Ottawa Hospital
Research Institute

Institut de recherche
de l'Hôpital d'Ottawa

2013 RESEARCH DAY




Program and Abstracts

Thursday, November 14, 2013
7:30 a.m. – 5:05 p.m.

St. Elias Centre
750 Ridgewood Ave.
Ottawa, ON

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The 2013 IMPACT Award is generously supported by:

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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)
Dr. Angela Crawley
Dr. Anouk Fortin
Paddy Moore
Dr. Luc Sabourin
Dr. Jay Baltz
Dr. Jim Dimitroulakos
Jennifer Ganton
Ms. Alessandra Pasut
Dr. Duncan Stewart
Jane Canniff
Dr. Dean Fergusson
Dr. Ian Lorimer
Dr. Tim Ramsay
Dr. Cathy Tsilfidis

Volunteers

Greg Canham
Melanie Genereaux

WELCOME TO RESEARCH DAY

I am pleased to welcome you to the 13th annual Research Day of the Ottawa Hospital Research Institute.

Today we are celebrating and showcasing the outstanding work of our young researchers, who are so vital to our organization. They provide insight, enthusiasm and dedication in our pursuit of scientific excellence and are critical to our success as one of Canada's top research hospitals — recently ranking 3rd in terms of CIHR funding and 4th for total research funding.

Today's program is designed to promote scientific interaction in a friendly environment for our trainees. Whether or not you are involved in the judging of posters or oral presentations, I strongly encourage you to ask questions of our trainees. More than just an important training exercise, today is a fantastic opportunity for us all to learn about the exciting research projects taking place at OHRI.

I would also like to draw your attention to the 3rd 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 a larger effort at OHRI to foster a culture that is proactive in translating research into benefits for Canadians.

Duncan Stewart, MD, FRCPC

CEO & Scientific Director, Senior Scientist in the Regenerative Medicine Program,
Ottawa Hospital Research Institute

Vice-President, Research, The Ottawa Hospital

Evelyne and Rowell Laishley Chair

Professor, Department of Medicine, Faculty of Medicine, University of Ottawa

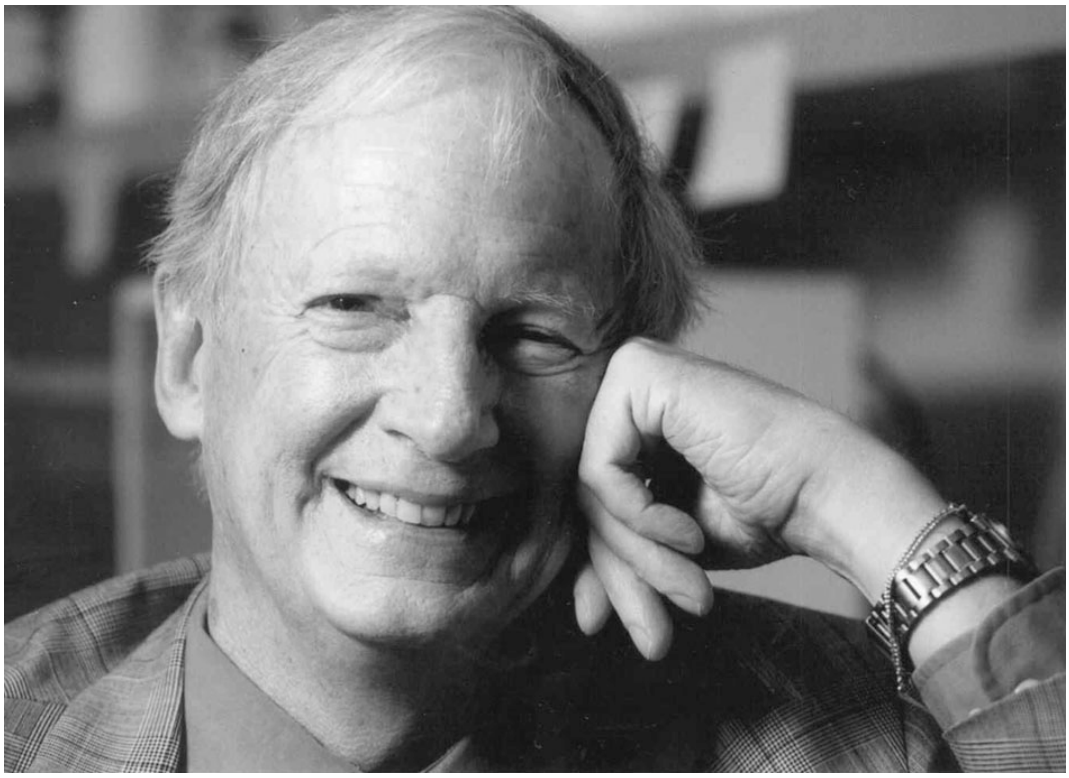
Also recognizing that the range of research we undertake spans from the bench to the bedside, this year we have two keynote speakers on the program.

Dr. Sharon Straus is a leader in knowledge translation and implementation and is spearheading Candian efforts to put knowledge at work to improve patient care. Her presentation will explore how clinical practice can be used to generate answerable research questions, as well as ways of developing feasible approaches to answer those questions, and is titled, *"Using your practice as a research collaborator."*

Most of us know Dr. Mona Nemer as Vice-President, Research at the University of Ottawa. Today, we have the opportunity to hear about her ground-breaking research, which focuses on molecular mechanisms involved in cellular growth and differentiation, particularly as this relates to heart failure and congenital heart diseases. Her work has been published at the highest levels and has garnered international recognition. Her presentation is titled, *"Heart generation and regeneration: a multi-partner affair.*

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.

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 OHRI 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 OHRI. He is also remembered through the Dr. J. David Grimes Research Chair at the University of Ottawa and through OHRI's Dr. J. David Grimes Research Career Achievement Award, which is awarded annually at The Ottawa Hospital Gala.

2013 DR. J. DAVID GRIMES LECTURE

"Heart generation and regeneration: a multi-partner affair"

Dr. Mona Nemer

Dr. Mona Nemer is the Vice-President, Research at the University of Ottawa where she is also a Professor in the Faculty of Medicine and the Director of the Cardiac Development Lab. Dr. Nemer's research interests focus on heart formation and function, particularly on the mechanisms of heart failure and congenital heart diseases. She is renowned, amongst other things, for her pioneering work on the regulation of natriuretic heart hormones and the identification of several genes essential for heart development. Her work has been published in prestigious scientific journals and has contributed to the development of diagnostic tests for heart failure and the genetics of cardiac birth defects.

KEYNOTE LECTURE

"Using your practice as a research collaborator"

Dr. Sharon Straus

Dr. Sharon E. Straus is a geriatrician and clinical epidemiologist who trained at the University of Toronto and the University of Oxford. She is the Director of the Knowledge Translation Program of St. Michael's Hospital; Director of the Division of Geriatric Medicine, University of Toronto; and a Professor in the Department of Medicine, University of Toronto. She has authored more than 300 publications including systematic reviews on mentorship. She holds more than $23 million in peer-reviewed research grants as a principal investigator. She has also received awards for mentorship from St. Michael's Hospital and the Department of Medicine, University of Toronto.

DR. GOODMAN COHEN SUMMER STUDENT AWARDS

Every year, OHRI holds the summer student seminar series, which gives students at the institute the opportunity to present their research to other students. This year, 55 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, OHRI's Associate Scientific Director responsible for Trainees, would like to thank and recognize Dr. Jennifer Collins and Dr. Yevgeniya Le for their excellent job running the summer student program this year.

2013 Winners of the Dr. Goodman Cohen Summer Student Award

Senior Award

Will Foster (Dr. Stewart)

Junior Award

Joanne Joseph (Dr. Tsai) **and Rebecca Xu** (Dr. Kothary) tied and will share the prize.

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
Mabel Ao
Michael Atkins
Marc Thomas Avey
Alina Beliavsky
Dylan Burger
Lauren Carter
Lana Castellucci
Chao Chang
Natasha Chang
Ketul Chaudhary
Jennifer Collins
Heather Colquhoun
Marc-Olivier Deguise
Sarah Dick
Mary-Anne Doyle
Nicolas Dumont
Debra Eagles
Jason El Bilali
Mehdi Eshraghi
Laura Evgin
Hervé Faralli
Taylor Ferrier
William Foster
Sylvain Fraineau
Kyla Garbuio
Vanessa Garcia
Feras Mustafa Ghazawi
Alexander Griffith
Mansoureh Hakimi
Emile Hashem
Steven Hawken
Kendra Hodgkinson
Crystal Holly
Carolina Ilkow
Anna Jezierski
Dan Kobewka
Kessiri Kongmanas
Samantha Kornfeld
Mary Kwakyepeprah
Melanie Lacaria
Luke Lavallée
Marissa Lithopoulos
Serena Liu
Christina Ly
Olebogeng Harold Majane
Monique Marguerie
Hannah Mazier
Arran McBride
Emily Rose McFall
John Paul Michalski
Amber Molnar
Sunita Mulpuru
Ryan O'Meara
Markian Pahuta
Parmvir Parmar
Alessandra Pasut
Sheetal Patel
Nischal Ranganath
Michael Reaume
Katherine Reilly
Andre Richard
Samantha Richard
Paul Ronksley
Melissa Rousseau
Dominic Roy
Erica Schmitz
Kenny Schlosser
Michael Shamy
Demetrios Simos
Jeffrey Smirle
Amanda Smith
Colin Suen
Lee-Hwa Tai
Peter Tanuseputro
Nhung (Rose) Vuong
Qi Wang
Yu Xin Wang
Carmen Wong

OHRI RESEARCH DAY PROGRAM

November 14, 2013, St. Elias Centre, Ottawa

7:30 AM **Registration / Poster Setup / Continental Breakfast**

8:15 AM **Opening Remarks**
Dr. Jack Kitts, Dr. Duncan Stewart, Dr. Bernard Jasmin, Dr. Fraser Scott

8:30 AM **Metabolic Perturbations in Chronic Disease**
(Talks: 9 minutes plus 3 minutes discussion)
Moderators: ***Arran McBride*** *and* ***Jean-Francois Thibodeau***

- **Kevin Courtney** (Xiaohui Zha Group) *Cholesterol transbilayer distribution in mammalian cells: mechanisms and functions*
- **Chet Holterman** (Christopher Kennedy Group) *Nox5 exacerbates filtration barrier damage and increases blood pressure in a mouse model of diabetes*
- **Hang Yin** (Michael Rudnicki Group) *Brown adipose determination in adult skeletal muscle satellite cells is regulated by microRNA-133*
- **David Felske** (Alexander Sorisky Group) *Extra-thyroidal effect of TSH on insulin-stimulated lipogenesis and Akt phosphorylation*

9:20 AM **Clinical Research** (Talks: 9 minutes plus 3 minutes discussion)
Moderators: ***Jelena Ivanovic*** *and* ***Peter Glen***

- **Jason Fernades** (Jonathan Angel Group) *Impaired IL-23 signaling and Th17 dysfunction in HIV infection*
- **Katherine Atkinson** (Kumanan Wilson Group) *Release of a virtual immunization record in Ontario, Canada: Uptake and opportunities for improving public health*
- **Diana Ghinda** (Eve Tsai Group) *Review of spine injuries over time to guide primary injury prevention: falls and leisure related injuries are increasingly responsible*
- **Ching Yeung** (Christina Addison Group) *Discordance in estrogen receptor, progesterone receptor and Her-2/neu status between primary and recurrent breast cancer: A systematic review and meta-analysis*

10:10 AM **Refreshment Break** (15 minutes) ***Sponsored by Allphase Clinical Research***

10:25 AM **Poster viewing / Judging of posters presented by PhD, MSc, BSc honours, and co-op students, and IMPACT Award finalists** (60 minutes)

11:25 AM **Cancer: Stem Cells, Microenvironment and Immunology**
(Talks: 9 minutes plus 3 minutes discussion)
Moderators: ***Nicole Forbes*** *and* ***Katherine Clark-Knowles***

- **Almohana Alkayyal** (Rebecca Auer Group) *Oncolytic rhabdo virus MG1-IL12 enhances anti-tumour immunity*
- **Carolina Ilkow** (John Bell Group) *Crosstalk between cancer cells and cancer-associated fibroblasts promotes oncolytic virus infection in tumours*
- **Curtis McCloskey** (Barabara Vanderhyden Group) *Characterization of a stem cell-like population in a spontaneously transformed syngeneic model of high-grade serous ovarian cancer*
- **Alexander Gont** (Ian Lorimer Group) *Investigating the role of tumour suppressor Lgl1 in glioblastoma tumor initiating cells*

12:15 PM **Buffet Lunch** (60 minutes)

1:15 PM **Keynote Lecture** (35 minutes plus 10 minutes discussion)

Using your practice as a research collaborator

Dr. Sharon Straus, Director of the Knowledge Translation Program, Li Ka Shing Knowledge Institute at St. Michael's Hospital and University of Toronto

Moderator: Dr. Dean Fergusson

2:00 PM **Poster viewing / Judging of posters presented by postdoctoral fellows, clinical fellows, research associates and residents** (60 minutes)

3:00 PM **Growth and Differentiation** (Talks: 9 minutes plus 3 minutes discussion)

*Moderators: **Jeffrey Smirle** and **Mohamad Taha***

- **Matias Alverez-Saavedra** (David Picketts Group) *Neural network assembly is governed by Snf2h-dependent transcriptional activation of the clustered protocadherin genes*
- **Kessiri Kongmanis** (Nongnuj Tanphaichitr Group) *Proteomic analyses of sperm anterior head plasma membrane reveal acrosomal protein involvement in acquisition of egg binding ability to sperm*
- **Samantha Richard** (Jay Baltz Group*) Glycine-Dependent Cell Volume Control in Mouse Oocytes: The Role of Gap Junctions in the Antral Follicle*
- **Janet Manias Rothberg** (William Stanford Group) *Pcl2 is critical for normal hematopoiesis*

3:50 PM **Refreshment Break** (15 minutes)

4:05 PM **Dr. J. David Grimes Lecture**

(35 minutes plus 10 minutes discussion)

Heart generation and regeneration: a multi-partner affair

Dr. Mona Nemer, Vice-President, Research, University of Ottawa

Moderator: Dr. Duncan Stewart

4:50 PM **Poster, Oral and IMPACT Award Presentations, and Closing Remarks**

Dr. Duncan Stewart and Dr. Fraser Scott

5:05 PM **Reception and Cash Bar**

ORAL PRESENTATIONS

Metabolic Perturbations in Chronic Disease (8:30 to 9:20)

Moderators: Arran McBride and Jean-Francois Thibodeau

1-1 Cholesterol transbilayer distribution in mammalian cells: mechanisms and functions

Kevin Courtney[1,2] , Xiaohui Zha[1, 2]

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

Background
Phospholipids and proteins in the plasma membrane (PM) bilayer are well established to be both laterally and transversely asymmetric. However, cholesterol transbilayer distribution within PM remains inconclusive. A fluorescent sterol, dehydroergosterol (DHE), is well-documented to primarily reside in the inner leaflet of PM (~80%). It remains unclear if native cholesterol shares this surprising asymmetry.

Objective
The purpose of this study is to first determine cholesterol transbilayer distribution (CTD) in PM. We will then examine how an asymmetric CTD influences lateral lipid microdomain formation in mammalian PM.

Methods
We developed a protocol that is capable of analyzing cholesterol in a leaflet-specific manner using ß-cyclodextrin (ßCD).

Results
In symmetric large unilamellar vesicles (LUVs), we found that cholesterol flip-flop is rapid at 37°C, leading to 100% extraction/exchange by ßCD. However, at 0°C, ßCD is only able to remove exactly 50% cholesterol, indicating a complete inhibition of cholesterol flip-flop. We then applied this protocol to erythrocytes and found that only 20-25% cholesterol is accessible by ßCD at 0°C, although 100% is accessible at 37°C. Therefore, most cholesterol resides in the inner leaflet of PM in mammalian cells.
We then investigated the role of phospholipid transbilayer asymmetry on cholesterol asymmetry. We found that, only in the asymmetric LUVs with long chain (22 carbon) sphingomyelin in the outer leaflet, could we observe cholesterol enrichment in the inner leaflet. Similar experiments with short chain (16 carbon) sphingomyelin and phosphatidylcholine (16:0/18:1) failed to influence cholesterol distribution.

Conclusion
We therefore conclude that, like DHE, cholesterol is enriched in the inner leaflet of PM and that this asymmetry is regulated by the phospholipid asymmetry and, more specifically, by long chain sphingolipids. We suggest that the current lipid raft model may need to be revised to reflect this cholesterol asymmetry.

1-2 Nox5 Exacerbates Filtration Barrier Damage and Increases Blood Pressure in a Mouse Model of Diabetes

Chet Holterman, PhD[*3], Chelsea Towaij[3], Mark Cooper, MBBS, FASN[1], Rhian Touyz, MD, PhD[2] and Chris Kennedy, PhD[3].

1. Baker IDI Heart & Diabetes Institute, Australia;
2. University of Glasgow, United Kingdom and
3. Kidney Research Centre, Ottawa Hospital Research Institute, Ottawa, Canada.

Background: We have previously demonstrated that Nox5 is upregualted in human diabetic kidney and contributes to ROS–induced podocyte damage and filtration barrier dysfunction. While several studies have implicated Nox1, Nox2 and Nox4 in diabetic nephropathy, nothing is known regarding the role of Nox5 partially due to its abscence from the mouse genome. Here we establish that podocyte specific Nox5 expression in a mouse model of STZ (type 1) diabetes results in an earlier and more severe albumin leakage, increased systolic blood pressure, and interstitial fibrosis.

Methods: Transgenic mice (Nox5Pod+) expressing Nox5 specifically in podocytes were subjected to low dose streptozotocin injection daily for five days. Urinary albumin levels from 24 hour urine collections were assessed by ELISA. Systolic blood pressure was measured weekly by tail-cuff plethysmography. Kidney damage was further assessed by PAS and Mason-Trichrome staining and electron microscopy to determine morphological changes, glomerular sclerosis, and foot process effacement.

Results: Urinary albumin levels in Nox5pod+ trended higher at 4 and 8 weeks post-STZ injectionand were significantly higher 16 weeks post injection as compared to non-transgenic littermates. Systolic blood pressure was not increased in Nox5pod mice at 4

weeks post-stz but showed increases over non-transgenic littermates at 8 and 16 weeks. PAS and Mason-trichrome staining revealed increased glomerular sclerosis and interstitial fibrosis 16 weeks post STAZ in Nox5pod+ animals.

Conclusions: These novel data identify Nox5 as an important NADPH oxidase isoform in the development of diabetic nephropathy. Nox5 may emerge as a novel therapeutic target for reducing the progression of this disease.

