuated in Angpt2OVR mice, as evidenced by decreased BAL IgM and albumin levels at 24 and 48 h. Pretreatment of Angpt2OVR mice with a specific Angpt2 inhibitor reversed the pro-inflammatory effects and abrogated the protective actions of Angpt2 overexpression on lung vascular leak in LPS-injured mice. Overexpression of Angpt2 had no effect on LPS-induced terminal morbidity. LPS injury caused time-dependent changes in the relative proportion of Angpt1 and Angpt2, which coincided with changes in lung Tie2 activation.

Conclusions: Angpt2 plays a dynamic role to fine-tune the biological response to lung injury in a time, concentration, and ligand-dependent process.

113 **Induced Pluripotent Stem Cells (iPSCs) Prevent Hyperoxia-Induced Lung Injury**

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Background: Bronchopulmonary dysplasia (BPD), a chronic lung disease characterized by arrested lung growth, is the most common complication in extreme premature infants. There is no treatment for BPD. Alveolar epithelial type 2 cells (AEC2) are putative distal progenitor cells. AEC2s are impaired in BPD. We hypothesized that iPSCs prevent lung injury in experimental BPD.

Methods: Newborn mice were exposed to room air or 85% oxygen (BPD model) for 10 days. Airway delivery of iPSCs was performed at day 4 (prevention) or day 14 (repair). Lung structure and function were assessed 2 weeks after treatment.

Results: Both, undifferentiated miPSCs and episomal hiPSCs prevented, but did not rescue hyperoxia-induced arrested alveolar growth as assessed by lung function testing and lung morphometry. At long term follow-up (18 months), 3/5 miPSCs recipients developed malignant tumours while at 7.5 months post hiPSC injection, only 1/5 mice developed a lung teratoma. We then established a highly efficient method to differentiate hiPSCs into a homogenous population of AEC2 using 5% hypoxic condition, signaling pathways agonists and Fibronectin matrix. hiPSCs acquired an AEC2 phenotype and were positive for NKX2.1, SPB, SPC. NKX2.1+ cells (day 12) were treated with PluriSIn#1 to eliminate undifferentiated iPSCs. Fully differentiated cells (day 25) were also purified through FACS using Lysotracker-green. Airway delivery of differentiated and purified hiPSCs improved lung function.

Conclusion: Undifferentiated iPSCs prevent oxygen-induced lung injury, but form tumors. hiPSCs can be differentiated into AEC2 and improve lung function in this model. Long term follow up will determine if differentiated lung iPSCs is safe and efficient.

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114 **The Proteostasis Function of the *Saccharomyces cerevisiae* metacaspase Yca1**

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**Background**

The activation of caspase/metacaspase proteases has been largely regarded as a detriment to cell viability owing to the nascent ability of these proteins to induce programmed cell death/apoptosis. Despite these prevailing assumptions, our laboratory has shown that both mammalian caspase and yeast metacaspase activities are required for normal cell function, independent of regulating cell death. In the yeast model system we that the metacaspase, Yca1p, regulates critical checkpoints associated with cell cycle progression to enhance overall fitness and cell survival; a novel non-death function. Furthermore, Yca1 co-localizes with markers of protein aggregates and acts to limit the accumulation of protein aggregates as well as dictate the composition of the insoluble proteome. However, the mechanism by which Yca1 exerts its function remains largely unknown.

**Objective**

Investigate the structure-function relationships within Yca1 and elucidate its functioning mechanism.

**Methods**

Using a panel of Yca1 truncation mutants we aim to identify the structure-function relationships within the protein. Immunoprecipitation analyses coupled with 2D LC-MS/MS were conducted to identify regions of interest within Yca1 as well as identify targets/substrates of Yca1. Interactions will be validated by generating of antibodies against targets as well as by conducting in vitro cleavage assays. Vacuolar morphology within these mutant strains by way of FM4-64 staining as well as distribution between the soluble and insoluble fraction by way of immunoblotting will be assessed. Further, the insoluble protein content will be assessed using membrane filtration assay.