1-3 Brown Adipose Determination in Adult Skeletal Muscle Satellite Cells is Regulated by microRNA-133

Hang Yin[1], Alessandra Pasut[1], Vahab D. Soleimani[1], C. Florian Bentzinger[1], Ghadi Antoun[2], Stephanie Thorn[3], Patrick Seale[4], Pasan Fernando[4,5], Wilfred van IJcken[6], Frank Grosveld[6], Robert A. Dekemp[3], Robert Boushel[7], Mary-Ellen Harper[2], and Michael A. Rudnicki[1]

1. Regenerative Medicine Program, Ottawa Hospital Research Institute, Ottawa, ON K1H 8L6, Canada
2. Department of Biochemistry, Microbiology, and Immunology, Faculty of Medicine, University of Ottawa, Ottawa, ON K1H 8M5, Canada
3. University of Ottawa Heart Institute, Ottawa, ON K1Y 4W7, Canada
4. Institute for Diabetes, Obesity, and Metabolism, University of Pennsylvania School of Medicine, Philadelphia, PA 19104, USA
5. Nordion, Ottawa, ON K2K 1X8, Canada
6. Department of Cell Biology and Genetics, Erasmus MC, Dr. Molewaterplein 50, 3015 GE Rotterdam, The Netherlands
7. Department of Biomedical Sciences, University of Copenhagen, Department of Anaesthesia, Bispebjerg Hospital, 2400 Copenhagen NV, Denmark

Background:
Obesity poses a great challenge for global health care. Brown adipose tissue (BAT) is an energy-dispensing thermogenic tissue that plays an important role in balancing energy metabolism. Lineage-tracing experiments indicate that brown adipocytes are derived from myogenic progenitors during embryonic development. However, adult skeletal muscle stem cells (satellite cells) have long been considered uniformly determined toward the myogenic lineage.

Objective:
We sought to determine whether adult satellite cells can give rise to brown adipocytes and the molecular mechanisms that govern the lineage switching of satellite cells.

Results:
By lineage tracing and clonal analysis, we found ~1% of adult satellite cells can differentiate into brown adipocytes. We further discovered that microRNA-133 regulates the choice between myogenic and brown adipose determination by targeting the 30UTR of Prdm16. Antagonism of microRNA-133 during muscle regeneration increases uncoupled respiration, glucose uptake, and thermogenesis in local treated muscle and augments whole-body energy expenditure, improves glucose tolerance, and impedes the development of diet-induced obesity. Furthermore, we demonstrate that miR-133 levels are downregulated in mice exposed to cold, resulting in de novo generation of satellite cell-derived brown adipocytes. Finally, we performed a screening for small molecule compounds that regulate microRNA-133 expression in skeletal muscle cells, which implicates a role of p53/PARP pathway in brown fat/skeletal muscle lineage switching.

Conclusion:
Lineage reprogramming of skeletal muscle satellite cells via microRNA-133 antagonism represents a novel therapeutic approach for the treatment of obesity.

1-4 Extra-thyroidal effect of TSH on insulin-stimulated lipogenesis and Akt phosphorylation

David Felske[1,2], AnneMarie Gagnon[1,2], Alexander Sorisky[1,2]

1. Chronic Disease Program, Ottawa Hospital Research Institute, Ottawa, ON. Canada
2. Biochemistry, Microbiology & Immunology, University of Ottawa, Ottawa, ON. Canada

Background:
In subclinical hypothyroidism (SH), high levels of circulating thyroid stimulating hormone (TSH) maintain normal thyroid hormone levels, despite mild thyroid failure. SH is associated with cardiovascular disease and insulin resistance, although the underlying pathophysiology is not fully understood. Functional TSH receptors are expressed on adipocytes, and may interfere with insulin action. We hypothesized that TSH may inhibit insulin action in adipocytes.

Objectives:
Using human differentiated adipocytes, we will:

1. Determine if TSH inhibits insulin-stimulated lipogenesis.
2. Determine if TSH inhibits the insulin signaling pathway.

Methods:
Abdominal subcutaneous adipose tissue samples were obtained (approved by the Ottawa Hospital Research Ethics Board) from 12 weight-stable patients (11 female), undergoing elective abdominal surgery. Mean age was 50+/-10, and mean body mass index (BMI) was 32.5+/-10 kg/m^2 (+/-SD). Stromal preadipocytes were isolated, placed in culture, and differentiated into adipocytes (~60%) over 14 days. To determine if TSH could inhibit insulin-induced lipogenesis, we stimulated the adipocytes with 5 mU/ml TSH and/or 100 nM insulin for 4 hrs and measured lipogenesis based on incorporation of 14C-glucose into cellular triacylglycerol. To understand the molecular mechanism, differentiated adipocytes were acutely stimulated with 5 mU/ml TSH and/or 100 nM insulin using a co-stimulation (0-30 mins) or pre-incubation (1 hr TSH followed by 5 min insulin stimulation) paradigm. Consecutively, immunoblot analysis of ser-473 Akt phosphorylation was performed.

Results:
Our data indicate that TSH inhibits insulin-stimulated lipogenesis (up to 36.6%), but depends on BMI. In non-obese patients, a significant negative correlation ($r^2=0.9661$; $p < 0.05$) was observed between BMI and the inhibition of insulin-stimulated lipogenesis by TSH (n=4). When co-stimulated with TSH and insulin for 30 mins, immunoblot analysis reveals a TSH-dependent suppression of insulin-stimulated Akt phosphorylation when normalized to insulin response (n=5; $p = 0.01$). When adipocytes are pre-incubated with TSH for 1 hr prior to insulin addition, TSH suppresses insulin-stimulated Akt phosphorylation at an earlier time point of 5 min (n=4; $p <0.05$).

Conclusions:
Our data show that TSH inhibits insulin-induced lipogenesis in a BMI-dependent manner and suppresses Akt-phosphorylation in human differentiated adipocytes. This raises the possibility that other insulin-regulated adipocyte responses may also be susceptible to TSH, which could help explain how TSH may play a role in insulin resistance. Future studies will investigate if inhibition of TSH signaling alleviates TSH-dependent suppression of insulin action.

Clinical Research (9:20 to 10:10)

Moderators: Jelena Ivanovic and Peter Glen

2-1 **Impaired IL-23 signaling and Th17 dysfunction in HIV infection**

JR Fernandes[1], A Kumar[2,3], JB Angel[1,3,4]

1. Chronic Disease Program, Ottawa Hospital Research Institute
2. Pathology, Children`s Hospital of Eastern Ontario Research Institute
3. Faculty of Medicine, University of Ottawa
4. Division of Infectious Diseases, The Ottawa Hospital

Background: Th17 cells maintain gut homeostasis by co-ordinating a variety of innate and adaptive immune responses. HIV infection causes a profound depletion of gut Th17 cells, contributing to loss of mucosal barrier function and microbial translocation, thus driving microbial translocation and systemic immune activation. Despite normalization of circulating CD4+ T cell counts with highly active antiretroviral therapy (HAART), Th17 frequency and function often remain impaired. The IL-12 family cytokine member IL-23 plays a crucial role in maintaining normal Th17 cell function. We hypothesize that HIV inhibits IL-23 signalling in Th17 cells, resulting in Th17 dysfunction.

Objective: The objective of this work is to determine the mechanism(s) of persistent Th17 deficiency in HIV-infected individuals.

Methodology: Th17 cells were isolated from peripheral blood of HIV-seronegative donors by magnetic selection and infected with HIVCS204, a dual-tropic clinical isolate for 24 hours. Expression of IL-17 was examined by ELISA and flow cytometry. Levels of Th17-associated transcription factors STAT3 and retinoic acid orphan receptor C (RORC) mRNA expressed in response to IL-23 stimulation were quantified by qRT-PCR. Phosphorylation of STAT3, the primary transducer of IL-23 signalling, in response to IL-23 stimulation was assessed by flow cytometry. Th17 cells were then isolated from untreated and HAART-treated HIV-infected individuals, and STAT3 phosphorylation (pSTAT3) in response to IL-23 was examined. Expression of the IL-23 receptor protein IL23R on Th17 cells was examined by flow cytometry.

Results: In vitro HIV infection significantly inhibited IL-17 production in response to TCR and mitogenic stimulation. In vitro HIV infection also significantly reduced IL-23-induced pSTAT3 and expression of STAT3 and RORC mRNA. Th17 cells isolated from untreated and HAART-treated HIV-infected individuals showed complete loss of IL-23 responsiveness. IL-6 induced pSTAT3 was unaffected by in vitro and in vivo infection, suggesting that HIV infection results in specific inhibition of IL-23 signalling. Expression of the IL-23 receptor protein IL23R was comparable between HIV-seronegative controls, untreated, and HAART-treated HIV-infected individuals.

Conclusions: These results demonstrate that in vitro and in vivo HIV infection results in impaired IL-23 signalling which is not reversed by HAART and is not a result from reduced receptor expression, demonstrating that HIV interferes with IL-23-activated

signaling pathways. These findings may explain the inability of HAART to restore Th17 frequency and function and the resulting persistent chronic immune activation observed in HIV-infected individuals.

2-2 Release of a virtual immunization record in Ontario, Canada: Uptake and opportunities for improving public health

Katherine Atkinson[1], Kumanan Wilson[1,2], Michael Pluscaukas[3], Cameron Bell[4]

1. Clinical Epidemiology Program, Ottawa Hospital Research Institute
2. Departments of Medicine and of Epidemiology and Community Medicine,
3. Better Outcomes Registry and Network
4. McGill University

BACKGROUND

ImmunizeON is a free iPhone app that was designed at the Ottawa Hospital Research Institute to help parents in Ontario track their children's vaccination records. The app reminds parents of when their child's vaccinations are due, according to the province of Ontario's Immunization Schedule. It allows parents to keep track of the dates that their child was vaccinated, and stores the information directly on their iPhone. The app also has direct links to official sources of information about vaccines, and will let the user know if there are outbreaks of vaccine-preventable diseases in their area.

OBJECTIVES

To describe our experience developing ImmunizeON. Based on usage data, and feedback from both users and public health officials, we present key messages for the development of virtual immunization records and their utility for the development of integrated vaccine information systems.

METHODS

We used 'iTunes Connect to track downloads and updates through the iTunes app store. We tracked media mentions on social media sites (Twitter, Facebook) as well as traditional media sources (Canadian daily newspapers, etc.). We used Urban Airship to track app opens and time spent in app. Feedback from end-users came through a feature embedded in the app, and from comment sections on the iTunes app store.

RESULTS

As of September 15th, 2013 there have been 4634 downloads. The most downloads occurred the week of December 03-09 with 1138 downloads. As of Sept 15th, 2013, 60% (2815/4634) of app downloads had been updated. 57% (2670/4634) of downloads occurred within 8 weeks of the initial app release (Nov 26-Jan27). On average there were 52.6 app opens per day and 125.56 seconds spent per open. We observed that downloads of the app were strongly correlated with traditional media coverage. Usage of the app has remained constant over time which suggests individuals are utilizing the app to input or acquire information.

Feedback has been helpful to identify problem areas of the app to improve usability/ interface desirability. Key obstacles to the utility of the app were concerns about privacy of health information and accessibility amongst lower socioeconomic status individuals. Opportunities include the development of a national version for all platforms and the potential to integrate barcode scanning, include mobile adverse event following immunization reporting and integrations with immunization registries.

CONCLUSIONS

The ImmunizeON prototype is proof of principal of a mobile app being a key component of a vaccine information system.

2-3 Review of spine injuries over time to guide primary injury prevention: falls and leisure related injuries are increasingly responsible

Diana Ghinda, Eve Tsai, Catherine Jones, Jackie Dunne [1]

1. Department of Neurosurgery, University of Ottawa

INTRODUCTION:

Spinal injuries occur far too frequently and are often associated with significant disability and mortality. To improve the injury prevention programs in our region, we reviewed the injury trends over the past 5 years.

METHODS:

From the prospectively obtained data in our trauma database, we selected patients with spine fracture and/or spinal cord injuries that occurred between January 2007 and January 2012. The cases were reviewed for age, sex, cause of injury, and extent of injury.

RESULTS:

A total of 861 patients were admitted for traumatic spinal fractures. Over time, there was no major change in the sex ratio of the patients, yet the average age is steadily increasing (mean age=50; male:female=7 :3). There was also no change in associated brain injuries (more than half of patients had associated brain injuries) and level of injury (most fractures occurred at the cervical level and caused incomplete SCI). While the absolute number of injuries remained stable over time, road traffic accidents (RTA)

decreased from 51% to 33% from 2007 to 2011. However, the proportion of falls increased from 31% to 39% over the same period. The proportion of sports and leisure related trauma also increased over time from 10% to 15%.

CONCLUSION
The preliminary data show a decrease in spine injures due to RTA, however, there was an increase in the proportion of injuries due to falls. Prevention strategies should thus be implemented in order to target these risk groups to prevent significant spinal cord injuries due to falls and to leisure related activities.

2-4 **Discordance in estrogen receptor, progesterone receptor and Her-2/neu status between primary and recurrent breast cancer: A systematic review and meta-analysis**

Ching Yeung[1], Mark Clemons[2,3], Fatima Haggar[3], Christina L. Addison[3], Brian Hutton[3], Iryna Kuchuk[2], Xiao Zhu[2], Sasha Mazzarello[2], Angel Arnaout[1,3].

1. Department of General Surgery, The Ottawa Hospital, University of Ottawa, Ottawa, Canada
2. Division of Medical Oncology, Department of Medicine, University of Ottawa, Ottawa.
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BACKGROUND: Systemic treatment choices for recurrent breast cancer are in part based on the estrogen receptor (ER), progesterone receptor (PR), and epidermal growth factor receptor (HER2) status of the primary cancer. Receptor discordance between primary and recurrent/metastatic disease is well recognized and can have important therapeutic implications.

OBJECTIVE: A systematic review was conducted to assess the extent of discordance in the biomarker status between primary breast cancers and their paired metastases.

METHODS: EMBASE (1980-2012), Medline (1946-2012) and Cochrane Central Register of Controlled Trials (CENTRAL, 2012 issue 1) were searched for English-language cohort studies reporting ER, PR, and HER2 expression for matching primary breast cancer and recurrent sites. Using pre-defined criteria, two reviewers performed data extraction of the included studies, as well as methodologic quality assessment (QUADAS-2) and risk of bias assessment. Median discordance and interquartile range (IQR) of discordance of each of the biomarkers (ER, PR, HER2) between primary breast cancer and their paired recurrence were calculated. Association between extent receptor discordance and the site of recurrence was also explored.

RESULTS: Of 7539 citations identified, 46 studies (representing a total of 3,155 matched primary and recurrent pairs) met the inclusion criteria. Studies were generally small with a median number of matched pairs of 42. Median discordance rates between primary and paired recurrence for ER, PR, and HER2 expressions were 18% (IQR10–29%), 23% (IQR16–43%),and 9% (IQR 4–14%) respectively. Discordance rates were higher if the metastatic site was a lymph node [ER: 42% (42–60%); PR: 48% (46–50%); HER2: 25% (11–38%] compared to all other sites (bone marrow, lung, liver, brain and gastrointestinal tract) [ER: 25% (14-42%); PR: 36% (19-45%); HER2: 13% (5-21%)].

CONCLUSION: The results of this systematic review suggest that discordance rates vary with the biomarker and site of recurrence. When discordance occurs, loss of a biomarker expression is more common than gain, and is observed most frequently with PR. Given this finding, future research on the impact of this discordance on patient outcomes and management is recommended.

Cancer: stem cells, microenvironment and immunology (11:25 to 12:15)

Moderators: Nicole Forbes and Katherine Clark-Knowles

3-1 **Oncolytic Rhabdo Virus MG1-IL12 Enhances Anti-tumour Immunity**

Almohanad Alkayyal [1,3,6] ,Lee-Hwa Tai[1], Jiqing Zhang[1,2] , Christiano T de Souza[1] , Charles Lefebvre[5], Andrew P. Makrigiannis[3], John C. Bell[1,3], David F. Stojdl[3,5] and Rebecca C. Auer[1,4].

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Background: Oncolytic viruses (OV) were originally designed to selectively infect and replicate in tumours, 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 this is critical for its 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 antitumour immunity.

Hypothesis: We hypothesize that a replication competent OV expressing IL-12 will generate high intra-tumoural levels of IL-12, enhance anti-tumoural immunity and result in improved cancer efficacy.

Methods and Results: We used a clinical candidate OV, an attenuated version of the Rhabdovirus Maraba (MG1) with mutations in M protein L123W and G protein Q242R. This negative-sense single stranded RNA virus encodes five proteins; large protein (L), glycoprotein (G), nucleoprotein (N), phosphoprotein (P) and matrix protein (M). The murine IL-12 gene was inserted downstream of G. And the plasmid was sent for sequencing to confirm the presence and orientation of IL-12. The rescued virus has equivalent in vitro cytotoxicity to the parental MG1 virus on a panel of murine and human cell lines including B16, SNB75, OVCAR8, and A549. We also confirmed high levels of supernatant IL-12 twelve hours following infection of Vero cells with MG1-IL12. In vivo expression of IL-12 in tumour bearing mice was also confirmed following infection with MG1-IL12. MG1-IL12 was able to induce NK cell IFN-? secretion in an ex vivo infected cell vaccine (ICV) model. It also induces NK cell chemotaxis in the presence of supernatant from DCs co-cultured with MG1-IL12 infected B16 tumour cells. Human PBMCs' cytotoxicity was significantly increased after eighteen hours co-culture with K562 cells infected with MG1-IL12. Currently we are evaluating the efficacy of MG1-IL12 in two murine models of disseminated malignancy: (1) a therapeutic infected cell vaccine (B16 melanoma) and (2) systemic administration resulting in an in situ tumour vaccine (CT26 colon cancer).

Conclusions: these data support that MG1-IL12 is more effective than MG1 at inducing antitumour therapeutic responses through increase NK cell cytotoxicity.

3-2 Crosstalk between cancer cells and cancer-associated fibroblasts promotes oncolytic virus infection in tumours

Carolina S. Ilkow[1], Monique Marguerie[1], Cory Batenchuck[1], Daniela Ben Neriah[1], Sophie Cousineau[1], Theresa Falls[1], Christiano Tanese de Souza[1], Fabrice LeBoeuf[1], Lawton Stubbert[1], Rozanne Arulanandam[1], and John C. Bell[1].

1. Cancer Therapeutics Program, Ottawa Hospital Research Institute
2. University of Ottawa

"Normal" cells of the tumour microenvironment (e.g. fibroblast, endothelial cells, and immune cells) play a critical role in promoting both tumour growth and metastasis as well as modulating the effects of a variety of therapeutics. Oncolytic viruses have re-emerged as promising therapeutic agents for the treatment of cancer as a result of recent clinical success. Currently, several viruses have been successfully engineered to selectively replicate and kill cancers; however very little research has focussed on the tumour microenvironment as a separate cancer-associated entity that may be targeted by oncolytic viruses (OVs) and / or may modulate virotherapy. The aim of our research is to understand how tumour stromal cells, in particular cancer-associated fibroblasts (CAFs) impact OV therapy.

Our in vitro and in vivo data shows that responses of cancer cells to OV infection are not exclusively determined by their intrinsic characteristics, but are also controlled by signals derived from CAFs. To assess the ability of CAFs to modulate replication and spread of OVs within different compartments of the tumour, we measured the impact of various growth factors and cytokines typically secreted by CAFs on oncolytic vesicular stomatitis virus (VSV) and oncolytic Vaccinia virus (VV) replication and cell killing activity. Interestingly, we found that various stroma-secreted factors exclusively enhance VSV and VV infectivity in several cancer cell lines and in in vivo xenograft models of pancreatic and ovarian cancers. Finally, we investigated the molecular mechanism(s) by which stromal factors specifically enhance OV replication in cancer cells and cancer-associated fibroblasts. Notably, our data shows that stroma-derived factors are able to modulate early anti-viral responses in cancer cells.

Our research provides compelling evidence that the tumour stroma is an important regulator of cancer-OV sensitivity. Moreover, our approach of uncovering signalling pathways that enhance OV replication within different compartments of the tumour will undoubtedly contribute to a better design of OV.

3-3 Characterization of a stem cell-like population in a spontaneously transformed syngeneic model of high-grade serous ovarian cancer

Curtis W. McCloskey[1,2], Reuben L. Goldberg[1,2], Lauren E. Carter[1,2], Lisa F. Gamwell[1,2], Olga Collins[1,2], Elizabeth A. Macdonald[1,2], Kenneth Garson[1,2], Manijeh Daneshmand[2,5] Euridice Carmona[3], and Barbara C. Vanderhyden[1,2,4].

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Improving screening and treatment options for patients with epithelial ovarian cancer has been a major challenge in cancer research. Development of novel diagnostic and therapeutic approaches, particularly for the most common subtype, high-grade serous ovarian cancer (HGSC), has been hampered by controversies over the origin of the disease and a lack of spontaneous HGSC models to resolve this controversy. Over long-term culture in our laboratory, an ovarian surface epithelial (OSE) cell line spontaneously transformed (STOSE). The objective of this study was to determine if the STOSE cell line is a good model of HGSC. STOSE cells grow faster than early passage parental M0505 cells with a doubling time of 13h and 48h, respectively. STOSE cells form colonies in soft agar, an activity for which M0505 cells have negligible capacity. Microarray analysis identified 1755 downregulated genes and 1203 upregulated genes in STOSE compared to M0505 cells, many associated with aberrant Wnt/ß-Catenin and Nf-?B signaling. Upregulation of Ccnd1 and loss of Cdkn2a in STOSE tumours is consistent with changes identified in human ovarian cancers by The Cancer Genome Atlas. Intraperitoneal injection of STOSE cells into SCID and syngeneic FVB/N mice produced pan-CK+, WT1+, inhibin- and PAX8+ tumours, a histotype resembling human HGSC. Based on evidence that a SCA1+ stem cell-like population exists in M0505 cells, we examined a subpopulation of SCA1+ cells that is present in STOSE cells. Compared to SCA1- cells, SCA1+ STOSE cells have increased colony-forming capacity and form palpable tumours 8 days faster after intrabursal injection into FVB/N mice. This study has identified the STOSE cells as the first spontaneous murine model of HGSC and provides evidence for the OSE as a possible origin of HGSC. Furthermore, this model provides a novel opportunity to study how normal stem-like OSE cells may transform into cancer stem cells.

3-4 Investigating the role of tumour suppressor Lgl1 in glioblastoma tumour initiating cells

Alexander Gont[1,2], Jennifer E L Hanson[1] and Ian AJ Lorimer[1,2,3]

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2. Department of Biochemistry, Microbiology and Immunology, University of Ottawa, Ottawa, Canada
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Background: Glioblastoma multiforme (GBM) remains an incurable form of adult brain cancer. A subpopulation of cells within the tumour, deemed glioblastoma tumour initiating cells (GTICs), are resistant to therapy and are responsible for the secondary relapses. GITCs share commonalties with neural stem cells as they exist in an undifferentiated state and have multi-lineage differentiation potential. I have previously shown that tumour suppressor Lgl1 was inhibited as a result of the most common genetic alteration in GBM, the loss of PTEN. Lgl1 is inactivated by protein kinase C iota (PKCi) phosphorylation as a result of aberrant phosphoinositide 3-kinase (PI3K) signalling.

Objective: To investigate tumour suppressive role of active Lgl1 in glioblastoma tumour initiating cells.

Methods and results: Glioblastoma tumour initiating cells were acquired from a patient undergoing a surgical procedure for primary glioblastoma. Deemed PriGO8A, they were confirmed to have PTEN loss and EGFR copy number gain. In vitro differentiation was assessed by increases of GFAP (astrocytic lineage) or TUJ1 (neuronal lineage) positivity by immunofluorescence microscopy. Expression of PTEN, knockdown of PKCi and expression of non-phosphorylatable Lgl1 (Lgl3SA) led to increases in TUJ1/neuronal lineage differentiation. In vitro motility was assessed by cell migration through a porous membrane. Expression of non-phosphorylatable Lgl3SA led to a significant decrease in cell migration. To investigate the in vivo effects of Lgl3SA expression, primary patient derived glioblastoma cells were transduced with a doxycycline inducible Lgl3SA lentiviral vector. Cells were injected intrastriatal into immune deficient SCID/Beige mice and grown for one month prior to transgene expression via doxycycline chow introduction for a further month. By immunohistochemical analysis, cells of human origin were detected by a human-specific STEM121 antibody and Lgl3SA expression was confirmed by positivity for the N-terminal flag epitope. Co-expression analysis of human cells (STEM121) and neuronal lineage specific marker TUJ1 revealed a significant increase in neuronal lineage differentiation. Lgl3SA expression also led to decreases in intracranial invasion as detected by positive pixel counts in the uninjected hemisphere.

Conclusions: Here I show that the most common genetic alterations in glioblastoma lead to repression of neuronal differentiation and increase in invasion via downstream phosphorylation and inactivation of Lgl1. These effects are evident in multiple primary cultures in vitro and in an in vivo animal model suggesting that inactivation of Lgl1 is a key event in gliomagenesis.

Growth and Differentiation (3:00 to 3:50)

Moderators: Jeffrey Smirle and Mohamad Taha

4-1 **Neural network assembly is governed by Snf2h-dependent transcriptional activation of the clustered protocadherin genes**

Matías Alvarez-Saavedra[1,2,6], Emile Hashem[1,2], Doo Yang[3], Keqin Yan[1], Yves De Repentigny[1], Danton Ivanochko[1], Gregory O. Cron[4], Rashmi Kothary[1,3], Teruyoshi Hirayama[5], Takeshi Yagi[5], Ilya Ioshikhes[3] and David J. Picketts[1,3,6].

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Genetic abnormalities affecting cortical development convey circuitry assembly alterations that underlie cognitive disorders. This is because the neocortex provides the brain with the cellular diversity and connectivity controlling higher order cognitive functions. However, the epigenetic mechanisms involved in neural circuitry development are unknown. Here we show that the chromatin remodeler Snf2h drives embryonic cortical expansion through FoxG1 and Satb2 gene regulation; and postmitotic neural arborization by clustered protocadherin gene activation. Telencephalon-specific Snf2h cKO embryos have impaired FoxG1 and Satb2 expression, resulting in hypoproliferation, increased cell death and cortical hypoplasia. However, adult Snf2h cKO mice display neural arborization deficits, clustered protocadherin gene hypoactivation and partial agenesis of the corpus callosum, resulting in cognitive dysfunction. We unravel that the epigenetic control of the clustered Pcdh genes is achieved by Snf2h, CTCF and Satb2 genetic epistasis at target loci. Thus, a single nucleosome remodeler triggers progenitor and postmitotic gene expression programs to control the three-dimensional wiring of the mammalian cortex.

4-2 **Proteomic analyses of sperm anterior head plasma membrane reveal acrosomal protein involvement in acquisition of egg binding ability to sperm**

Kessiri Kongmanas[1,2], Hathairat Kruevaisayawan[1, 4], Clarissa Sugeng[1,2], Puneet Souda[5], Kym F. Faull[5], Ken Kitajima[6], R. John Aitken[7], Julian Whitelegge[5], Daniel Hardy[8], Trish Berger[9], Mark A. Baker[7], and Nongnuj Tanphaichitr[1,2,3]

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9. Department of Animal Science, University of California, Davis, California, USA

Introduction: 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. Our objective was to compare APM proteins before and after capacitation with the hopes to gain an insightful understanding of the capacitation process.

Methods: We have performed quantitative mass spectrometry based proteomic analyses of APM vesicles isolated from non-capacitated and capacitated pig sperm by nitrogen cavitation. 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.

Results and Conclusion: Protein levels in APM vesicles of capacitated sperm were significantly higher than those of non-capacitated samples. Proteomic analyses revealed that proteins with significance in male fertility were the most abundant group in the APM vesicles and many of them showed higher levels in the capacitated APM samples. BN-PAGE revealed a number of HMW protein complexes in both non-capacitated and capacitated APM samples, and far western blot analysis showed that the capacitated sperm APM complexes (700-1300 kDa) had stronger ZP affinity than those of the non-capacitated counterpart. Corroborating this, proteomic analyses revealed increased levels of ZP-binding molecules, along with molecular chaperones and cytoskeletal proteins, in the capacitated APM protein complexes. Significantly, zonadhesin, known as an acrosomal protein, ranked

the highest in the capacitated APM HMW complexes, whereas its level was 1.7 fold lower in the non-capacitated samples; this suggested that zonadhesin was targeted to the sperm anterior head surface during capacitation. Our immunofluorescence validated this postulate; live capacitated sperm showed distinctive zonadhesin staining on the periacrosomal surface in contrast to non-capacitated sperm, showing only background staining. The significance of zonadhesin in APM-ZP binding was further revealed by two lines of results. First, immunoblotting bands of zonadhesin superimposed the HMW complex bands that showed ZP affinity. Second, pretreatment of APM HMW protein complexes with anti-zonadhesin IgG prior to pig ZP3 incubation resulted in decreased ZP3 binding by the complexes. This study provided a new perspective that sperm gained their fertilizing competence through acquisition of acrosomal proteins with inherent ZP affinity into the APM HMW complexes during capacitation.

4-3 Glycine-Dependent Cell Volume Control in Mouse Oocytes: The Role of Gap Junctions in the Antral Follicle

Samantha Richard[1], Dr. Jay Baltz[1,2]

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2. Cellular and Molecular Medicine, University of Ottawa

Background
Pre-implantation embryos are very sensitive to changes in osmolarity and utilize volume regulatory mechanisms to maintain normal cell volume. Following ovulation, detachment of the oocyte-ZP adhesion results in decreased cell volume and initiation of cell volume regulation through the intracellular accumulation of organic osmolytes, primarily glycine. Glycine accumulation is mediated by the GLYT1 transporter, a mechanism unique to eggs and early embryos.

Objective:
Determine what mechanism is responsible for the maintenance of GLYT1 quiescence in the follicle.

Hypotheses:

1. The mural granulosa and/or follicular fluid components are required for the maintenance of decreased GLYT1 activity.
2. Both connexin (Cx) 37 and connexin (Cx) 43 gap junctional communication is required for the continued maintenance of decreased GLYT1 activity and closure of these junctions will result in GLYT1 activation within the follicle.

Methods:
Intact antral follicles, punctured antral follicles, and cumulus-oocyte complexes (COCs) were maintained in culture. GLYT1 activity was assessed by measuring the rate of [3H]-glycine uptake. Specific gap junction inhibition was tested using connexin mimetic peptides.

To test hypothesis 1, intact and punctured antral follicles were incubated and measured for GLYT1 activity. GLYT1 activity in oocytes incubated in COCs and antral follicles were compared.

To test hypothesis 2, the efficacy of the connexin mimetic peptides were first tested by their ability to induce germinal vesicle breakdown in meiotically arrested COCs in culture. To determine whether these gap junctions are required for maintenance of decreased GLYT1 activity in follicles, punctured follicles were incubated in the presence of connexin mimetic peptides and GLYT1 activity measured.

Results/Conclusion
The ability of cultured antral follicles to prevent GLYT1 activation in vitro suggests that a factor from the follicle is necessary for the maintenance of GLYT1 quiescence. Inhibition of specific gap junctions resulted in GLYT1 activation within cultured follicles which further suggests that an inhibitory factor from the mural granulosa and/or follicular fluid is required to maintain decreased GLYT1 activity at the level of the oocyte. Understanding the mechanisms involved in this method of volume regulation is important in providing optimal conditions for oocyte and 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.

4-4 **Pcl2 is critical for normal hematopoiesis**

Janet Manias Rothberg[1,2], Harinad Maganti[1], Christopher J. Porter[1], Gareth A. Palidwor[1], Theodore J. Perkins[1], Caryn Ito[1], William L. Stanford[1,2]

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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 (Walker, 2010). Knockdown of Pcl2 in ESCs causes defects in differentiation and increased self-renewal characteristics.

Pcl2 is expressed at its highest level during development as well as in some adult tissues, such as spleen, thymus, lymph nodes and hematopoietic stem cells. We have generated a Pcl2 knockout mouse model using 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. Based on flow cytometric analysis and peripheral blood smears, Pcl2-/- mice have significantly fewer enucleated erythrocytes, suggesting Pcl2 is necessary for definitive erythropoiesis. Transcriptomic analysis in mouse erythroid progenitors reveals changes in cell cycle and migration genes upon knockout of Pcl2. Using a colony-forming assay to assess progenitor cell function, Pcl2-/- mice formed more CFU-GEMM colonies, indicating that cells lacking in Pcl2 are in a more primitive/progenitor state. HSCs are also affected by a misregulation of Pcl2, since Pcl2-/- fetal liver cells fail to reconstitute the hematopoietic system of recipient mice in secondary transplants. We propose that Pcl2 is critical for normal development of the hematopoietic system.

Funding provided by CIHR and CCSRI

POSTER PRESENTATIONS

OHRI IMPACT Award

(Identification of Marketable Products, Applications and Commercializable Technologies)

1 **Development of antimicrobial peptides into vaginal contraceptives with anti-STI properties**
Jeffrey Smirle, Nopparat Srakaew, Charlene Young, Arpornrad Sae-wu, Nongnuj Tanphaichitr

2 **Improving the completeness of reporting of research articles: implementing an innovative electronic system**
Larissa Shamseer, David Moher, James Galipeau, Jason Roberts, Tim Houle, Pierre-Olivier Charlebois, Chris Ivey

3 **Pharmacologic Modulation of Skeletal Muscle Stem Cells**
C. Florian Bentzinger, Julia von Maltzhn, Danny A. Stark, Nicolas A. Dumont, Yu Xin Wang, Jerome Frenette, D. D. W. Cornelison, Michael A. Rudnicki

Cancer Therapeutics Program

4 **The role of Ldb1 in Neu-induced Tumorigenesis**
Sarra M. Ahmed[1,2], Chris J. Storbeck[1], Luc A. Sabourin[1,2]
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 downsream of HER2/ErbB2/Neu. Focal adhesion kinase (FAK) signalling is required for Neu-mediated SLK activation. SLK activity is required for focal adhesion turnover and cell migration downstream of FAK signalling. Previously, LIM binding domain protein 1, Ldb1, was identified as an important regulator of SLK kinase activity and cell migration. Based on in-vitro analysis, Ldb1 knockdown has been shown to result in a substantial increase in SLK kinase activity accompanied by an increase in cellular motility. Global knockout of Ldb1 in mice results in mid-gestation lethality. To investigate the role of Ldb1 in Neu-induced tumorigenesis, mammary tissue specific Ldb1 knockout mice were generated for this study.

Objective:
To determine the effect of Ldb1 conditional knockout on mammary gland development, to investigate the role of Ldb1 in Neu-induced tumorigenesis and to study the effect of Ldb1 knockout on SLK kinase activity and the metastatic potential of mammary tumour cells.

Methods/Results:
Efficient Ldb1 knockout has no significant impact on overall survival. Also, no significant effects on mammary tumour onset, tumour proliferative status and total tumour volume (TTV) at endpoint were observed when Ldb1 was ablated. Ldb1 knockout increases SLK kinase activity in vivo. The next step would be to determine the effect of Ldb1 knockout on the metastatic potential of mammary tumour cells.

Conclusions:
Ldb1 conditional knockout in the mammary gland doesn't affect overall survival and tumour growth.
Ldb1 conditional knockout in the mammary gland increases SLK kinase activity.

5 **Role of PAX2 in the etiology and progerssion of ovarian cancer**

Ensaf Alhujaily[1,2], Yong Tang[1], Kenneth Garson[1,2], Barbara Vanderhyden[1,2].

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2. Department of Cellular and Molecular Medicine, University of Ottawa.

Background: Epithelial ovarian cancers originate from multiple tissues, including the ovarian surface epithelium (OSE). The cancers develop through a process that includes the formation of a transitional, preneoplastic structure characterized by increased proliferation of precursor cells as well as anchorage-independent growth, growth signal sufficiency and resistance to cell death. Recent characterization of ovarian cancers implicates altered expression of the transcription factor PAX2 in the preneoplastic structures and suggests that its induction in OSE is an early event in the formation of low-grade cancers. We hypothesize that the early events of transformation leading to low-grade cancers are due to PAX2 expression in OSE with effects on proliferation, migration, survival, and KRAS/Myc-associated transformation of these cells.

Objectives: To identify the biological consequences of the expression of PAX2 in normal and malignant epithelium of the ovaries.

Methods: To define the contribution of PAX2 to early ovarian carcinogenesis, we are studying in vitro models of cancer progression: mouse OSE cells that are normal, spontaneously transformed (STOSE) and K-Ras/Myc-transformed (mOSE-RM). To determine the functional consequences of PAX2 in those cells, overexpression of PAX2 was induced using lentiviral infection and empty vector as control.

Results: In normal mOSE cells, Pax2 expression led to increased proliferation and motility. Pax2 expressing STOSE are more proliferative and resistant to cisplatin. Also, they showed more epithelial morphology. STOSE-PAX2 cells also became less motile and formed fewer and smaller colonies in soft agar. In the highly aggressive cancer cells (RM), PAX2 enhanced proliferation and colony formation. When injected into mice, RM-PAX2 cells formed larger tumours and animals died earlier than those injected with RM cells.

Conclusion: Expression of PAX2 has different biological effects depending on the cellular context.

6 **Induction of epithelial-mesenchymal transition in mouse oviductal epithelial cells enhances stem cell characteristics**

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2. Department of Obstetrics and Gynecology, University of Ottawa, and
3. Centre for Cancer Therapeutics, Ottawa Hospital Research Institute

Introduction: Tubal fimbria, the distal part of fallopian tube, have been reported to be an origin of ovarian cancer based on the discovery of p53 expression in preneoplastic structures. It is unknown whether other processes that promote cancers, such as epithelial-mesenchymal transition (EMT), are involved. Since induction of EMT in mammary epithelial cells increases stem cell features, we hypothesize that oviductal epithelial cells (OVE) can undergo EMT which enhances their 'stemness' and susceptibility to tumour formation.

Objective: We aim to show that, in the presence of TGFß, OVE will become more mesenchymal and that induction of EMT increases characteristics associated with stem cells.

Methods: Oviducts were isolated from FVB/N female mice and OVE cells were extracted and cultured. OVE cells were treated with 10 ng/ml TGFß for 7days and collected for counting, RNA extraction, reverse transcription and qPCR, and for western blot analysis.

Results: TGFß induces a morphological transition from epithelial to spindle shape in OVE cells and reduces their proliferation. Vimentin and Snail mRNA and protein are up-regulated, whereas E-cadherin is down-regulated. Investigation of stem cell marker expression by qPCR shows that TGFß-treated OVE cells up-regulate CD44, Sca-1. TGFß also suppresses expression of Pax2, a marker of differentiated oviductal epithelial cells.

Conclusions: TGFß inhibits the proliferation of oviductal epithelial cells, promotes an epithelial-mesenchymal transition, enhances the expression of mRNAs encoding for stem cell markers, and may suppress OVE differentiation. These results suggest that induction of EMT in OVE promotes cellular responses associated with susceptibility to transformation.