**Results**

The N-terminal prodomain of Yca1 is important for establishing protein interactions.

Loss of the prodomain leads to reduced number of multivacuolated cells post stress induction and leads to faster processing of Yca1 post stress.

**Conclusion**

Together, these results suggest that the N-terminal prodomain of Yca1 influences various aspects of Yca1 function.

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115 **Periostin Induces Pancreatic Regeneration**

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We found that the secreted protein Periostin (Postn) is highly induced following partial pancreatectomy in regenerating areas containing mesenchymal stroma and tubular complexes. Importantly, following partial pancreatectomy Postn-deficient mice exhibit impaired mesenchymal formation and reduced regeneration specifically within the pancreatic  $\beta$ -cell compartment. Furthermore, Postn-deficient mice demonstrate an increased sensitivity to streptozotocin. Notably, injection of Postn directly into the pancreas stimulated pancreatic stellate cells to form a mesenchymal stroma that induced regeneration within a localized area. Intraperitoneal injection of Postn resulted in increased numbers of islets and long-term glucoregulatory benefits with no adverse affects found in other tissues. Delivery of Postn throughout the pancreas via the common bile duct resulted in increased numbers of small insulin-expressing clusters and a significant improvement in glucose tolerance. Therefore, Postn is novel molecule capable of potentiating pancreatic  $\beta$ -cell regeneration.

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116 **Strain-Specific Differences in Right Ventricular Adaptation and Survival in the Rat Model of Severe Pulmonary Arterial Hypertension**

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**INTRODUCTION:** Inhibition of VEGFR2 with SU5416 (SU), in combination with chronic hypoxia (CH), causes severe, irreversible pulmonary arterial hypertension (PAH) in rats associated with the development of complex intimal and plexiform lesions. In this

study, we explored the potential importance of differences in genetic background for the development of severe PAH by comparing the response of Sprague-Dawley (SD) to Fischer rats to SU/CH.

**METHODS :** SU5416 (SU: 20mg/kg), or vehicle (Control) was subcutaneously injected in 6-week-old Sprague-Dawley (SD, Harlan, USA) or Fischer rats (CDF, Charles River) followed by a 3-week exposure to CH (10% oxygen).

**RESULTS:** In SD (n=6) and Fischer (n=13) rats, SU+CH resulted in similar, marked elevation in RVSP ( $104 \pm 13$  and  $102 \pm 6$  mmHg, respectively; NS) and RV hypertrophy (RV/LV+septum:  $68 \pm 5\%$  and  $72 \pm 6\%$ , respectively, NS). Despite comparable hemodynamic abnormalities, there was a remarkable difference in mortality with 100% of SD and only 27% of Fischer rats surviving to 8 weeks ( $p < 0.01$ ). At 4 weeks after SU/CH, both Fischer and SD rats showed similar increases in RV wall thickness and decreased PA acceleration time by echocardiography. However, at 7 to 8 weeks, Fisher (but not SD) rats exhibited evidence of RV enlargement, determined by the ratio of RV to LV internal diameter (RVID/LVID). The RVID/LVID was markedly elevated in the early mortality group of Fischer rats ( $1.5 \pm 0.1$ ) vs SD survivors ( $0.82 \pm 0.05$ ) and Fischer survivors ( $0.82 \pm 0.16$ ,  $p < 0.01$ , early mortality vs. survivor), consistent with the onset of RV dysfunction. Indeed, RV dilatation was seen only in the Fischer rats that showed early mortality, suggesting that a deficiency in RV adaptation contributed to poor survival in this background strain.

**CONCLUSIONS:** These data identify important strain differences that impact on the outcomes in a clinically relevant model of severe PAH. Although Fischer rats showed similar hemodynamic responses compared with SD rats in the SU/CH model, they exhibited much higher mortality, due to differences in RV adaptation in response to the increased hemodynamic load. Therefore, this model provides a unique opportunity to explore the mechanisms underlying RV failure in severe PAH and develop strategies to restore RV adaptation.