7 **Essential role for the SLK protein kinase in embryogenesis and placental tissue development**

Khalid Al-Zahrani[1,2], Prabjhot Sekhon[3], Daniel Tessier[3], Julien Yockell-Lelievre[3], Benjamin Pryce[1,2], Kyla Baron[1,2], Grant Howe[1,4], Roshan Sriram[1,2], Kate Daniel[1], Marlene McKay[1], Vivian Lo[1], Jennifer Quizi[1], Christina Addison[1,4], Andree Gruslin[2,3,5] and Luc Sabourin[1,2].

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3. Chronic Disease Program, Ottawa Hospital Research Institute
4. Biochemistry, Microbiology and Immunology, University of Ottawa
5. Obstetrics and Gynecology, University of Ottawa

Background
Over the last decade, the Ste20-like kinase SLK, has been implicated in a number of signaling processes. SLK repression has been shown to impair cell cycle kinetics and inhibit FAK-mediated cell migration. To date, no global knock-out SLK models have been established.

Objective
To generate the first SLK global knock-out mouse model and to characterize the role of SLK in normal murine development.

Methods
Using a gene trapped allele, we have generated mice expressing a truncated form of the SLK kinase. By caesarian section we have harvested embryos at different timepoints during gestation. Biochemical and histological analysis was performed on these embryo samples in order to assess the role of SLK in development.

Results
Our results show that an SLK-LacZ fusion protein is expressed in embryonic stem cells and in embryos throughout development. We find that the SLK-LacZ fusion protein is less efficient at phosphorylating substrates resulting in reduced cell proliferation within the embryos and angiogenic defects in the placentae of the homozygous mutant animals at E12.5. This results in marked developmental defects and apoptotic lesions in the embryos by E14.5.Homozygotes expressing the SLK-LacZ fusion protein present with an embryonic lethal phenotype occurring between E12.5 and E14.5.

Conclusions
Overall, we demonstrate a requirement for SLK kinase activity in the developing embryo and placenta.

8 **PRD1-BF1 drives VEGF-mediated innate immune suppression in tumour vasculature - a key determinant of oncolytic virus therapy**

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Background: Following the discovery of VEGF as a driving factor in tumour angiogenesis, efforts focused on disrupting the tumour vasculature through VEGF/VEGFR inhibitors have resulted in transient clinical benefit. Oncolytic viruses (OVs) are promising multimodal therapeutics which in part exert their anti-neoplastic activity by directly infecting tumour endothelial cells leading to vascular collapse in patients, while leaving normal vessels untouched.

Objective: Our in vitro studies strongly suggest that overexpression of VEGF in the tumour microenvironment may be the critical factor that sensitizes vascular endothelial cells to infection. We propose that the key antiviral cytokine, interferon (IFN), and pro-angiogenic growth factors may have evolved antagonistic signaling properties and therefore predict that VEGF treatment of endothelial cells could suppress interferon responses leading to productive OV infection.

Methods and Results: In an attempt to characterize the molecular mediators of vessel infection within the tumour microenvironment, we observed that conditioned media from cancer cells could increase OV replication over ten fold in cultured endothelial cells and that this effect could be antagonized by known VEGFR inhibitors. In contrast, confluent, growth-arrested monolayers of endothelial cells were less responsive to VEGF and resistant to infection. We reveal that VEGF/VEGFR2 induced signaling through MAPK and Stat3 leads to a dose dependant increase in the transcriptional repressor, PRD1-BF1. siRNA knockdown of PRD1-BF1 in endothelial cells prevents VEGF-mediated sensitization to OV infection. Microarray analysis demonstrates that PRD1-BF1 negatively impacts the antiviral response. Furthermore, 60% of the genes repressed by VEGF are rescued following PRD1-BF1 knockdown, thereby identifying it as a novel and critical mediator of VEGF–mediated innate immunosuppression in endothelial cells. In vivo, murine window chamber models reveal tumour-specific vessel infection following intravenous administration of oncolytic vaccinia virus, an advanced clinical candidate. Immunohistochemistry performed on these tissues demonstrates strong PRD1-BF1 staining specifically within the tumour endothelial cells, suggesting that VEGF-mediated

activation of PRD1-BF1 could be a key factor in immunotherapies as well as a predictor of the response to OVs.

Conclusions: These findings elucidate a mechanism explaining the preferential infection of the tumour vasculature by OVs. Furthermore, while the role of IFN in suppressing angiogenesis is fairly well understood, our results identify VEGF as a novel inhibitor of IFN production, pointing towards a potential bi-modality between the two signalling molecules. Understanding and harnessing these microenvironment alterations will allow the optimization and customization of the next generation of cancer therapeutics.

9 **TGFß1 acts through Wnt signaling to increase expression of stem cell markers in ovarian surface epithelial cells**

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Background: The ovarian surface epithelium (OSE) is a monolayer of epithelial cells surrounding the ovary and is ruptured during ovulation. After ovulation, the ovulatory wound is repaired, although the processes involved in this repair and the consequences of improper healing are poorly understood. The OSE is one tissue of origin of ovarian cancer and ovulation is the primary non-hereditary risk factor for this deadly disease. We have shown that the OSE contains Stem Cell Antigen1 (SCA1) expressing cells that, when isolated, exhibit stem cell characteristics. Treatment with Transforming Growth Factor Beta 1 (TGFß1) at concentrations found in follicular fluid increases the stem cell characteristics, indicating that these cells may play a role in ovulatory wound repair.

Objective: To determine the molecular mechanism(s) by which ovulation regulates OSE stem cells.

Methods and Results: To study the effects of ovulation on the OSE, a microarray was performed to compare gene expression in OSE cells collected three days after superovulation (SOV) vs. cells from normally cycling mice. Superovulation altered the expression of several components of the Wnt signaling pathway: Proliferin, Wnt4 and Wnt5a were increased (12.1-fold, 1.5-fold and 1.4-fold, respectively) while the expression of Secreted frizzled-related sequence proteins 1-4 (Sfrp1-4) decreased (2.1-fold, 11.3-fold, 10.6-fold, and 2.0-fold, respectively) in OSE from SOV mice. This suggests that Wnt signaling is activated in the OSE after ovulation. Since TGFß1 is present in the follicular fluid during ovulation, we examined if TGFß1 can activate Wnt signaling. TGFß1 treatment increased ß-catenin protein levels and Proliferin, Wnt4 and Wnt5a mRNA expression (5-fold, 1.5-fold and 1.4-fold, respectively), and decreased Sfrp1-4 mRNA expression (0.5-fold, 0.3-fold, 2-fold, and 1.5-fold respectively). This suggests Wnt signaling is increased in OSE cells after ovulation, and may be responsive to TGFß1. Since we have previously shown that TGFß1 increases stem cell characteristics in OSE cells, and the Wnt signaling pathway is known to regulate stem cells, we determined if the Wnt pathway increases expression of Sca1 and/or CD44, stem cell markers found in the OSE. Activation of the Wnt pathway increased CD44 mRNA expression (3-fold). In addition, the TGFß1-mediated increase in OSE CD44 mRNA expression was reduced 1.5-fold when inhibiting the Wnt pathway. These data suggest that TGFß1 increases the expression of stem cell markers in OSE cells by upregulating Wnt signaling.

Conclusion: The results suggest that TGFß1 acts through Wnt signaling to exert its effects on promoting OSE stem cell characteristics to repair the ovulatory wound.

10 **SLK Is Required for TGFß-Mediated EMT in Normal Mammary Epithelium**

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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 tumours 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 signalling pathways downstream of the HER2 receptor.

Objective: Our aim is to explore possible links between SLK and EMT signalling considering that both pathways have been linked to cell migration.

Methods: Immunofluorescence and western analyses as well migration and invasion assays were used to elucidate the potential relationship between SLK and the EMT process.

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 signalling. 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 ß.

Conclusions: This demonstrates that SLK knockdown either prevents cell migration, or prevents cells from transitioning into a mesenchymal phenotype. This study identifies SLK as a molecular target in TGFß-induced epithelial-to-mesenchymal transition.

11 **Exploration of epigenetic viral sensitization**

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Background
Viral therapeutics are key to modern healthcare due to their ability to target specific cell types, deliver genetic material, and to promote immune responses to their targets. However, the balance between creating a safe attenuated virus and an effective treatment can be problematic.

CT26 cells (mouse colon fibroblast) are highly resistant to oncolytic viruses and are very difficult to treat. While creating a CT26 LacZ variant, a clonal selection resulted in a CT26 derived from the wild-type, that is very sensitive and easily treated by oncolytic viruses.

Objective
To explore differences in gene expression and chromatin remodeling that resulted in CT26 sensitization to viral infection.

Methods
Our lab has gathered microarray expression datasets, and Chip-seq histone methylation, of CT26 Wild-Type and CT26 LacZ under control and viral infection conditions.

We have performed GO-term Gene set enrichment analysis of microarray data from CT26 Wild-Type and CT26 LacZ to determine effected processes and functions, and mapped the fold-change on pathways of interacting proteins. We will be combining this with Chip-seq analysis of histone methylation and transcription factor mapping.

Results
Gene set-enrichment and methylation data has identified key affected pathways and proteins, including changes in the type 1 interferon signaling cascade. Furthermore, there is some support that the sensitization may be cause by epigenetic factors and changes to the chromatin structures. Several sites have been identified where both expression and histone methylation differ greatly between CT26 Wild-Type and CT26 LacZ.

Conclusions
We hope that the knowledge gained from this investigation will provide us with novel genetic targets and methods of epigenetic cellular sensitization. From this we aim to design and develop novel viral sensitizer drugs to aid treatment with oncolytic viruses.

12 **Oncolytic viruses disrupt vasculature and potentiate bystander cell death: a role for macrophages?**

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Background: Oncolytic viruses (OVs) exert their anticancer effects through direct infection followed by cell lysis. In addition, there is a Bystander Effect, where uninfected cancer cells are rapidly killed through the virus' ability to induce an inflammatory response that results in acute vascular disruption, apoptosis and necrosis of the tumour core. Vascular disruption was observed in multiple preclinical models with two viruses, VSV and Vaccinia, and in patients systemically treated with JX-594 vaccinia virus.

Objective: To characterize the mechanism by which OVs disrupt intratumoural vascular supply and to examine variations between different types of cancer.

Methods: The kinetics of inflammatory features and bystander cell killing in response to OVs was characterized by histological analyses of tumours before and after IV injection of VSV. The role of inflammatory macrophages and other immune cells was

determined through cell depletion experiments. A panel of different tumours derived from different human and mouse cancer cell lines were analyzed to determine which were sensitive to this bystander effect.

Results/Conclusions: The kinetic analysis reveals that macrophage distribution is rapidly altered 3 hours post VSV administration. Macrophages redistribute from the tumour periphery to the centre of the tumour. Coincident with this change, blood vessels become disrupted and intravascular coagulation is rapidly initiated. By 9 hours after infection, widespread apoptosis of uninfected tumour cells is observed along with the emergence of neutrophils that surround areas of necrosis. Similar changes were observed following JX-594 treatment. Depletion of neutrophils and macrophages using an antibody to Gr1, abrogates bystander cell death indicating that these cells are important in the chain of events between viral infection and bystander killing.

In our survey of cancer models, two mutually exclusive patterns of killing were observed. While the response of some tumours to OVs is rapid, resulting in widespread death of uninfected cells, other tumours support robust replication of OVs without bystander cell killing. Preliminary results suggest that homogeneous tumours, with few stromal elements, tend to be susceptible to bystander cell killing. Understanding how and when this robust anticancer effect occurs may provide opportunities to augment oncolytic viruses for therapeutic benefit.

13 Novel small molecule viral sensitizing technology enhances vaccine production

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Background: Vaccines are currently the most effective way to prevent the spread of infectious viral diseases. For vaccine safety, vaccine virus strains are genetically modified or serially passaged to obtain attenuated strains that no longer cause disease. These attenuated viruses are problematic for vaccine manufacture, as they replicate sub-optimally. Recently, Diallo et al. discovered a series of small molecules that sensitized virus-producing host cells and resulted in up to 1000-fold increase in virus yield. We have since found that viral sensitizing technology (VST) can be used more generally to enhance the production of several viral vaccines. Notably, these viruses belonged to evolutionarily distinct families. We hypothesize that VST compounds exert their activity through the suppression of multiple components of the cellular antiviral response.

Objective: Demonstrate the applicability of VSTs for the manufacture of viral vaccines.

Methods: Several promising VST compounds were evaluated for enhanced viral vaccine production (in relevant cell lines in vitro, first in microplate format, and subsequently using a larger scale (e.g. roller bottles, macro-carrier bioreactors). Plaque assay, ELISA, and real-time PCR were used to quantify virus yield. To ensure viral vaccines produced in the presence of promising VST compounds are not biologically distinguishable from unaltered vaccine products, we analyzed resulting viral gene sequences and performed mass spectrometry on the final product. Finally, in vivo assays were used to confirm equivalent vaccine immunogenicity in presence and absence of promising VST compounds.

Results: Several of the viral sensitizing technology compounds were able to enhance production of virus vaccines. The compound was not detected in the final vaccine product, which protected mice from wild-type virus challenge.

Conclusions: Viral sensitizing technologies have the potential to significantly improve viral vaccine production, however effective treatment concentrations, virus production substrates, and level of enhancement vary with each given vaccine candidate.

14 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 tumours remain resistant to OV infection. Our group has previously shown that VSVd51 spread and replication can be enhanced in tumours 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.

15 **Investigating the role of GREB1 in ovarian cancer progression in a mouse model**

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Background:
Estrogen therapy increases the risk of developing ovarian cancer, and 17ß-estradiol (E2) promotes proliferation and decreases apoptosis in ovarian cancer cell lines. We have shown previously that E2 promotes ovarian cancer progression in mouse models, and hypothesize that these effects are caused by E2 altering transcription of one or more key genes.

Objective:
To investigate the mechanisms underlying the decreased survival caused by E2 in mouse models of ovarian cancer

Methods:
We used a xenograft model in which SCID mice were injected with mouse ovarian cancer cells. The MASE cell line was derived from the ascites of a transgenic tgCAG-LS-TAg mouse, a model of ovarian cancer. Mice were treated with E2 pellets and monitored until loss of wellness. Survival was compared and tumours were examined by microarray to identify genes altered by E2. QPCR confirmed expression of several genes of interest, and lentiviral shRNA was used to investigate function of a particularly interesting target, gene regulated by estrogen in breast cancer-1 (GREB1).

Results:
Exogenous E2 decreased survival of both the transgenic and xenograft mouse models. Microarray analysis of tumours from E2-treated mice showed upregulation of 196 genes and downregulation of 55 genes compared to tumours from placebo-treated mice. In addition to known ER targets such as Pgr, several novel genes were identified which are involved in angiogenesis, proliferation or differentiation, including GREB1. Knockdown of GREB1 in MASE cells decreased proliferation and migration in vitro and slowed tumour progression and decreased metastasis in vivo.

Conclusions:
GREB1 may play a role in ovarian cancer progression. Investigation of GREB1 expression in human tumours is underway. Characterization of the function of E2-targeted genes such as GREB1 will aid the identification of mechanisms by which E2 increases the risk of ovarian cancer.

16 **Investigating the Immunogenicity of Oncolytic Viruses**

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Background: Oncolytic viruses (OV) specifically replicate in and lyse tumour cells. In addition to their direct cytotoxicity, OV can stimulate the immune system and facilitate the generation of anti-tumour immune responses by releasing tumour-associated antigens (TAA), danger signals and pro-inflammatory cytokines into the tumour microenvironment. The generation of anti-tumour immunity following oncolytic virotherapy has shown to be critical for successful therapy in numerous pre-clinical models. Vaccinia Virus (VACV) is an oncolytic virus known to adopt numerous methods to evade the immune system, allowing the virus to successfully replicate and spread undetected; however this aspect of VACV biology may not be advantageous in terms of developing an anti-tumour immune response. Objective: This study aims to generate a VACV with increased immunogenicity in an aim to augment VACV oncolytic virotherapy and generate long term anti-tumour T cell responses. Using a human in vitro priming assay in conjunction with other immunological readouts we aim to develop an immunogenic VACV candidate for clinical development.

Method: In order to address whether oncolytic viruses can generate anti-tumour immune responses in people a human in vitro priming assay was developed. Dendritic cells (DC) are cultured with tumour cells infected with an oncolytic virus, the potentially

antigen loaded DC are removed and cultured with autologous T cells permitting DC cross presentation and T cell expansion. A second round of T cell stimulation is performed following which anti-tumour T cell responses are measured by CD107 degranulation, killing assays and tetramer/pentamer staining. Measuring the anti-tumour T cell responses will enable quantification of immune responses developed following viral infection of different VACV and may identify an immunogenic candidate for further assessment

17 **Novel VSe1 Derivatives for Improved Oncolytic Virotherapy and Drug Target Identification**

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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 significant subset of tumours highlights areas for improvement in OV-based therapeutics. Combining OV therapy and viral sensitizer drugs is a promising strategy to enhance OV activity in resistant tumours. To this end, we have previously identified the synthetic compound VSe1 using a high-throughput pharmacoviral approach. VSe1 was found to vastly improve infection of resistant tumour cells by several OVs in vitro and led to improved efficacy in vivo by targeting the innate antiviral response. Through a medicinal chemistry approach, we have recently generated a panel of chemical derivatives of VSe1, some of which show significantly improved activity, potency, physico-chemical and toxicological properties over the parental VSe1 compound. In addition, some of these novel derivatives were used in target-identification experiments through ligand affinity capture and mass spectrometry. Validation of identified putative targets is ongoing.

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

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Background

Surgical 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 prophylactic TAA encoded vaccine, is rendered ineffective post-surgery in a 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 murine B16 melanoma model of surgical stress.

Methods

A C57BL/6 murine B16 melanoma model was used to demonstrate the surgical impact on adaptive T cell immunity. Mice were actively immunized (intramuscular) with AdhDCT, a non-replicating adenovirus expressing human dopochrome totaumerase (hDCT), a melanoma TAA that effectively produces a robust anti-tumoural response. Seven days post-AdhDCT injection, mice were either untreated or underwent a laparotomy and left nephrectomy to induce surgical stress. The function and number of DCT-specific CTLs was evaluated by IFNy, TNFa and Granzyme B production post-surgery in the spleen using flow cytometry and IFNy secretion by ELISpot. 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 stains.

Results

Surgical stress significantly attenuates proportion ($p<0.001$) and absolute numbers ($p<0.05$) of total IFNy+ DCT-specific CTLs by over 2-fold. Similar results were detected for TNFa and Granzyme B. ELISpot of isolated CTLs confirmed a postoperative decrease in DCT peptide stimulated production of IFNy by 2-fold. Preliminary results from the extra-cellular tetramer stain indicate surgery does not affect the number of DCT-specific CTLs. Furthermore, there was no significant difference among apoptotic CTLs in untreated mice and those undergoing surgery. T cell migration and proliferation assays are currently underway.

Conclusion

Surgical stress abrogates cytokine production and cytotoxicity of antigen-specific T cells following antigen binding rather than a reduction in T cell numbers due to apoptosis following surgery. Understanding the mechanisms of T cell dysfunction following surgery will facilitate the development of targeted immunotherapies to reverse this effect in the postoperative period.