## 117 **Sirtuin 1 plays a crucial role in modulating the pulmonary and systemic hypoxic response in mice**

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**Background:** Pulmonary Hypertension (PH) is caused by occlusive remodelling of pulmonary arterioles leading to increased pulmonary vascular resistance, right ventricle hypertrophy and eventually failure. Sirtuin (Sirt)-1 is an NAD<sup>+</sup>-dependent deacetylase that has been strongly implicated in maintaining endothelium homeostasis in systemic vessels, but little is known about its role in hypoxia sensing and the lung vasculature. The goal of this study was to understand the mechanisms by which Sirt1 regulates the pulmonary vascular and systemic response to chronic hypoxia (CH)-induced PH.

**Methods/Results:** Mice lacking Sirt1 catalytic activity (sirt1Y/Y, H355Y) and their wild type (WT) littermates were exposed to chronic hypoxia (10% O<sub>2</sub>) for 1, 7, 14 or 21 days. Sirt1Y/Y exhibited an exaggerated increase in right ventricle systolic pressure (RVSP), apparent within the first week of hypoxic exposure (Day 7:  $33 \pm 2$  sirt1Y/Y vs.  $27 \pm 2$  WT; n=8-10,  $p < 0.05$ ) which progressively increased over the 3 week CH exposure period ( $41.5 \pm 1.8$  sirt1Y/Y vs.  $29.7 \pm 0.8$  WT; n=27 both,  $p < 0.001$ ). Right ventricular (RV) hypertrophy, assessed by the RV/LV+S weight ratio was also increased (Day 7:  $0.52 \pm 0.01$  sirt1Y/Y vs.  $0.40 \pm 0.02$  WT, n=8-10;  $p < 0.0001$ ). Hematocrit levels were similar in Sirt1Y/Y and WT mice at baseline; however, there was a delayed increase after three weeks of CH in Sirt1Y/Y mice relative to WT mice (Day 7:  $55 \pm 2\%$  sirt1Y/Y vs.  $52 \pm 2\%$  WT, n=8-9) (Day 21:  $71 \pm 2\%$  sirt1Y/Y vs.  $63 \pm 1\%$  WT, n=17 both;  $p < 0.001$ ). Plasma levels of erythropoietin (EPO), a hypoxic responsive protein responsible for hematopoiesis, were assessed by ELISA at each time point. EPO was markedly increased at Day 7 in Sirt1Y/Y vs. WT mice ( $492 \pm 240$  vs.  $81 \pm 16$  pg/mL, respectively; n=7-9,  $p < 0.05$ ) but normalized at later time points (Day 21:  $75 \pm 9$  sirt1Y/Y vs.  $96 \pm 12$  pg/mL WT; n=4 both).

**Conclusions:** Our data supports a role for Sirt1 in modulating the response to hypoxia, with loss of deacetylation activity leading to an exaggerated pulmonary and systemic induction of the hypoxic response genes.

## 118 **Atypical PKC-CBP Pathway Regulates Murine Adult Neurogenesis**

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Our previous studies have shown that CBP phosphorylation at serine 436 (S436) by atypical PKC (aPKC) is important for CBP to promote embryonic neural precursor differentiation in culture (Dev Cell, 2010). Recently, intriguing findings that CBP level and/or its activity is also required to regulate adult neurogenesis led us to ask further whether CBP aPKC phosphorylation at S436 is a key

modulator for adult neurogenesis. We used two knock-in mouse models to target the aPKC-CBP pathway, CBPS436A and p300G422S, where an aPKC phosphorylation site was modified in CBP and p300 alleles in order to generate phosphorylation-defective (CBPS436A) and phosphorylation-competent (p300G422S) mouse models. By using BrdU in vivo labeling technique, we showed that total number of hippocampal newborn neurons (BrdU/NeuN+ cells) was significantly decreased in CBPS436A mutants, while total number of hippocampal newborn neurons (BrdU/NeuN+ cells) was significantly increased in p300G422S mutants at the age of 3-month. More interestingly, we observed that the proportion of double labeled BrdU/NeuN + neurons over total BrdU + cells was only reduced in the hippocampi of CBPS436A mutants at the age of 6-month, associated with the increased proportion of double labeled BrdU/Sox2 + neural stem cells (NSCs) in the same hippocampi. However, total number of immature doublecortin (DCX) + neurons was not changed in CBPS436A mutants at the age of 6-month. These data suggest that aPKC-mediated CBP phosphorylation is important for appropriate adult neurogenesis, and it modulates rate of differentiation of NSCs and maturation of newly-born neurons in the hippocampus in an age-dependent fashion. In addition, our co-immunoprecipitation experiments showed that the association of CBP with CREB in CBPS436A hippocampal tissues was abolished at the age of 6-month, but not at the age of 3-month, suggesting that CREB might be a downstream signaling of the aPKC-CBP pathway to regulate adult hippocampal neurogenesis in an age-dependent manner. To probe the upstream kinase that regulates the aPKC-CBP pathway to promote adult neurogenesis, we cultured subventricular zone (SVZ) adult neurospheres and showed that AMPK activators (metformin and AICAR) promoted adult neurogenesis in culture and the increased neurogenesis by AMPK activators was abolished in cultures derived from CBPS436A mice, but potentiated in cultures derived from p300G422S mice. In summary, the aPKC-CBP pathway regulates adult hippocampal neurogenesis in an age-dependent manner and the aPKC-CBP pathway is essential to mediate AMPK-induced adult neurogenesis in culture.

### 119 **Aurora kinase A determines cellular asymmetry in muscle stem cell self-renewal**

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**Background:** In muscle regeneration, the activation of resident muscle satellite cells to proliferate, differentiate, and fuse is necessary to lead to formation of new myofibers. However, prolonged regenerative states due to degenerative disorders, such as Duchenne muscular dystrophy, lead to the depletion the satellite cell population and attenuate regenerative capacity. Our lab has identified the satellite stem cell population (Kuang et al., 2007; Cell) and demonstrated that increasing the tissue content of these cells results in a direct enhancement on regeneration kinetics (Le Grand et al., 2009; Cell stem cell). However, in vitro culture of satellite cells leads to their spontaneous differentiation into myoblasts; thus the maintenance and regulation of satellite stem cells is not well understood.

**Objective:** To study the cell-signalling network controlling the homeostatic levels of satellite stem cells and their response in regeneration within their native niche.

**Methods:** We have developed a novel in-niche molecular screening platform to examine the proliferation kinetics of satellite stem cells on ex-vivo cultured muscle fibres.

**Results:** Screening against well-characterized library of compounds that have defined therapeutic targets (>600 compounds), we have identified compounds capable of modulating the activation/proliferation kinetics of satellite stem cells and their committed progeny. Using pathway analysis, we have identified families of compound targeting known regulatory pathways of satellite stem cell homeostasis. As well, we identified the Aurora kinase A pathway as a novel modulator of asymmetric satellite stem cell divisions. Ex vivo perturbation of Aurora kinase A activity by pharmacological inhibition or by siRNA knockdown reduces the capacity of satellite stem cells to perform asymmetric divisions, defaulting to symmetric divisions that expand the stem cell pool. Concurrently, temporal inhibition of Aurora kinase during the early stages of muscle regeneration increase the satellite stem cell pool and produces a 50% increase in the number of Pax7+ satellite cells towards the completion of regeneration. Genetic deletion of Aurora kinase A in satellite cells by conditional Pax7CreERT2 recombination followed by muscle injury also led to an 80% increase in tissue satellite cell content, confirming the specificity of the drug target.