19 **Exploiting the immunogenic mutanome to enhance oncolytic virus therapy**

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Over the last decade oncolytic viruses (OVs) have been shown to be very effective anti-cancer agents - able to target tumour cells while leaving normal cells intact. We have recently published that an infected cell vaccine (ICV) can lead to robust anti-tumour immunity. In theory the ICV presents a number of different tumour and viral antigens to which the immune system responds. We would like to determine which tumour epitopes are lending to the therapy's success and whether the presentation of a few strong epitopes in the presence of an oncolytic virus may lend to greater anti-tumour efficacy than the presentation of many.

A new area of research has emerged which focuses on vaccinating with mutated sequences of the cancer exome to stimulate anti-cancer immune responses. While vaccinating with self epitopes holds the risk of inducing autoimmunity, vaccinating with non-self "mutanome" epitopes should not. A number of B16F10 mutanome epitopes have been identified which are suspected to be the most stimulatory within the B16F10 cell genome. We have confirmed the presence of these mutated sequences within our B16F10 melanoma cells and have confirmed the ability of these epitopes in generating strong anti-tumour immune responses. We have also interrogated the roles of these epitopes in the immunity generated by our ICV platform as well as for their ability to augment the immune stimulatory properties of the OV platform.

We are currently working to include these immune-stimulatory mutanome sequences in oncolytic viruses to further enhance the anti-tumour immune response generated as a result of oncolytic virus therapy. Through work with these mutanome peptides in a variety of cancer models, we hope to gain a better understanding of how to couple personalized mutanome vaccinations with oncolytic virus therapy.

20 **Role of the Ste20-Like Kinase in Muscle Development and Regeneration**

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Background

The Ste20-Like Kinase is highly expressed in developing neuronal and myogenic tissue. SLK co-localizes with Myf5 and Pax7 positive satellite cells. The expression of a truncated/kinase dead form of the kinase results in decreased fusion of C2C12 myoblasts. To date, no knockout muscle models of SLK have been established.

Objectives

To establish SLK conditional knockout mice Myf5 and Pax7 Cre will be crossed with SLK flox transgenic mice. To validate our in vivo observations primary myoblast cultures will be established from knockout mice.

Methods

Caesarian section of Myf5 and Pax7 Cre transgenic females will be performed at several gestational time points in order analyze the effect of SLK in embryonic muscle development. Adult mice will be sacrificed at several time points and muscle will be taken for histological analysis. Biochemical analysis will also be performed on muscle an embryonic samples. Primary myoblast cultures will be established from hindlimb muscle for each genotype to assess the impact of SLK deletion on myoblast fusion.

Results

Muscle specific deletion of SLK via the Myf5 Cre model results in the generation of mice with reduced body mass, as well as a reduction in fiber number in skeletal muscles. SLK knockdown in C2C12 myoblasts leads to a marked reduction in myoblast fusion without affecting differentiation marker expression. Mass Spec analysis revealed ? catenin as a novel binding protein of SLK. Localization of ? catenin is affected after knockdown.

Conclusion

SLK is responsible for proper formation of muscle tissue, as well as localization of beta catenin. Further work is required in order to elucidate the mechanism by which SLK re-localizes beta catenin, and if this interaction is responsible for the fusion phenotype in the knockdown.

21 **A Novel RNAi Screen to Identify Host Factors that Modulate Oncolytic Virus Replication**

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Background

Oncolytic viruses (OVs) infect and kill tumour 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 tumour specificity. This strategy leads to enhanced replication in tumour cells, which often possess defects in antiviral pathways, while sparing normal cells. However, not all tumours have defects in their antiviral defenses and thus OV replication in these tumours 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 tumours 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. Conduct a Sindbis virus shRNA library screen in cultures of various cancer cells. Cell selected virus populations will be analyzed by deep sequencing and bioinformatics analysis will be used to identify shRNAs that are enriched.
2. Selected shRNA sequences will be tested for enhancement of OV growth in cell culture as well as in vivo.
3. Identify the cellular mRNA targets of the enriched shRNAs.

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. Preliminary experiments suggest that the identified shRNAs increase replication of Sindbis virus, as well as Vesicular Stomatitis Virus (VSV), another oncolytic virus.

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 a modest 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 tumour and virus specific, and if shRNA-expressing viruses demonstrate enhanced anti-tumour activity in vivo.

22 **Utilizing the cancer mutanome in personalized heterologous prime:boost vaccines for pancreatic cancer**

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Background:

Pancreatic cancer is a significant health issue due to the diseases' high mortality rate and a lack of improvement in survival rates over the last three decades. Therefore we have set out to develop a novel vaccine treatment platform utilizing heterologous prime:boost targeting either a PanC02 tumour associated antigen (TAA) expressed in our tumour model, or the mutant peptides comprising the PanC02 mutanome.

Objective:

These studies will provide the proof-of-principle for moving forward with a more personalized approach to cancer therapies. While we are focusing on pancreatic cancer, the results from our studies can be translated to any cancer as the mutations utilized are specific to the cancer being studied and will provide critical information about the utility of personalized cancer vaccines using Adenovirus and Maraba virus heterologous prime:boost.

Methods:

The proposed studies will be carried out using an orthotopic murine pancreatic tumour model PanC02. The PanC02 genome was sequenced using both exome sequencing and RNA sequencing technologies to identify mutated genes differentially expressed in

the tumour cells compared to WT C57Bl/6 DNA. Adenovirus and Maraba virus constructs will be made expressing either PanC02 TAA or a multi-epitope construct expressing the mutations identified and validated to be immunogenic from the sequencing described above. These viruses will be tested in therapeutic vaccination platform treating orthotopic PanC02 tumours.

Results:
We have identified 188 expressed mutated genes in PanC02. Through in silico analysis, 99 peptides were identified to have the potential to bind MHC class I, a pre-requisite for the induction of immune responses. Moving forward, we intend to construct 8-10 Adenovirus vectors expressing multi-epitope constructs comprising the 99 potential epitopes and will use these viruses to prime animals and assess immune responses by ELISpot. Finally Adenovirus and Maraba virus vectors will be constructed expressing either PanC02 TAA or a multi-epitope construct containing validated mutant peptides. These viruses will be utilized to treat PanC02 to assess whether a more personalized approach utilizing the cancer mutanome is an effective treatment strategy.

Conclusions:
The studies described above have very recently been initiated as a collaborative effort amongst a number of Canadian oncolytic virus consortium researchers to find a new treatment modality for pancreatic cancer. We have currently begun validating the top 12 mutant epitope candidates in vivo utilizing peptide vaccination and will soon begin construction of recombinant viruses to validate the remaining 87 mutants in the PanC02 cell line.

23 **Modulation of tumour microenvironment utilizing deletion of Vaccinia virus B8R gene, an IFN gamma sequestering protein results in reduced number of metastases in a murine model of breast cancer.**

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Background: Oncolytic virus treatment represents a novel, safe and targeted cancer treatment approach avoiding non-specific toxicities associated with traditional chemotherapy and radiation. Vaccinia virus (VV) , a double stranded DNA poxvirus with a ~200 Kb genome encoding an armament of immune control genes has been widely used as a vaccine for smallpox and safely as a cancer therapeutic in patients. Oncolytic VV is thought to control tumours by three mechanisms: direct lysis and death of tumour cells, destruction of tumour vasculature leading to reduced nutritional support for tumour cells and stimulation of anti-tumour immunity. Enhancement of these mechanisms or new mechanisms of tumour destruction and control are being sought to improve VV as an oncolytic agent.

Objective: We investigated the effect of deleting the Vaccinia B8R gene (VV-B8R-) on mechanisms of tumour control and destruction. B8R encodes a protein capable of binding and inhibiting the action of IFN gamma, a cytokine with a multitude of immune stimulating and anti-tumour functions. Therefore, it was hypothesized that oncolytic infection of tumours in vivo with a vaccinia virus incapable of inhibiting the actions of IFN gamma may result in enhanced immune activity against the tumour.

Methods: We utilized the murine 4T1 metastatic breast cancer model to investigate the effects of a VV-B8R- compared with a control virus lacking the thymidine kinase (TK) gene (VV-TK-) as a control. Mice were treated at day 7 post tumour cell seeding with a single dose (1E7) of virus or PBS control, tumours excised at day 14 and lung metastases enumerated at day 28. Excised tumours were analyzed by immunohistochemistry (IHC). In addition, in vitro scratch wound migration assays were performed on 4T1 cells infected with VV-B8R- and VV-TK- viruses.

Results: Mice treated with VV-B8R- had significantly fewer lung metastases compared with VV-TK- or PBS control groups. In cell migration assays in vitro, VV-B8R- virus infected 4T1 cells or cells treated with supernatant from VV-B8R- infected cells resulted in slower migration compared with VV-TK- virus. Treatment of VV-B8R- infected supernatant with an antibody to IFN gamma but not a control antibody resulted in restoration of normal migration. IHC analysis of excised 4T1 tumours indicated reduced blood vessel density, no dramatic changes in CD8 T-cell or macrophage infiltration but increased phospo-STAT3 and phospho FAK nuclear accumulation.

Conclusions: Utilization of VV B8R- virus in a murine breast cancer model may result in reduced metastases through IFN gamma mediated inhibition of tumour cell migration.

24 Surgery-induced expansion of myeloid derived suppressor cells modulates natural killer cell phenotype and anti-tumour cytotoxicity

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Background: Surgical resection is the mainstay of therapy for patients with localized solid malignancies. Even with complete resection, many patients develop a metastatic recurrence and ultimately die of their disease. The immediate postoperative period provides an ideal environment for the formation of cancer metastases. We have shown that the suppression of natural killer (NK) cells occurs following surgery and promotes the formation of metastases in both preclinical models and human studies. Myeloid derived suppressor cells (MDSC) are a heterogeneous population of immune regulatory cells that have been shown to expand in cancer patients and suppress the effector function of NK cells. Despite these associations, the role of MDSC expansion in modulating postoperative NK function has not been studied.

Objective: We hypothesize that postoperative MDSC expansion is a key mechanism responsible for modulating NK cell phenotype and anti-tumour cytotoxicity following cancer surgery.

Methods and Results: Using our validated animal models of surgical stress and experimental metastases (B16 melanoma) and spontaneous metastases (4T1 breast carcinoma), we dissected the mediating role of MDSC in the spread of cancer cells after surgery. By flow cytometric analyses, we observed that major surgery resulted in spleen and lung MDSC expansion by 2 to 4-fold. Following depletion of MDSC, with Gr-1 antibody, we observed a significant 10-fold decrease in the number of lung tumour metastases. MDSC depletion also partially rescued the cytotoxicity defect of NK cells following surgery. Gene expression by microarray analysis of extracted RNA from surgically stressed NK cells showed an increase in the induction of scavenger receptors MSR1, CD36 and CD68 over no surgery controls. QRT-PCR and flow cytometric analyses validated these results by demonstrating increased induction and cell surface expression on surgically stressed NK cells. Functional assays verified that scavenger receptor high NK cells purified from surgically stressed mice demonstrated impaired NK cell cytotoxicity, but enhanced phagocytosis of lipid particles. In MDSC-NK cell co-culture assays, NK cell cytotoxicity was significantly impaired and scavenger receptor surface expression was increased in the presence of MDSC from surgically stressed mice compared to no surgery control MDSC.

Conclusions: Surgically-induced MDSC expansion modulates NK cell phenotype and anti-tumour cytotoxicity. The perioperative period presents an opportunity for therapeutic attenuation of MDSC with the potential to decrease metastatic disease.

25 Evaluating the role of anti-viral antibodies on a leukemia oncolytic virus vaccine

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Background
A feature of some oncolytic virus therapies is the induction of an anti-tumour adaptive immune response. Dr. David Conrad has developed a vaccine to elicit this type of immune response toward L1210 leukemia in a murine model. This vaccine platform has been termed iLOV. Thus far, we have determined that iLOV induces anti-L1210 immunity that is long term, specific and largely mediated by T-cells. Furthermore, vaccine derivatives have been made to elucidate which characteristics of the infected cell vaccine are essential. iLOV has been found to offer a clear survival benefit to immunized hosts while unimmunized or insufficiently immunized groups succumb to the L1210 leukemic challenge. Currently, our lab is studying the biological and immunological phenomena associated with iLOV, specifically in the presence of anti-viral immunity. As most viral vector vaccine platforms are hindered significantly by anti-viral humoral responses, this project aims to specifically evaluate the effect of anti-viral antibodies on iLOV vaccine efficacy.

Objective
We want to evaluate iLOV vaccine efficacy in a setting where the immune system has been previously exposed to MG1 rhabdovirus (the virus used to create iLOV).

Methods
Experimental tools used to evaluate this phenomenon include dendritic cell activation assays, phagocytosis assays, flow cytometry, in vitro immune response assays and survival experiments.

Results
Preliminary results have suggested that antiviral antibodies do not hinder this whole cell vaccine platform in activating antigen-

presenting cells or in survival outcomes in subsequent animal studies. One of our experiments have suggested that administering iLOV in the presence of anti-viral immunity actually increases survival. This result is currently being further explored.

Conclusions
Preliminary results suggest few differences between antibody-coated iLOV and iLOV alone. Homologous priming (where the same vaccine is given more than once) is difficult for viral vector based vaccines, as anti-viral antibodies neutralize the virus quickly. There would be a clear contrast between iLOV – an infected cell vaccine – and current viral vector based vaccines, if iLOV vaccine efficacy was not hindered by the presence of anti-viral antibodies, but rather potentiated.

26 **Rhabdovirotherapy reduces the risk of metastatic disease after cancer surgery by enhancing natural killer cell function**

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Background: Surgical stress promotes the formation of metastatic disease secondary to profound suppression of innate natural killer (NK) cells. Our group has recently reported that NK cells are highly activated following infection with several Oncolytic Viruses (OVs). We have further demonstrated that preoperative OV administration can reverse surgery-induced NK cell suppression and abrogate the prometastatic effect of surgery. Unfortunately, there are several theoretical barriers to the administration of a live, replicating virus immediately before surgery, including the potential for an overwhelming systemic inflammatory response and the risk of infection of operating room staff.

Objective: In this study we sought to characterize NK stimulation followed by MG1 infection and identify a safe, non-replicating version of MG1 that could stimulate NK cells and effectively prevent metastases when administered preoperatively.

Methods and Results: The replicating attenuated Maraba virus, MG1 was able to effectively infect and kill melanoma B16lacZ cells in-vitro and reduce metastases in B16lacZ lung metastases model. The efficacy, however, appeared to be independent of oncolytic activity, but dependent on intact NK cells. MG1 administration resulted in an immediate (24h) and intense activation of NK cells, as evidenced by significantly increased NK cell cytotoxicity and cytokine expression. In order to better understand the requirements for NK cell activation by MG1, we compared several variations of MG1: (1) live MG1- productive infection and replication; (2) a G-less version (MG1-Gless) - capable of a single-replication cycle of virus; (3) MG1 exposed to Ultraviolet (UV) for 2 minutes to 2 hours – replication incompetent confirmed by plaque assay. MG1, MG1-Gless, and MG1-UV2min exhibited significantly higher NK cell function compared to PBS control, and they effectively attenuated in vivoB16lacZ lung metastases to near identical levels. Furthermore, we characterized this panel of MG1 viruses in terms of virus morphological structure and cell associated interaction via Electron Microscopy, qRT-PCR and Westernblot and found that MG1-UV2min remains an intact virus particle (virus proteins, genetic materials) with cell associated interactions, corresponding to the highest NK activation and least lung metastases, among MG1-UV viruses. Finally, preoperative intravenous administration of MG1, MG1-Gless or MG1-UV2min overcame surgery induced NK suppression and attenuated the development of postoperative metastases in the B16lacZ model of implanted lung metastases, as well as in the breast 4T1 model of spontaneous lung metastases to equivalent levels as replicating MG1.

Conclusion: Our results suggest that the intact viral particle and cellular recognition, along with viral proteins and genomic RNA are essential for NK cell mediated antitumour responses. Non-replicating forms of MG1, including MG1-UV2min, are novel cancer therapies that can be safely used in the immediate preoperative period to prevent the formation of metastatic disease.

Chronic Disease Program

27 **Akt confers cisplatin chemoresistance in human ovarian carcinoma cells by modulating PPM1D content and function.**

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Background: Ovarian cancer is the most lethal gynecological malignancy. Cisplatin (CDDP) derivatives are first line chemotherapeutics and their resistance is a major hurdle in successful ovarian cancer (OVCA) treatment. Therefore, understanding the molecular dysregulation underlying chemoresistance is important for the development of rational therapeutic strategies. We have previously established that the oncogenic phosphatase protein phosphatase magnesium/manganese-dependent 1 D (PPM1D) confers CDDP resistance in OVCA cells by deactivating the tumour suppressor p53. Furthermore, we have also demonstrated that the oncogenic kinase Akt, an important downstream target of the PI3 kinase survival pathway, is a determinant of CDDP chemoresistance in OVCA cells. However, whether CDDP regulates intra-cellular PPM1D localization, if this regulation is different between chemosensitive and resistant OVCA cells, how CDDP induces PPM1D down-regulation, and whether Akt attenuates p53 activity by regulating PPM1D in the context of CDDP resistance in OVCA, has not been studied.

Hypothesis: PPM1D modulates CDDP sensitivity by decreasing p53 activity, a phenomenon promoted by the PI3K/Akt survival pathway.

Objective: to study the role and regulation of PPM1D in chemoresistance in OVCA cells and its regulation by the PI3K/Akt pathway.

Results:

1. CDDP induced PPM1D down-regulation through ubiquitin-mediated proteasomal degradation in sensitive OVCA cells;
2. CDDP induced PPM1D nuclear localization in resistant cells, and PPM1D nuclear exclusion in sensitive OVCA cells and xenografts;
3. Overexpression of active Akt in sensitive cells stabilized PPM1D content through inhibition of CDDP-induced PPM1D down-regulation;
4. Inhibition of Akt activity in resistant cells lead to decreased PPM1D stability and CDDP-induced down-regulation in resistant cells;
5. PPM1D is highly expressed in human ovarian tumour subtypes (serous, endometrioid, and clear cell).

Conclusion: In the present study we have examined the mechanism of PPM1D processing in OVCA cells, PPM1D intra-cellular localization in response to CDDP, and finally we have demonstrated the role Akt plays in regulating PPMID content and how this regulation influences CDDP sensitivity in OVCA cells. We have demonstrated for the first time that PPM1D intra-cellular localization is differentially regulated by CDDP between sensitive and resistant OVCA cell lines. Furthermore, PPM1D is a downstream target of Akt and the two cooperatively regulate CDDP sensitivity in OVCA cells.

28 **Transient Receptor Potential Melastatin 7 Cation Channel (TRPM7) Kinase Domain plays a role in Ang II-induced hypertension and vascular dysfunction**

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Background: Transient receptor potential melastatin 7 (TRPM7) cation channel is a unique protein that has the dual ability to act as a channel to regulate transmembrane Mg2+ transport and also as a kinase to promote cellular signaling. Despite increasing awareness of the importance of Mg2+ in cardiovascular biology little is known about TRPM7 and its kinase domain in the pathophysiology of hypertension. We previously demonstrated that Angiotensin II (Ang II) regulates TRPM7 in vitro.

Objective: Here we studied TRPM7 kinase-deficient mice to explore the role of the TRPM7 kinase domain in Ang II-induced hypertension.