**Conclusions:** Using a proprietary screening protocol to detect subtle changes to stem cells within a complex culture environment, we have uncovered the requirement for Aurora kinases in the determination of asymmetric self-renewal of muscle stem cells. Furthermore, pharmacological inhibition of Aurora kinases has the potential to stimulate the expansion of satellite cells in degenerative muscle diseases.

120 **The development of a cat model of retinal detachment**

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**Purpose:** Our laboratory has previously shown that XIAP gene therapy (via adeno-associated virus; AAV) can protect photoreceptor structure in a rat model of retinal detachment. Due to the size of the rat eye and the technical challenges associated with retinal surgery in rodents, we could not reattach the retina to determine whether the function of the photoreceptors was preserved. In order to follow-up on this work, we will conduct studies in a cat model of retinal detachment, which will allow natural reattachment of the retina and functional testing of the photoreceptors after reattachment.

**Objective:** Design a protocol for induction of retinal detachment in a feline. Rescue the dying photoreceptors due to the detachment with XIAP gene therapy.

**Methods:** Surgical tools and procedures identical to those used on humans in the clinic can be performed. Twelve wild type cats will receive either AAV-XIAP or AAV-GFP (as a control), injected into the subretinal space. Two weeks after viral delivery, a retinal detachment will be induced by administering a gas bubble into the subretinal space. The animals will be followed with seven-hexagon multifocal electroretinography (ERG; to determine photoreceptor function), fundus imaging and optical coherence tomography (OCT) biweekly. After six weeks, the eyes will be removed and prepared for sectioning. Histology will be performed to assess the presence of XIAP and to determine if retinal structure is preserved.

**Results:** Preliminary experiments with three adult wild type cats were employed to optimize the surgical technique of inducing a retinal detachment, to determine the timing of the naturally occurring reattachment, and to develop the protocols for mfERG, fundus and OCT analyses. Ideal detachment conditions involve a lensectomy followed by a two-port pars plana vitrectomy. Subsequently, C3F8 gas is injected into the subretinal space in the central retina with a 42G cannula, inducing a 16-20% retinal detachment. This procedure generates few vitreal bands, little intraoperative hemorrhage, and allows the surgeon to place the detachment in close proximity to the optic nerve. The retinal detachment resolves approximately 1 month post-surgery. Imaging is enhanced when using external lenses for OCT and mfERG fundus imaging, respectively, to accommodate for the removed lens.

**Conclusion:** Current experiments involve a total of twelve animals, employing the optimized protocols described here. Since retinal detachment is an acute disorder, often occurring without any prior warning, the above studies will provide important proof-of-principle for XIAP gene therapy efficacy.

121 **Differential Regulation of eNOS by Extracellular Matrix in Human Endothelial Cells and Blood Outgrowth Endothelial Progenitor Cells**

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**Background:** Endothelial nitric oxide synthase (eNOS) is an important regulator of vascular tone and loss of eNOS activity is a hallmark of endothelial dysfunction. Previously we demonstrated that eNOS overexpression enhances human blood outgrowth endothelial progenitor cells (BOECs) adhesion on extracellular matrices (ECM). However, the regulation of eNOS by ECM in BOECs remains unclear.

**Objective:** In this study, we investigated the regulation of eNOS expression by ECM in BOECs by comparing human umbilical vein endothelial cells (HUVECs).

**Methods and Results:** When grown on different ECM proteins (fibronectin (FN), type I collagen (Col. I) and type IV collagen (Col. IV)) we found decreased eNOS mRNA and protein expression in HUVECs ( $0.52 \pm 0.08$  fold change on FN,  $0.49 \pm 0.07$  on Col. I and  $0.69 \pm 0.07$  on Col. IV) compared to cells on polystyrene. However, in BOECs no significant change of eNOS expression was observed on either matrix even though the integrin-linked kinase level in both cell types was increased by cell-ECM interactions. The matrix mediated downregulation of eNOS in HUVECs was blocked by  $\beta 1$  integrin siRNA and focal adhesion kinase (FAK) siRNA transfection. In addition, rho-associated protein kinase (ROCK) inhibitors including fasudil and Y27632 blocked the effect of ECM on eNOS downregulation in HUVECs. Our preliminary results suggest that ECM proteins may post-transcriptionally regulate eNOS signaling by reducing its mRNA stability. In contrast, in BOECs eNOS protein expression was unchanged with  $\beta 1$  siRNA or ROCK inhibitors.



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Conclusions: Taken together, these findings suggest that signal transduction by multiple ECM proteins tightly regulates the expression of eNOS in mature endothelium by  $\beta$ 1 integrin/FAK/ROCK dependent pathways but is less important in regulating eNOS activity in BOECs.

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122 **Prevention of Pulmonary Arterial Hypertension in Monocrotaline-treated Rats by Mesenchymal Stem Cells Expressing Heme Oxygenase-1**

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**RATIONALE**

Pulmonary arterial hypertension (PAH) carries unacceptable mortality and morbidity despite the modern therapies. A number of preclinical studies have demonstrated the promise of cell-based therapy in experimental models of PAH. Recently, HMOX1-expressing MSCs were shown to reverse modestly elevated pulmonary pressures associated with chronic hypoxia in heme oxygenase-1 (HMOX1) knockout mice. However, the efficacy of HMOX1-enhanced MSCs in the more severe monocrotaline (MCT)-induced PAH model in a wild-type background has not been established.

**METHODS**

The over expression of heme-oxygenase in syngeneic rat MSCs (obtained from Invitrogen) was achieved using two methods. Both chemical induction of MSCs using cobalt protoporphyrin (CoPP), as well as transfection using minicircle DNA plasmids with JetPRIME (obtained from Polyplus) achieved transient but significant over-expression of HMOX1. PAH was induced in 5 week old SD rats by intraperitoneal (IP) injection of MCT. A single dose of cells (MSCs, chemically treated or genetically modified MSCs) or vehicle was infused through the internal jugular vein 3 days after MCT treatment. Echocardiographic parameters including pulmonary artery acceleration time (PAAT) and RV end-diastolic wall thickness (RVEDWT) were obtained on day 23. Invasive measurements of RV pressures as well as indices of RV hypertrophy were obtained on day 24.

**RESULTS**

CoPP pretreatment of MSCs resulted in 10 fold higher HMOX1 expression than with minicircle DNA transfection; however, the expression levels were more sustained in transfected cells. MCT significantly increased the RV systolic pressure (RVSP) from  $31 \pm 1$  mmHg to  $81 \pm 5$  mmHg (Saline/PBS: n=8, MCT/PBS: n=11). Injection of MSC at day 3 resulted in a modest and nonsignificant reduction in RVSP in MCT-treated SD rats (MCT/MSC: n=15, p=0.07). In contrast, transfected MSCs overexpressing HMOX1 significantly decreased RVSP (MCT/HMOX1: n=12, p=0.005), whereas CoPP-treated MSCs had no beneficial effect (MCT/CoPP: n=17, p=0.51). Similar findings were observed in terms of RV hypertrophy (RV/LV+Septal mass ratio), as well as the echocardiographic parameters of PAAT and RVEDWT.

**CONCLUSIONS**

Overexpression of HMOX1 potentiated the therapeutic effects of MSCs in the MCT model of PAH in rats. Pre-treatment of MSCs with CoPP, which resulted in substantial increases in endogenous HMOX1 expression, had no beneficial effects and even may have reduced the ability of MSCs to prevent PAH in this model. This may be due to the distinct temporal expression pattern of HMOX1 overexpression in CoPP-treated MSCs compared with transfection, or to off target effects of chemical induction on MSC activity.