Methods: TRPM7 kinase deficient mice (TRPM7+/-) and wild type (WT) were infused with Ang II (400 ng/kg/min; minipumps) for 4

weeks. Blood pressure (BP) was measured by tail cuff. Vascular reactivity and structure studies were performed by myography in mesenteric arteries. Expression of TRPM7 downstream targets were analyzed by Western blots.

Results: Baseline BP was similar between TRPM7+/- and WT mice (127 ± 6.0 vs 119 ± 2.2 mmHg). TRPM7+/- mice displayed earlier onset of BP increase by Ang II. After 4 weeks, BP was significantly higher in TRPM7+/- (174 ± 10 mmHg) than in WT mice (147 ± 8 mmHg). Ang II-induced hypertension was associated with cardiac hypertrophy, an effect that was exacerbated in TRPM7+/- mice (g/body weight; WT: 4.4 ± 0.1; WT-Ang II: 5.0 ± 0.2; TRPM7+/-: 4.5 ± 0.1; TRPM7+/-Ang II: 5.7 ± 0.1). Mesenteric arteries from Ang II-infused TRPM7+/- mice exhibited reduced relaxation to acetylcholine compared to WT mice (maximal relaxation; WT-Ang II: 88 ± 8% vs TRPM7+/-Ang II: 59 ± 10%). Consistently, Ang II-induced increase of eNOS expression in mesenteric arteries from WT (3.5-fold), but not from those of TRPM7+/- mice. Ang II induced a leftward shift in the stress-strain relationship for both WT and TRPM7+/- mice in a similar fashion. Plasma analysis revealed that TRPM7+/- mice were hypomagnesemic, and that Ang II increased Mg2+ levels to a greater extent in WT than in TRPM7+/- mice (mmol/L; WT: 0.65 ± 0.02; WT-Ang II: 0.74 ± 0.04; TRPM7+/-: 0.60 ± 0.01; TRPM7+/-Ang II: 0.64 ± 0.02). Ang II induced increase of annexin-1 (2.5-fold) and calpain (2.3-fold) expression in mesenteric arteries from WT mice, and this effect was blunted in TRPM7+/- mice.

Conclusion: Our findings demonstrate that hypertension, cardiac hypertrophy and endothelial dysfunction are exaggerated by Ang II in TRPM7+/- hypomagnesemic mice, suggesting a novel role for TRPM7 kinase domain in cardiovascular pathophysiology.

29 **Influence of Depot Origin on the Susceptibility of Adipose Progenitor Cells to Cell Death**

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Nutrient excess and a sedentary lifestyle lead to an accumulation of adipose tissue (obesity) through an increase in adipocyte size (hypertrophy) and number (hyperplasia). Hypertrophic obesity results in dysfunctional adipocytes associated with adipose tissue inflammation and insulin resistance. On the other hand, hyperplastic adipose tissue expansion is linked to preservation of insulin sensitivity. Metabolically functional adipose tissue expansion via hyperplasia may require an adequate number of responsive adipose progenitor cells. Distinct adipose tissue depots vary with respect to functional responses, such as lipolysis and adipogenesis. The aim of this study was to investigate the influence of depot origin on the susceptibility of adipose progenitor cells to cell death. Using serum deprivation alone or in the presence of 25nM tumour necrosis factor (TNF) a, visceral omental (OM) versus abdominal subcutaneous (SC) adipose progenitor cells displayed a 3- and 1.7-fold-increase in cell death, as assessed by by Hoechst staining, respectively ($p<0.05$, $n=3$ or 4). Similar results were obtained when cell death rates were evaluated by cell enumeration. The ratio of OM/SC cell death in response to serum deprivation was positively correlated with body mass index ($r=0.96$; $p<0.01$, $n=5$). The depot-specific difference in apoptosis susceptibility observed was lost when a stronger apoptotic stimulus (TNFa with cycloheximide) was used. Depot-related differences in apoptotic susceptibility of adipose progenitor cells may influence regional cellular remodeling during adipose tissue expansion and alter metabolic functionality in obesity.

30 **Urinary Podocyte Microparticles Identify Pre-Albuminuric Diabetic Glomerular Injury**

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Backgroud: Microparticles (MPs) are small (0.1-1.0 µm) vesicles shed from the surface of cells in response to stress. Whether podocytes produce MPs, and whether this reflects glomerular injury is unclear.

Objective: To assess podocyte MP formation in response to diabetic stress conditions.

Methods: We examined MP formation in cultured human podocytes (hPODs) and diabetic mice. hPODs were exposed to cyclical stretch, high glucose (HG, 25 mM), angiotensin II, or transforming growth factor ß. Urinary podocyte MPs were assessed in two mouse models of diabetic nephropathy: streptozotocin (STZ) and OVE26 mice.

Results: Cyclic stretch and HG increased MP release as assessed by flow cytometry ($P<0.01$ and $P<0.05$ respectively vs. controls). STZ-treated mice (8 weeks) exhibited increased urinary podocyte MPs compared to age-matched non-diabetics (18035±3813 vs. 42±34 MPs/mg creatinine, $P<0.001$). Similarly, in 16 week old OVE26 mice urinary podocyte MPs were elevated compared with wild-type littermates (20392±9646 vs. 35±29 MPs/mg creatinine $P<0.01$). At 1 week post-STZ, at a time when albuminuria was absent, urinary podocyte MPs were already significantly increased compared to non-diabetic mice (4052±900 vs. 5±4 MPs/mg creatinine, $P<0.001$).

Conclusions: Our results indicate that podocytes produce MPs that are released into urine. Podocyte-derived MPs are generated by exposure to mechanical stretch and high glucose, and could represent early markers of glomerular injury in diabetic nephropathy.

31 **Impaired IL-7 activity of CD8+ T cells in HCV infection: Implications for HIV-HCV Co-infection**

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BACKGROUND: Effective immune responses against hepatitis C virus (HCV) are dependent on CD8+ T cells, yet their function is impaired in chronic infection. CD8+ T cell impairment is also a feature of chronic HIV infection, where it is associated with decreased activity of IL-7. HCV is the most prevalent co-morbidity in HIV, yet the effect of HCV on HIV infection remains largely unknown. IL-7 is critical for CD8+ T cell development, and important for T cell homeostasis, memory cell generation, and cytolytic function. CD8+ T cell responses to IL-7 are dependent on the expression of IL-7 receptor alpha on the cell membrane (mCD127). Reduced mCD127 expression, increased plasma soluble CD127 (sCD127) levels, or cellular deficiencies in IL-7 signalling may contribute to impairment, as we reported in HIV infection.

OBJECTIVES: This study will determine how CD8+ T cell activity (specifically IL-7 activity) is impaired in chronic HCV infection. The hypothesis of this study is that HCV infection decreases CD8+ T cell activity, specifically IL-7 responsiveness, in both HCV and HIV-HCV infection.

METHODS: CD8+ T cells were isolated from healthy donors (controls) as well as individuals with untreated HCV infection and HAART-controlled (< 40 copies/ml HIV RNA) HIV-HCV co-infection. Expression of mCD127 on CD8+ T cells and plasma sCD127 levels were measured by flow cytometry and immunobead assays, respectively. IL-7-induced signalling (STAT5 phosphorylation), proliferation, and production of the anti-apoptotic molecule Bcl-2 were measured by flow cytometry. Dose responses were assessed by regression analysis ($P < 0.05$).

RESULTS: There was no significant difference in mCD127 expression on blood-derived bulk CD8+ T cells or plasma sCD127 levels between control, HCV and HIV-HCV infection. IL-7-induced STAT5 phosphorylation was significantly reduced ($p = 0.005$) in CD8+ T cells from HCV infection compared to controls, and similar to HIV-HCV co-infected individuals. Cell division of CD8+ T cells cultured with suboptimal amounts of T cell stimulator (PHA) was of lower magnitude in HCV infection than controls. Lastly, the production of Bcl-2 in response to IL-7 was significantly reduced in CD8+ T cells of HCV and HIV-HCV infected individuals compared to controls ($p<0.001$ and 0.04, respectively).

CONCLUSIONS: These results suggest that CD8+ T cell impairment in HCV infection is characterized by decreased responsiveness to IL-7, independent of mCD127 expression, in contrast to what is observed in HIV infection. The mechanism of CD8+ T cell impairment may be through IL-7-stimulated signalling, since we know Bcl-2 production is STAT5 dependent.

32 **Determining the optimal cell line for the transfection of pCMV-CD127 plasmid, for the purpose of studying IL-7 signaling in vitro.**

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Background: Interleukin (IL) 7 is an essential cytokine for CD8 lymphocyte differentiation, proliferation and homeostasis. IL-7 signals through a heterodimeric receptor complex composed of ? chain (CD132) that is shared with the IL-2 family of cytokines and a chain (CD127) that is specific to IL-7 signaling. 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 MS and rheumatoid arthritis (RA).

Objective: To determine how mutations within CD127 affect IL-7 signaling, expression, and regulation.

Methods: Anti-CD132-PE and anti-CD127-PE antibodies were used to measure surface expression of CD132 and CD127 respectively by flow cytometry on four different cell lines (A3, CEM, Jurkat, & SupT1). Cell lines with low levels of endogenous CD127 and high levels of CD132 were transfected with pMAX-GFP using LT1, 2020, TransIT, Nucleofector and Ingenio. Transfection efficiency was determined by measuring GFP+ve cells, by flow cytometry. Cell viability was measured by PI staining. STAT5 was detected by western blot.

Results: SupT1 and A3 cells have high levels of endogenous CD127 expression. In contrast, Jurkat and CEM cells have low levels of endogenous CD127, and high levels of endogenous CD132 compared to CD8 T-cells. In CEM and Jurkat cell lines, 2020 reagent provides higher transient transfection efficiencies, while keeping cell toxicity low. 2020 reagent provides transient transfection efficiencies of 40.53% in CEM and 19.01% in Jurkat, 24 hours after transfection. All cells have STAT5.

Conclusions: Jurkat cells were chosen for transfection of pCMV-CD127 plasmid, since they were more culturable than CEM cells. IL-7 treated transfected cells will next be analyzed by western blot for STAT5p. Jurkat cell line will be used to complete the objective,

only if signal transduction is detected. In accordance with the objective, mutations will be made within CD127 gene on a pCMV-CD127 plasmid. These mutations are based on identified SNPs (identified in GWAS) within the CD127 gene. These mutations have the potential to cause a gain of function or a loss of function with regards to IL-7 signaling. These plasmids will then be stably expressed in Jurkat cells and CD127 analyzed for regulated expression and signal transduction in response to IL -7.

33 **Determination of seminolipid's roles in testicular germ cell-Sertoli cell interactions during spermatogenesis**

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Introduction: Seminolipid is a glycolipid which is present abundantly and selectively on the surface of mammalian male germ cells. Mice genetically targeted to be incapable of producing seminolipid are infertile due to spermatogenesis disruption. Nonetheless, the mechanisms on how seminolipid regulates germ cell development are still unknown. Sertoli cells are somatic cells present in the seminiferous tubules, spanning from the basement membrane to the lumen; they provide both cyto-organizational and nutritional support to the developing germ cells. The dynamic and continuous interactions between Sertoli cells and germ cells are thus important for the completion of spermatogenesis. As a major glycolipid on the testicular germ cell surface, seminolipid is likely involved in this cell-cell interaction through its binding proteins on Sertoli cells. In fact, TLR-4 and SR-B1 expressed on the Sertoli cell surface are known for their affinity for seminolipid.

Objective: The objective of this study is to isolate and identify all seminolipid binding partners on the Sertoli cell surface and determine their roles in germ cell-Sertoli cell interactions.

Methods: Plasma membrane proteins were extracted from isolated mouse Sertoli cells with 2% octylglucoside. Proteins with specific affinity for seminolipid were affinity-captured with multilamellar liposomes containing seminolipid. The liposome-protein complexes were then ultracentrifuged and subsequently treated with chloroform/methanol to simultaneously solubilise liposomes and precipitate the proteins. The seminolipid bound proteins were subjected to SDS-PAGE/silver staining. To minimize non-specific binding to seminolipid liposomes, the Sertoli cell protein extracts were pre-incubated with liposomes containing phosphatidylcholine (PC), and/or phopshatidylserine (PS).

Results: Pre-incubation with PC or PC/PS liposomes removed many proteins through hydrophobic and/or electrostatic interactions as seen by silver staining and protein quantification (Bradford Assay). Binding of the remaining proteins to the seminolipid liposomes produced consistent protein profiles across multiple trials. The profile was significantly reduced from the total membrane extracts after incubation with the PC- and PS-liposomes. Specifically, prominent bands were seen at 25, 28, 35, 38, 41, and 55 kDa.

Conclusion: The liposome binding experiment has consistently isolated seminolipid binding proteins from the total extract of Sertoli cell membrane proteins. Proteomic analysis will reveal the identity of these proteins and allow further characterization through functional studies. Determining seminolipid binding partners will advance our understanding of spermatogenesis and may facilitate the development of male contraceptives and/or treatments for male infertility.

34 **Differential and contrasting functions of Interleukin-7 during homeostatic vs. cytotoxic responses**

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Background: IL-7 signaling is important for CD8 T cell function. In addition to ongoing homeostatic roles, IL-7 is an inducible factor upon viral infection and enhances CTL function. As a result, decreased IL-7 receptor (CD127) expression on CD8 T-cells as seen in HIV+ individuals may not only reduce cell survival but may also disrupt naïve CD8 T-cell activation in response to antigens.

Objective: To Better understand the function of this vital cytokine and how it is regulated holds importance in understanding HIV disease and developing future therapies.

Methods: Naïve CD8 T cells (CD8+ CD45RA+CCR7+CD45RO-CD56-CD57-) were isolated from PBMC obtained from healthy donors. Cells were stimulated in vitro with anti-CD3/anti-CD28 beads +/- IL-7. Flow cytometry was used to follow expression of a number of markers over 72 hours and cell division was measured with CFSE.

Results: Although IL-7 alone (5 ng/ml) had no effect on any surface markers measured with the exception of CD127, IL-7 significantly enhanced many TCR induced phenotypic changes including upregulation of CD25, CD56, CTLA4, CD69, HLADR,

CD62L, CCR7, CD8, TIM3, PD1 and PDL1. IL-7 also enhanced TCR induced proliferation as measured by CFSE dilution over 7 days and increased Ki-67 expression after 72 hours. Although IL-7 alone had no effect on the production of IFN? and perforin in naïve CD8 T cells, IL-7 significantly enhanced production of these cytotoxic molecules in the presence of TCR stimulation. Interestingly, IL-7 induced expression of Bcl2 was completely blocked in the presence of TCR stimulation. This was also reflected in a loss of IL-7 induced suppression in of AnnexinV on CD8 T cells after TCR stimulation.

Conclusions: Our data suggest that IL-7 signaling plays an important role in CD8 T-cell homeostasis and cell survival that changes dramatically following antigen stimulation. By enhancing expression of a number of activation markers and cytotoxic functions in naïve CD8 T cells, IL-7 may facilitate differentiation to the effector phenotype and increase CD8 T cell responsiveness to pathogens such as HIV. Decreased CD127 expression and impaired IL-7 signaling in HIV+ individuals may then affect CD8 T-cell differentiation and function in response to antigens. Reduced CD127 expression could also play a role in foiling potential HIV vaccination strategies, and IL-7 based therapies.

35 **Vaccinium angustifolium var. laevifolium House (Lowbush blueberry) leaf extract increases trophoblast migration.**

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Objectives: Leaf extracts from many species of Vaccinium, such as Vaccinium angustifolium Ait. (Lowbush blueberry), have been used for decades by indigenous populations of Canada in the context of diabetes. In other systems, Vaccinium has been shown to promote cell migration for wound healing. Many placenta-mediated diseases are associated with altered trophoblast migration. Since V. angustifolium has never been studied in the context of placental biology, we wanted to determine the effects of this extract on trophoblast biology.

Methods: Dose-response studies were performed by treating HTR-8/SVneo cells with V. angustifolium var. laevifolium House for 24 hours. Cell migration, proliferation, and viability were assessed using a Boyden chamber migration assay, BrdU incorporation assay, and trypan blue exclusion test, respectively. Western blot analyses of time-course studies were used to detect changes in ERK and AKT phosphorylation upon treatment. Mass spectrometry was used to identify the components in the extract.

Results: Cells treated with 20 ng/mL of the extract displayed a significant increase in migratory ability compared to the untreated group. At this concentration, the extract had no effect on cell proliferation or viability. There were no differences in ERK and AKT phosphorylation between control and treated groups. Phenolic acids, flavonoids, and terpenes were identified in the extract.

Conclusion: V. angustifolium leaf extract increases cell migration with no effect on proliferation or cell death. ERK and AKT phosphorylation are not affected with treatment. Preliminary data suggests that polyphenols are major components of the extract. Fractionation studies are underway in order to identify the active components of the extract.

36 **Influence of Chemerin on the Regulation of Mitochondrial Dynamics and Ovarian Cell Fate**

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Polycystic ovarian syndrome (PCOS) accounts for 75% of women with anovulatory infertility. The adipokine Chemerin and its receptor play a role in the regulation of follicular growth and steroidogenesis. Increased ovarian follicular expression of chemerin and its receptor its receptor as well as serum chemerin levels in a DHT-induced rat PCOS model suggest that chemerin and its receptor may be involved in the pathogenesis of PCOS.

Mitochondria are highly dynamic organelles, constantly dividing (fission) and elongating (fusion) to form a network, which maintains mitochondrial function including the regulation of cell death. Dysregulated mitochondrial fission and fusion has been linked to pathogenesis of human diseases. A number of intracellular intermediates are known to regulate mitochondrial fission (Drp1, Oma1) and fusion (Opa1). However, it remains unclear as to whether chemerin plays a role in the dysregulation of mitochondrial fission and fusion and if this process is causally related to PCOS.

The overall objective of this project is to assess the role of chemerin in early antral follicular growth. My overall hypothesis is that chemerin suppresses gonadotropin-induced follicular growth by promoting granulosa cell apoptosis through induction of mitochondrial fission, and that PCOS is due in part to increased chemerin-mediated, gene-regulated mitochondrial fission. My specific objectives are to (a) if chemerin induces granulosa cell apoptosis during early antral follicular growth and, if so, whether it

is mediated through increased mitochondrial fission and (b) study the influence of chemerin on the expression of mitochondrial fission (Drp1, Oma1) and fusion (Opa1), mitochondrial fission, and apoptosis in granulosa cells in vitro.

The influence of chemerin on granulosa cell apoptosis and mitochondrial dynamics will be examined by in vitro granulosa cell culture with chemerin treatment. Mitchondrial fission and fusion protein expression will be examined using standard cellular and molecular techniques such as Western blot, TUNEL, qualitative PCR and immunofluorescent confocal microscopy.? The proposed study will demonstrate for the first time the role of chemerin and mitochondrial fission in the regulation of granulosa cell apoptosis and their possible dysregulation in PCOS. They will provide greater understanding of the pathophysiology of this complex syndrome and offer new insights on future treatment strategy.