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## Ottawa-Carleton Institute for Biomedical Engineering (OCIBME)

### 123 Electrical Impedance Tomography for guiding ventilation settings

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**Background:** Patients with inadequate breathing require respiratory support (RS) to ensure adequate gas exchange. RS is a lifesaving therapy, but imposes significant risks, such as complications attributable to the endotracheal tube, positive pressure ventilation or sedation. With inadequate pressure support, lung tissue may collapse and be repeatedly forced to open and close with each breath, while overpressure imposes cardiovascular stress and overdistension of small lung units. Lung Protective Ventilation (LPV) seeks a patient ventilation strategy to maintain adequate gas exchange while avoiding complications. In practice, implementation of LPV is difficult. One promising technology to continuously monitor these patients is Electrical Impedance Tomography (EIT), which has recently seen a significant and growing clinical interest for its ability to provide real-time images of regional lung ventilation.

**Objective:** While EIT shows tremendous promise, EIT data are difficult to acquire and thus images can be noisy and misleading to interpret. To address these images we seek to develop image analysis technologies which calculate 1) diagnostic parameters from EIT images which are clinically relevant, and 2) assesses the circumstances under which these parameters are reliable.

**Methods:** During prolonged acquisition, EIT data are affected by the electrode contact quality, postural changes and electronic hardware drift. We have developed robust image reconstruction algorithms that account for electrode movement, patient body shape and internal anatomy as well as approaches to detect and compensate for missing and erroneous electrode data. Recently, this work has been extended to define data quality using a predictive model as a means to automatically reject unreliable measurements.

We have also defined of physiologically based EIT parameters, including regional opening pressures via ventilation delay, and the lung mechanical regional compliance and tissue time constants. Tissue regions may be classified from these parameters; for example, tidally-ventilated tissue has low compliance and long time constants, while overdistended also tissue has low compliance but short time constants.

**Results:** Data quality measures (DQM) were computed on data acquired on from ten PICU patients acquired over prolonged acquisitions (hours) and validated with the corresponding ventilator data. It was found that 1) DQM show abrupt changes, which typically correspond to known events, and 2) DQM behave differently and are sensitive to different effects.

**Conclusions:** EIT shows tremendous potential to help manage patient ventilation in ICU, but suffers from errors and difficulties in analysis. Our technical developments appear to show significant progress to make such analysis more robust and relevant.

### 124 Robotics and Biomechanics: Technologies to Promote Rehabilitation, Mobility, and Prevent Falls

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**Background:**

With the number of elderly, stroke victims, and people with disability, and shortage of healthcare resources in rehabilitation, acute care, and geriatrics, a technology push in robotics to develop a novel class of medical devices has recently started. Such devices can be used to facilitate gait training after stroke or orthopedic surgeries, prevent falls and promote early mobilization for acute care patients, or enhance balance in elderly.

**Objective:**

Three major areas of concern were taken into account and three devices were conceived: (1) The GaitEnable: a mobile robotic system which can act as a patient safety net in fall prevention, as an assessment tool allowing safe and dynamic gaits for patients, or as a rehabilitation tool allowing gait training in combination with other methods. (2) Virtual Gait Rehabilitation Robot (ViGRR): A robotic arm system for early stroke (subacute) patients that would act as a haptic system attached at the feet, enabling a walking practice/training exercise for this type of patients. This technology is currently being completed for basic healthy subject testing and case studies. (3) Balance Aid: aims at developing a novel technology to predict falls and provide tactile feedback to the user to adjust posture.

**Methods:**

From early stages, a collaborative patient-centric design approach was adopted involving medical experts. The approach was to only engage healthy subjects initially to allowed a fast design iteration cycle.

**Results:**

All three classes of devices have been partially or fully tested with healthy subjects to prove the technology at this stage. The GaitEnable system is not available commercially and is going to be used in the hospital for research (at CHEO). The initial data proves the device's ability to enable free gait and safe fall prevention. The ViGRR system's ability to develop ground reaction forces for patients to simulate walking exercise while in bed has been tested and verify a proper movement of CoP under the feet and similar foot trajectories to those of walking. The Balance Aid is at early stages and only pieces of technology have been tested to allow design revisions.

**Conclusion:**

A proper use of technology or combination of technologies is converging to address some of the most important aspects of healthcare: Fall prevention, rehabilitation, and mobilization.

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**125 Species-specific microRNA prediction for elucidation of freeze-tolerance**

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**Background:** MicroRNA (miRNA) are short expressed RNA sequences that are known to play an important role in regulating the expression of proteins. Current miRNA de novo prediction methods are largely trained and evaluated on human sequences. When these methods are applied to unannotated genomes of non-human species, their sensitivity is maintained, but their specificity (i.e. ability to reject false positives) drops dramatically. This is a serious problem since the prevalence of miRNA among all hairpin structures within the genome is expected to be extremely low, leading to a class imbalance of approximately 1:1000.

**Objective:** In the present study, we aim to develop a framework for creating species-specific de novo miRNA prediction systems. Our initial motivation for creating such a framework is to study the unannotated genome of the turtle *C. Picta Bellii*, as our collaborators (Ken Storey and Kyle Biggar) seek to understand its freeze-tolerance ability.

**Methods:** The framework first clusters known miRNA sequences from all species by sequence similarity. Positive training data is formed from the representative sequence within each cluster that derives from the species most closely related to the target species. Negative data is taken from miRNA-like hairpin sequences from within mRNA from a species closely related to the target species. To address class imbalance during training, over-sampling of the positive data is applied. In the present study, support vector machines are trained using a variety of features derived from RNA sequence and structure. However, it should be noted that our framework is applicable to any form of machine learning.

**Results:** Species-specific classifiers are built for four diverse test species (*Anolis carolinensis*, *Drosophila melanogaster*, *Arabidopsis thaliana*, Rhesus lymphocryptovirus). Precision-recall curves are used to compare the accuracy of our species-specific classifiers to two state-of-the-art prediction systems (the human-trained microPred system and the multi-species-trained heteroMirPred classifier). Results are dramatically improved when using our proposed framework.

**Conclusions:** By developing species-specific classifiers and accounting for class imbalance during both training and testing, we have increased classification recall at a precision of at least 50% by over 300%. This framework is now being applied to a number of scientifically important species, including *C. Picta Bellii* (turtle) and *B. glabrata* (snail). Independent RNAseq experiments have validated a number of novel predicted miRNA that would not have been found through homology to known miRNA in other species.

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## Evaluation Instructions

Once again , the Ottawa Hospital Research Institute is offering prizes for the best trainee presentations at Research Day. Participants will be judged in the following categories:

**Best Poster** (\$500 for 1st, \$250 for 2nd and \$100 for 3rd in each category)

- Master's
- PhD
- Postdoctoral

**Best Oral Presentation** (\$500 for 1st, \$250 for 2nd and \$100 for 3rd)

The following criteria have been given to all the evaluators to guide their judging.

All presentations will be evaluated with a score from zero to 100 for each of the following categories:

- Introduction (clearly presented rationale and hypothesis)
- Methodology (sufficiently clear with appropriate details)
- Results (quality and clearly explained)
- Discussion (summary, interpretation and relevance)
- Visual appearance of poster/slides
- Ability to answer questions (Not leaving enough time for questions will result in a mark of zero for this section)

The scale should be applied as follows:

0	Unable to asses
50 – 59	Below average: unclear methodology and results
60 – 69	Average: many presentations will fall into this category
70 – 79	Good: most presentations will fall into this category
80 – 89	Very good: clearly above average; only a few fall into this category
90 – 100	Excellent: Best possible!! Wow!! Top 5%.

## Some of our research trainees who hold salary awards



For a full list of trainees with salary awards, please see page 5.





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