37 **Potential role of PRAJA2 in TSH-stimulated lipolysis human differentiated adipocytes**

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Background:
Thyrotropin (thyroid-stimulating hormone; TSH) binds to TSH receptors on thyrocytes to regulate development and growth of the thyroid gland, and to stimulate thyroid hormone production. TSH can also act in an extra-thyroidal fashion, and engage TSH receptors on adipocytes to induce lipolysis (release of free fatty acids). In both cell types, cAMP-dependent kinase (PKA) is activated. Recently, PRAJA2, a novel E3 ubiquitin ligase that acts on the regulatory subunits of PKA (PKAr) was identified in thyrocytes. The ubiquitin-dependent proteasomal degradation of PKAr by PRAJA2 prolongs the activity of PKA's catalytic domain (PKAc). In adipocytes, TSH-stimulated PKA is required for lipolysis.

Objectives:

1. To characterize the expression of PRAJA2 in primary human adipocytes.
2. To determine the effect of PRAJA2 expression on primary human adipocyte lipolysis.

Methods:
Adipose tissue from patients undergoing elective abdominal surgery is processed to obtain primary human preadipocytes. These cells are then differentiated into adipocytes, over the course of 14 days. They are then stimulated with TSH (or isoproterenol, beta-adrenergic agonist) and readouts of lipolysis (NEFA/glycerol), PRAJA2 mRNA expression (qPCR) and PRAJA2 protein expression (Immunoblot) are analyzed.

Results
Preliminary data show TSH and isoproterenol stimulate lipolysis in our cellular model. PRAJA2 is expressed at the mRNA and protein level in primary human preadipocytes and differentiated adipocytes. There is no notable change in PRAJA2 mRNA or protein expression in adipocytes upon stimulation with TSH or isoproterenol.

Conclusions:
PRAJA2 is present in our human adipocyte cellular model; mRNA and protein levels are not altered with TSH (or isoproterenol) stimulation. Future studies will examine changes in phosphorylation state (activation) of PRAJA2 upon TSH (or isoproterenol) stimulation. PRAJA2 expression will be modulated (by overexpression or knockdown) to determine if TSH-stimulated lipolysis is altered.

38 **Effect of glucose on human adipogenesis and its regulation by macrophages**

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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. Glucose is a dietary factor that activates macrophages and little is known about the effects of high glucose concentrations on the anti-adipogenic effect of macrophages.

Objective: To determine whether exposure of macrophages to high glucose levels increases their secretion of anti-adipogenic factors that inhibit human adipogenesis.

Methods: Human monocyte derived macrophages (MDM) were derived from peripheral blood mononuclear cells (PBMCs) isolated from blood donated by 5 (3 male, 2 female) healthy local volunteers (approved by the Ottawa Hospital Research Ethics Board),

where age was 33+/-8.4 (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 5 (1 male, 4 female) patients undergoing elective abdominal surgery (approved by the Ottawa Hospital Research Ethics Board), where age was 43+/-16, and body max index was 25+/-2.5 (mean+/-SD).

Results: Macrophage-conditioned medium (MacCM) generated in the presence of HG inhibited triacylglycerol (TG) accumulation and protein expression of sterol regulatory element-binding protein 1 (SREBP-1), fatty acid synthase (FAS), and peroxisome proliferator-activated receptor ? (PPAR?) by 54+/-4.8%***, 40+/-13%**, 37+/-14%*, and 50+/-11%* respectively (mean+/-SE) (n=5, ***p<0.001, **p<0.01, and *p<0.05) compared to control. There were no changes in responses to NG-MacCM (n=5, p= n.s for these markers).

Conclusions: These preliminary results suggest that dietary factors such as glucose may influence adipose tissue function by modulating anti-adipogenic factors produced by macrophages.

39 **Ubiquitin C-terminal hydrolase L1 deletion ameliorates glomerular injury in mice with ACTN4-associated focal segmental glomerulosclerosis**

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Background:
Renal ubiquitin C-terminal hydrolase L1 (UCHL1) is upregulated in a subset of human glomerulopathies, including focal segmental glomerulosclerosis (FSGS), where it may serve to promote ubiquitin pools for degradation of cytotoxic proteins.

Objective:
In the present study, we tested whether UCHL1 is induced in a mouse model of ACTN4-associated FSGS. We hypothesize that deletion of UCHL1 in a mouse model of ACTN4-associated FSGS reduces indications of filtration barrier injury and glomerular lesions.

Methods:
UCHL1 expression in kidneys of K256E-ACTN4pod+ / UCHL1+/+ mice was confirmed by western immunoblotting, immunohistochemistry and qPCR. UCHL1+/- and K256E-ACTN4pod+ mice were intercrossed to generate K256E-ACTN4pod+/UCHL1-/- mice. Albuminuria (albumin/creatinine) was determined by ELISA and Coomassie brilliant blue staining of urine samples resolved by SDS-PAGE. Glomeruli were scored for sclerosis and glomerular collapse using PAS stained kidney sections. Tail cuff plethysmography was used to measure systolic blood pressure. Podocyte foot process effacement was analyzed by electron microscopy of kidney sections. TUNEL-staining of glomerular and cortical renal paraffin-embedded sections was performed. Immunofluorescence using anti-WT1 confirmed podocyte numbers in glomeruli of OCT-embedded frozen kidney sections. Finally, renal poly-ubiquitinated protein levels and K256E-a-actinin-4 levels were determined by western immunoblot.

Results:
UCHL1 protein was detected in glomeruli of K256E-ACTN4pod+ / UCHL1+/+ mice. UCHL1+/- mice were intercrossed with K256E-ACTN4pod+ mice and monitored for features of glomerular disease. 10-week-old K256E-ACTN4pod+ / UCHL1-/- mice exhibited significantly ameliorated albuminuria, glomerulosclerosis, tubular pathology and blood pressure. Interestingly, while UCHL1 deletion diminished both tubular and glomerular apoptosis, WT1-positive nuclei were unchanged. Finally, UCHL1 levels correlated positively with poly-ubiquitinated proteins but negatively with K256E-a-actinin-4 levels, implying reduced K256E-a-actinin-4 proteolysis in the absence of UCHL1.

Conclusions:
Our data suggest that UCHL1 upregulation in ACTN4-associated FSGS fuels the proteasome and that UCHL1 deletion may impair proteolysis and thereby preserve K256E/wt-a-actinin-4 heterodimers, maintaining podocyte cytoskeletal integrity and protecting the glomerular filtration barrier.

40 **Role of Clusterin During Epididymal Sperm Maturation and Sperm Capacitation**

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Background: Clusterin (CLU) is a ubiquitously expressed sulfated glycoprotein involved in various biological processes. Uniquely, CLU can act as a chaperone preventing precipitation of stress-induced proteins. CLU is found at a high amount in the epididymal fluid due to the secretion from epididymal epithelial cells. A number of zona pellucida (ZP) binding proteins (e.g., SED1, arylsulfatase A), present also in the epididymal fluid, tend to oligomerize with a possible consequence to precipitate. CLU may function in chaperoning these ZP binding proteins onto the sperm surface. To date, information on the amounts and functions of epididymal CLU is not available. We therefore characterized the CLU distribution in mouse epididymal fluid and sperm. Since CLU has an affinity for cholesterol and cholesterol efflux occurs during capacitation, we asked whether CLU on the sperm surface relocalized during this event.

Methods: Mouse caput epididymal sperm were centrifuged through a 45% Percoll solution to remove loosely bound components. Motile caudal sperm were prepared by Percoll gradient (45/90%) centrifugation. Epididymal fluid was centrifuged (14,000g) to remove subcellular particles. Caudal epididymal sperm were capacitated in medium containing 0.3% BSA. Immunofluorescence for CLU was performed on various sperm types, and CLU from epididymal fluid and sperm was quantified by immunoblotting/densitometric analyses. Anti-CLU antibody against CLUa peptide (Glu209-Glu222) was used in all immunodetections.

Results and Conclusions: Caput epididymal fluid contained 9x higher CLU than caudal fluid (5.24 versus 0.61 µg in each epididymus). In contrast, CLU was present only on caudal sperm. This was possibly due to the change of plasma membrane properties of caudal sperm thus selectively allowing CLU deposition on their surface. Significantly, the amount of CLU on total number of sperm in each caudal epididymus was 0.55 µg, comparable to the CLU amount in the caudal epididymal fluid (0.61 µg), a result indicating a CLU homeostasis between the fluid and sperm. Immunofluorescence revealed CLU staining patterns in the caudal sperm head. Interestingly, sperm CLU re-localized during capacitation. On non-capacitated caudal sperm, the CLU staining pattern was punctate over the entire ventral head region; following capacitation, CLU staining became distinct only at the hook of the sperm head. These results suggested that CLU may be involved in capacitation. Possibly, its relocalization to the hook region reflected its dissociation from the ZP-binding proteins, which then were free to bind to the ZP. These CLU associated properties on sperm may serve as bioindexes of sperm maturation and capacitation.

41 **Release of soluble IL-7 receptor α by CD8$^+$ T cells and human thymocytes**

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Background: Interleukin-7 (IL-7) has a crucial role in the development, proliferation, survival and cytotoxic (CTL) activity of T-cells. IL-7 downregulates the expression of the IL-7 receptor α subunit and the release of a soluble form of CD127 (sCD127) is thought to contribute to this effect. The soluble form of CD127 alters IL-7 activity and sCD127 plasma concentrations are increased in HIV infection. Despite the potential biological role of sCD127 in IL-7 activity in health and HIV infection, the mechanisms of its production and release have not been described.

Methods: Primary human CD8$^+$ T cells and freshly isolated thymocytes were stimulated with IL-7 and either T-cell stimulation (anti-CD3/CD28 antibodies) or phytohaemagglutinin (PHA). The concentration of sCD127 in culture supernatants was measured by a sCD127-specific ELISA every 24 hours for up to 96 hours. To determine the potential mechanisms of sCD127 release, the roles of IL-7 signaling (using Jak and STAT5 inhibitors) and proteolytic cleavage (using serine, cysteine and Matrix metalloproteinases protease inhibitors) were investigated.

Results: Stimulation of CD8$^+$ T cells with IL-7 significantly enhanced anti-CD3/CD28 antibody- or PHA-induced sCD127 release after 72 hours of culture and this occurred in part by direct loss of membrane CD127 (mCD127). Inhibition of Jak signaling blocked the release of sCD127. Inhibition of MMP, but not of serine and cysteine proteases alter sCD127 release. Stimulation with IL-7/anti-CD3/CD28 did not induce release of sCD127 from thymocytes, but the basal (unstimulated) production of sCD127 did increase overtime in culture.

Conclusions IL-7 and T cell stimulation induced sCD127 release from CD8+ T cells but not from thymocytes. The mechanism of release of sCD127 by CD8$^+$ T-cells is in part dependent on IL-7 signaling and MMP cleavage, indicating that CD127 might be shed directly from the surface membrane upon IL-7 and TcR co-stimulation. Ongoing studies are investigating the signaling pathways

more closely as well as alternative mechanisms of soluble receptor expression such as exosomal release.

42 The antimicrobial host defence peptide, LL-37, 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 membranes following its interaction with surface anionic phospholipids. ALL-38 (an LL-37 close analog: LL-37 + Ala at the N-terminus) is produced in the vagina 2-6 h post-intercourse from its precursor hCAP-18, a seminal plasma component. At this time, motile sperm, already swum into the uterine cavity, are not exposed to ALL-38. 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.

Objective: To test the contraceptive effects of LL-37.

Methods: Mouse/human sperm treated with LL-37 in a physiological concentration range were assessed in vitro for SGG-dependent LL-37 binding, and parameters relevant to fertilizing ability- motility and intactness of the sperm acrosome and plasma membrane. Ability of mouse sperm to fertilize eggs in vitro was also evaluated. Each study was performed with =3 different sperm samples. The efficacy of LL-37 to inhibit sperm fertilizing ability in vivo was determined in mice transcervically injected with sperm + LL-37 and assessed for pregnancy (n=26 for LL-37 treatment and n=26 for no treatment). Sperm motility was assessed by videomicroscopy and the acrosomal status by Coomassie staining of acrosome intact mouse sperm or CD-46 exposure for acrosome reacted human sperm. Sperm membrane disruption was assessed by the incorporation of Sytox Green, and electron microscopy. Mouse in vitro fertilization was scored by the presence of two–pronuclei in eggs 6 h post-insemination.

Results: LL-37 bound to both mouse and human sperm and the binding was partially dependent on sperm surface SGG. Mouse and human sperm lost their motility and became prematurely acrosome reacted upon treatment with LL-37. The initial action of LL-37 on both mouse and human sperm appeared to be through permeabilization/disruption of sperm surface membranes evidenced by the Sytox Green staining and EM revealing ultrastructural damage. LL-37-treated mouse sperm were incapable of fertilizing eggs in vitro or generating any pregnancy in all 26 inseminated females. Females inseminated with LL-37 treated sperm showed no abnormal damage to their reproductive tract and were capable of resuming their fecundity in subsequent mating with fertile males.

Conclusions: Our results reveal selective inhibitory effects of LL-37 on sperm fertilizing ability without apparent damaging the female reproductive tract. LL-37

43 Peri-ovulatory putrescine supplementation reduces midgesation embryo loss in older mice

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Women's fertility diminishes in their late 30s or early 40s, 10-15 years before menopause. It is well known that egg aneuploidy is the main factor. Recently, we have demonstrated that deficiency of ornithine decarboxylase (ODC) in older mice causes egg aneuploidy, which can be rescued by exogenous supplementation of putresince, the direct product of ODC activity. The present study was undertaken in order to determine ovarian polyamine dynamics during ovulation in young mice, and polyamine level change in older mice, as well as in older mice receiving oral putrescine supplemeation. We utilize ion chromatograph system to analyze polyamines with an online anion suppressor so only cations are detected by a built-in conductivity detector. We found

that ovarian putrescine level had a clear rise during ovulation, peak time at 5 h after hCG, concurrent with ovarian ODC activity peak. Older mice had reduced putrescine peak, corresponding to the reduced ODC activity peak reported previously. Exogenous putrescine supplementation during peri-ovulatory time by drinking water reduced the embryo/fetus resorption site significantly at 12 dpc in older mice, from 14% to 8% (P=0.0124). Furthermore, mice herozygous for the odc gene had higher ratio of resorption sites compared age-matched wildtype littermates. It is concluded that putrescine exerts protective function during oocyte maturation to reduce the incidence of egg aneuploidies, resulting in reduction of midgestation embryo deaths. Peri-ovulatory putrescine supplementation may be an effective fertility treatment for older women.

44 A Novel Model of Advanced Diabetic Kidney Disease in Mice

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Background:
Insight into the pathogenesis of diabetic nephropathy (DN) has been limited by a lack of animal models which recapitulate key features of human DN. While the majority of currently available rodent models exhibit characteristics of early DN (hyperfiltration, mesangial expansion, albuminuria), features of advanced DN (hypertension, GFR decline, tubulointerstitial fibrosis) are absent or require a significant time investment for full phenotype development. A possible explanation for resistance to renal complications of diabetes in rodents is the lack of a concomitant increase in blood pressure.

Objective:
Accordingly, the aim of the present study was to develop a mouse model of advanced DN with hypertension superimposed.

Methods:
Type 1 diabetic OVE26 mice were crossed with a mouse model of angiotensin-dependent hypertension utilizing mice transgenic for active human renin cDNA under the control of the transthyretin promoter (TtRhRen). TtRhRen mice exhibit angiotensin-dependent hypertension and progressive cardiac hypertrophy beginning at 6 weeks of age.

Results:
At 20 weeks of age, OVE26 mice exhibited modest albuminuria as compared with wild-type and TtRhRen mice. However, albuminuria was significantly more pronounced in OVE26/TtRhRen mice. Additionally, OVE26/TtRhRen mice displayed renal and glomerular hypertrophy and evidence of tubulointerstitial fibrosis. Interestingly, beginning at 10 weeks TTRhRen/Ove26 mice were severely hypertensive, significantly exceeding that of TTRhRen littermates.

Conclusions:
In summary, our results suggest that TtRhRen/OVE26 mice are a robust model of type I DN that recapitulates key features of human disease. Such a model may be of significant interest in the analysis of DN pathogenesis and in the assessment of putative therapeutics.

45 Chemerin and its processing in polycystic ovarian syndrome

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Background: Polycystic ovarian syndrome (PCOS) is a heterogeneous disorder of reproduction and metabolism with potential systemic sequelae (such as diabetes and obesity) and associated with abnormality of follicle growth, dysregulated sex hormone profile, hyperthecosis and insulin resistance. An adipokine chemerin is associated with obesity and metabolic syndrome and its plasma concentration increases in obese women and in PCOS subjects. Our previous findings indicated that chemerin suppresses FSH-induced follicular growth and granulosa cell steroidogenesis in vitro and its serum and ovarian levels are elevated in a chronic rat PCOS model. Although chemerin is shown to be cleaved at its C-terminus by various proteases in vitro, its processing pattern in ovarian follicular fluid, is poorly understood. Moreover, whether the dysregulation of chemerin expression and processing in the ovary contributes to the pathogenesis of PCOS is unknown.

Methods: Serum and follicular fluid are collected from PCOS subjects and matched controls from the infertility clinic. The levels of chemerin in human serum and follicular fluid are determined by Western blot. Chemerin is enriched by immuneprecipitation using specific antibody and its levels and isoforms are analyzed by Western blot and mass spectrum.

Results: Serum chemerin levels was higher in obese subjects than non-obese controls; however its level in PCOS groups was slightly but not significantly higher than those in non-PCOS subjects. Chemerin is present in human follicular fluid and its

concentration are 8-10 folds higher than those in serum. The levels of chemerin in follicular fluid were significantly higher in PCOS subjects compared to the controls. The clinical treatment of metformin to PCOS patients dramatically reduced the chemerin levels.

Conclusion: Our findings will offer useful pathophysiologic insights into this complex syndrome and may provide important clue to the clinical treatment of PCOS.

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46 **Maternal serum levels of FGL2: A screening tool for preeclampsia?**

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Background: Fibrinogen-like protein 2 (FGL2) is a 260 kDa tetrameric cell-surface prothrombinase previously linked to spontaneous abortions through a process of leukocyte activation. It was shown to be highly expressed in trophoblasts, and its ability to induce coagulation suggested it might play a role in the pathogenesis of PE. It was demonstrated that FGL2 deficient mice were resistant to lipopolysaccharides (LPS)-induced abortions but that these same mice had smaller litters under normal conditions. This observation could be explained by the fact that the soluble form of FGL2 (sFGL2) possesses immunosuppressive activity by binding to the Fc gamma receptor (FcgR)IIB expressed on the surface of antigen presenting cells.

Objectives: We measured the circulating levels of FGL2 in first trimester maternal serum and compared normal pregnancies to those which were later complicated by PE. We confirmed the presence and localization of FGL2 in the placenta. We assessed the levels of FGL2 expression and secretion by trophoblast cell lines.

Methods: First trimester serum samples from the OaK birth cohort were analysed. The samples from mothers who developed PE (n=13) were compared to samples from healthy pregnancies (n=30). The diagnosis of PE was made according to ACOG guidelines. FGL2 was quantified by ELISA and Western blot. The placental localisation of FGL2 was analyzed by immunohistochemistry and immunofluorescence. HTR-8/SVneo and BeWo cells were cultured in RPMI 1640 medium +10% FBS.

Results: There is no difference in first trimester levels of circulating FGL2 between women undergoing normal pregnancies and those who will later develop PE. However, a 65 kDa fragment recognized by the FGL2-specific antibody is present in lower concentration in women destined to develop PE. The immunohistochemistry analysis shows that FGL2 immunoreactivity is more pronounced at the maternal-fetal interface. The cell line analysis shows that HTR-8/SVneo cells, a model of human extravillous trophoblasts, express and secrete more FGL2 than BeWo cells.

Conclusions: Since soluble FGL2 has immuno-suppressive effects, it is possible that higher levels of circulating FGL2 during pregnancy could have a protective effect by attenuating the maternal immune response towards the semi-allogenic fetus. The 65 kDa fragment present in lower concentration in the serum of women destined to develop PE could represent the monomeric form of FGL2, which has been found to carry a stronger immunosuppressive activity than the tetrameric form in vitro. High levels of expression of FGL2 at the maternal-fetal interface could also play a role in local regulation of the maternal immune response.

Clinical Epidemiology Program

47 **Increased emergency room visits or hospital admissions in females after 12-month MMR vaccination, but no difference after vaccinations given at a younger age.**

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Background:
Previous studies have suggested that a child's sex may be a predictor of vaccine reactions.

Objective: To examine the association between sex and health services utilization following standard pediatric immunizations, defined as emergency room (ER) visits or hospitalizations, during a pre-specified risk period after vaccination.

Methods:
We used a self-controlled case series design, an extension of retrospective cohort methodology which controls for fixed confounders using a conditional Poisson modeling approach. We compared a risk period immediately following vaccination to a control period farther removed from vaccination in each child, and estimated the relative incidence of emergency room visits and/or hospital admissions following the 2-, 4-, 6-, and 12-month vaccinations to investigate the effect of sex on relative incidence. All infants born in Ontario, Canada between April 1, 2002 and March 31, 2009 were eligible for study inclusion.

Results:
In analyses combining immunizations at 2, 4 and 6 months and examining these vaccinations separately, there was no significant relationship between the relative incidence of an event and sex of the child. At 12 months, we observed a significant effect of sex, with female sex being associated with a significantly higher relative incidence of events (P=0.0027). The relative incidence ratio (95% CI) comparing females to males following the 12-month vaccination was 1.08 (1.03 to 1.14), which translates to 192 additional events in females as compared to males for every 100,000 infants vaccinated.

Conclusions:
As the MMR vaccine is given at 12 months of age in Ontario, our findings suggest that girls may have an increased reactogenicity to the MMR vaccine which may be indicative of general sex differences in the responses to the measles virus.

48 **Maternal and perinatal outcomes in cesarean section on maternal request in a tertiary hospital: a retrospective cohort study in China**

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Background: The substantial increase in cesarean section rates in China were largely driven by maternal request. However, limited information is available on the associated risks of cesarean section on maternal request.

Objective: To assess maternal and perinatal outcomes in cesarean section (CS) on maternal request.
Methods A retrospective cohort study was performed in a teaching hospital in China. Healthy women underwent a CS on maternal request or a spontaneous vaginal delivery were included to compare maternal and perinatal outcomes. Multiple linear regression and multiple logistic regression analyses were used to adjust for potential confounding.

Results: Of the 8723 women delivered in Nan Fang Hospital during the study period, 3913 (44.9%) were delivered by CS. Of the CS cases, 1126 (29%) were performed on maternal request, and 487 of the 1126 CS on maternal request had no any detectable pregnancy complication or medical disease. Compared with spontaneous vaginal deliveries for healthy women with the same exclusion criteria as CS, women who delivered by cesarean section had increased risk of puerperal febrile morbidity (relative risk (RR): 2.46, 95% confidence interval (CI): 1.08-5.60), prolonged hospital stay (5.1 vs 3.1 d) and increased hospital cost (7376 vs 3454 RMB). On the other hand, CS was associated with decreased risks of meconium stained fluid (RR: 0.53, 95% CI: 0.40-0.69) and neonatal jaundice (RR: 0.48, 95% CI: 0.30-0.75).

Conclusions: CS on maternal request for healthy women is associated with increased risk of pueperal febrile morbidity, prolonged hospital stay, and higher hospital costs.

49 **Partial Versus Complete Fundoplication for the Control of Gastroesophageal Reflux Disease in Children: A Systematic Review and Meta-Analysis**

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Background: Gastroesophageal reflux disease (GERD) is a common problem for infants and children. Symptoms range from pain and discomfort, to life-threatening aspiration events. For some patients symptoms resolve spontaneously, or with use of medication. Many require fundoplication, a procedure in which the stomach is wrapped around the lower esophagus to buttress the anatomic valve at the gastro-esophageal junction, correcting GERD. While laparoscopic approach to fundoplication is standard of care, there is debate as to what extent of "wrap" is required: a complete 360 degree wrap (Nissen) or a partial wrap of less than 360 degrees (e.g. Thal or Toupet).

Objective: Conduct systematic review and meta-analysis to determine if a complete fundoplication gives better control of symptoms than partial fundoplication.

Methods: A systematic search of MEDLINE and Scopus databases was conducted to identify studies of fundoplication in children. Titles and abstracts were reviewed to identify studies comparing partial and complete fundoplication. Full text articles were abstracted by independent reviewers on standardized forms. Risk of bias assessed with a modified Cochrane Risk of Bias tool. Data was analyzed using RevMan 5 software, odds ratios for surgical success were generated using a random effects model, and meta-analysis performed for RCTs.

Results: 2289 abstracts identified using search, screening identified 2 RCTs and 12 retrospective cohort studies that met eligibility. Definition of surgical treatment success varied between studies. Two RCTs measured success using clinical scores and symptom relief. A meta-analysis of the two studies showed no significant difference between partial and complete fundoplication in recurrence of GERD, with an OR of 1.33 [0.67,2.66]. Of the 12 cohort studies, 3 (25%) used an objective technique for determining success (pH study, UGIS, or manometry) along with symptoms. Of the 3 studies, 1 showed greater GERD recurrence with partial fundoplication, however an unadjusted OR was not reported. The remaining 9 cohort studies (75%) used subjectively reported outcomes. 2 of these studies found greater improvement with partial fundoplication and 1 with complete, although unadjusted OR were not provided by the authors. Across all 14 studies, 31 types of complications were reported. Most commonly reported were dysphagia, gas-bloat syndrome, and death. Overall study quality was poor.

Conclusions: 2 RCTs and 12 observational trials have been published comparing partial and complete fundoplication. The methodical and analytical quality and reporting was poor. As such, there is no definitive evidence to suggest one technique is more effective at controlling reflux.

50 **The Impact of Influenza Vaccination and Influenza illness on an individual's risk of acquiring Guillain-Barré Syndrome: An evidence-based simulation study**

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Background: Influenza immunization is an important intervention for the reduction of morbidity, mortality and costs to the healthcare system from seasonal influenza. There is accumulating evidence to suggest that both influenza illness and influenza vaccination may increase the risk for Guillain-Barré Syndrome (GBS), a serious autoimmune disorder.

Objective: To assess the impact of receiving the seasonal influenza vaccine on an individual's age- and sex-specific absolute risk of developing GBS.

Methods: Using a probabilistic decision tree modelling approach, we constructed simulation models to quantify the risk of GBS in individuals who either do or do not receive seasonal influenza vaccination. Using published evidence, we modelled the impact of age, sex, influenza incidence and vaccine effectiveness, as well as the attributable risk of GBS with influenza vaccination and influenza illness. We used point estimates and standard errors to conduct 1000,000 probabilistic simulations for each scenario considered. Using this approach we were able to calculate absolute risk difference with respect to GBS for a vaccinated versus unvaccinated individual based on a single decision point to receive or not receive influenza vaccination. We also calculated 95% credible intervals for each estimated risk difference by calculating the 2.5th and 97.5th percentile points of the 1000,000 simulations conducted for each scenario.

Results: In the base-case analysis, for a 45-year old female base case and a 75-year old male base case respectively, excess GBS risk for influenza vaccination versus no vaccination was calculated to be -0.36 (95% Credible Interval [CrI], -1.22-0.28) and –0.42 (95%

CrI,–3.68-2.44) per million vaccinations, representing a small absolute reduction in GBS risk. Based on multiple sensitivity analyses, we determined that under most typical conditions, vaccination was protective against GBS. The only exceptions noted were for the 75-year old male base case when influenza incidence approached a low of 2% and when vaccine effectiveness was only 20%.

Conclusions: Influenza vaccination reduces individual risk of GBS except under conditions of low influenza incidence and/or low vaccine effectiveness. Even where the absolute risk of GBS may be raised by influenza vaccination, the excess risk is small (in most cases, less than the generally quoted estimate of one in a million). These findings should strengthen confidence in the safety of influenza immunization, and allow health professionals to better put the risk of GBS in context when communicating risks and benefits to potential vaccinees.

51 The Efficacy of Phone Follow-up in Reducing Adverse Events in the Emergency Department: A Pilot Project

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Background: Patients who are triaged as high acuity and discharged from the Emergency Department (ED) are potentially vulnerable to adverse events (AEs), unintended injuries that result from health care. Currently, there is no systematic way to identify these patients.

Objective: To determine whether computerized telephone follow-up of high acuity patients discharged from the ED reduces the occurrence of AEs.

Methods: Before and after study at the Ottawa Hospital Civic Campus from 2011-2012. Phase one: we retrospectively assessed for the occurrence of AEs among 500 consecutively discharged high acuity ED patients. Phase two: a telephone follow-up using an interactive voice response system (IVRS) for 504 discharged high acuity patients was implemented. Patients were contacted by IVRS within 48 hours of ED discharge, and were asked if they would like to speak with a nurse regarding health concerns. Health records for all study participants were searched for flagged outcomes: death, admission to hospital, return ED visit, and visit to a healthcare provider within 14 days. Case summaries of flagged outcomes were independently reviewed by 3 trained emergency physicians. AE determinations were made using a structured piloted process. Descriptive statistics were used to analyze the results.

Results: We enrolled 500 patients in the pre-phase and 495 in the post-phase (9 were excluded due to unavailable or restricted phone number). Of the 495 eligible post-phase patients, 393 were successfully contacted by IVRS with 89 patients requesting contact with a nurse. We identified 159 (31.8%) and 153 (30.9%) flagged outcomes in the pre- and post-phase cohorts respectively. For patients with flagged outcomes in the pre-phase cohort the average age was 56 (+/- 21) years, 48% were female, and 96.8% had a CTAS score of 3 or less. These characteristics were almost identical in the post-phase cohort. In the pre-phase cohort, adverse events were identified in 31 (19.5%) cases. In the post-phase group, 21 cases (13.7%) with adverse events were identified. Overall adverse event severity (n=52) included: death (n=1, 1.9%), hospital admission (n=25, 48.1%), return ED visit (n=23, 44.2%), and clinic visits (n=2, 3.8%).

Conclusions: This study highlights the feasibility of an IVRS to screen for potential adverse events in high acuity patients who were discharged from the ED. IVRS may be a useful tool for post-discharge follow-up for patients ultimately enhancing patient safety across Canada.

52 Quality of Life at the End of Life in Men Diagnosed with Prostate Cancer: Results from the CaPSURE Database

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Introduction and Objectives:
The purpose of this study was to evaluate the impact of local therapies on health related quality of life in men dying with a previous diagnosis of prostate cancer.

Methods:
We studied patients from CaPSURE, a large longitudinal observational cohort of men that have biopsy proven prostate cancer.

Patients were included in the analysis if they were diagnosed with non-metastatic prostate cancer, completed a minimum of 2 quality of life questionnaires after diagnosis, and subsequently died of any cause. Quality of life was measured using the RAND 36-Item Health Survey and the UCLA -- Prostate Cancer Index. The primary outcome of this study was the association between prior local therapy and quality of life. Men were stratified based on baseline characteristics, primary treatments, and cause of death (prostate cancer vs. other).

Results:
2296 men in CaPSURE were diagnosed with non-metastatic disease and have subsequently died. Of these, 1076 (47%) completed a minimum of two quality of life questionnaires. The mean age of the study population was 71.6 years (SD 8). Local treatment was used in 662 (62%) patients with 292 (27%), 320 (30%), and 50 (5%) undergoing prostatectomy, radiotherapy, or cryotherapy, respectively. Mean follow-up from the time of local therapy was 71 months (SD 37). Prostate cancer was identified as the cause of death in 150 (16%) individuals. In univariate analysis, prior local therapy significantly improved several quality of life outcomes including; physical function, vitality, and general health ($p<0.05$). Local therapy did not have a statistically significant impact on pre-death urinary function, sexual function, or pain outcomes.

Conclusions:
Local therapy for prostate cancer provides benefit in some, but not all, quality of life domains at the end of life. Further elucidating how quality of life is impacted by local therapy will provide important prognostic information to patients.

53 **Time is of the essence: How do Internal Medicine residents devote their time?**

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Background: Since the death of 18 year-old Libby Zion in 1984 resident duty hours have been under scrutiny as a cause for unsafe patient care. There is a need to study the current system of resident workflow and education activities so that teaching hospitals can determine how to effectively optimize the distribution of resident duty hours.

Objectives: To determine how Internal Medicine residents allocate their time while on duty. This study tests the feasibility of using a new system of workflow analysis to evaluate the impact of changing work patterns including new restrictions to resident duty hours with regard to efficiency, physician patient interactions and education. The identification of patient safety and quality of care concerns will be assessed in light of shorter “shifts”, and more frequent handovers. Analysis of results will assist in identifying methods to enhance the learning environment while simultaneously reducing the risk of adverse events that may be caused by extraneous duty hours.

Methods: Twenty consenting Internal Medicine residents from two tertiary care sites were shadowed on the Clinical Teaching Units during six-hour blocks of time during the day, overnight call, and weekend shifts. All resident activities were tracked using an Apple iPad application that recorded the type and duration of each task. Tasks were categorized as Direct Patient Care, Indirect Patient Care, Transit, Education, Communication, Documentation, Administration, Non-physician tasks, or Personal. Descriptive analyses were conducted using SAS (version 9.3).

Results: From June 19th to August 20th 2013, 61 six -hour blocks were included for study A total of 17,709 tasks were recorded, resulting in 521 hours of observation. The majority of resident tasks were in Direct Patient Care (27.50%), followed by Communication (22.65%), Personal tasks (14.57%), Documentation (13.24), Education (11.52%), Transit (5.80%), Indirect care (3.78%), Administration (0.58%), and Non-physician tasks (0.35%).

Conclusions: This study demonstrates the feasibility of a time motion software application to facilitate workflow analysis, as well as to determine the impact of new restrictions on resident duty hours. This methodology can be used to accurately determine the service to education ratio of a specific resident shift or rotation. As well our study demonstrated the additional value of observer insights about serious or recurrent issues around resident confidence or patient safety. This work will inform the development of new workflows and education to optimize resident knowledge and patient physician relationships, while simultaneously reducing the risk of adverse events that may be caused by extraneous duty hours.

54 **Design and pilot of a theory-based knowledge translation intervention to improve physician hand hygiene practice**

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Background:
The prevalence of health care-associated infections (HAIs) in Canada is high compared to other high-income countries throughout the world, with these infections being the most serious complication of hospitalization and the fourth leading cause of death. According to the World Health Organization, proper hand hygiene is the most effective method for preventing HAIs. However, hand hygiene compliance among healthcare workers, more specifically physicians, is consistently suboptimal.

Objective:
This study aims to identify the barriers and enablers to physicians hand hygiene compliance and to use this knowledge to develop and pilot a theory-based knowledge translation (KT) intervention to increase compliance.

Methods:
This study consists of three phases. In Phase 1, we identified the barriers and enablers to physician hand hygiene compliance using key informant interviews with physicians and residents in Medicine and Surgery, focus groups with hand hygiene experts, and non-participant observation of hand hygiene audits. The interviews were conducted using a semi-structured interview guide informed by the Theoretical Domains Framework (TDF); a behavior change framework designed to simplify psychological theories related to behavior change. Intervention mapping occurred in Phase 2, to develop a theory-based KT intervention. In Phase 3 (in progress) we are piloting our hand hygiene KT intervention in medicine and surgery at The Ottawa Hospital. We are conducting pre and post hand hygiene audits and a process evaluation to assess feasibility of the intervention.

Results:
After completing 42 key informant interviews, 2 focus groups, and non-participant observation, 9 of 14 TDF domains were found to be important to physician hand hygiene. Our pilot intervention targets 5 of these domains: environmental context and resources; knowledge; memory, attention, and decision processes; skills; and social influences. The intervention is being delivered using rapid education sessions presented at resident orientation, team rounds, academic half days, and division meetings depending on the service targeted (i.e. medicine and surgery).

Conclusions:
A better understanding of the barriers and enablers to physician hand hygiene has allowed us to develop a theory-based KT intervention to address physician hand hygiene compliance. Increasing hand hygiene compliance in physicians will lead to better care for patients and a safer hospital environment for healthcare workers and patients, and their families. Upon completion of this study, we will refine our piloted intervention so it can be tested in a multi-site cluster randomized controlled trial.

55 **“Dr. Cochrane”: An Innovative Approach to Continuing Medical Education using Cochrane Reviews**

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Background:
The Italian Cochrane Centre and the Canadian Cochrane Centre are usingCochrane Reviews addressing gastrointestinal and musculoskeletal conditions to develop a comprehensive suite of online continuing educational and professional development (CEPD) modules targeting Canadian family physicians and other healthcare professionals.

Objectives:

- To promote evidence-based management of common gastrointestinal and musculoskeletal conditions.
- To strengthen the availability of high quality information resources for Canadian family physicians and other healthcare professionals (especially those in remote/rural settings who have difficulties accessing traditional continuing professional

development activities).

- To build Canadian capacity to take a global leadership position in the further development of Cochrane educational activities.

Methods:
The modules will include questions and answers corresponding to a fictional vignette featuring “Dr. Cochrane” and based on published Cochrane Reviews. Vignette topics are chosen by the Cochrane Review Groups, family physicians, and specialists according to quality, relevance, and potential impact. The modules will be produced by The Cochrane Collaboration, Wiley-Blackwell Publishing and the University of Ottawa CME Office.

Results:
The Review Groups have identified module topics, and vignette writing is underway. The modules have been approved for credit with the Accreditation Council for Continuing Medical Education. We anticipate that the modules will be launched online in 2013.

56 Multiple Medical Conditions and Driving Outcomes: Preliminary results from the Candrive Prospective Older Driver Study

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Background: The elderly population in Canada is predicted to double in the next 25 years, estimated at being 9.9 – 10.9 million. As people age the number of concurrent medical conditions and subsequent functional impairments also increase. While there is much research done on individual diagnoses and there effects on driving, much less is known about the relationship between multiple medical conditions and driving.

Objective: This study aims to examine whether drivers with multiple medical conditions differ on driving outcomes such as risk of crash, traffic violations, driving practices and perceived ability to drive. It is hypothesized here that persons with more than 2 conditions with a moderate disability rating according to an expanded Cumulative Index Rating Scale will show a higher risk of collision, different driving practices and lower perceived ability to drive.

Methods:The CIHR study Candrive II is a 5 year longitudinal study of older drivers aged 70 and over. Data for 928 participants collected over the first three years were considered in this study. Utilizing an expanded version of Millers 14 item CIRS scale, participants self-reported their conditions. Trained research assistants applied Hudon’s 2005 CIRS scoring guidelines. Outcomes: Ministry reported collisions and violations were collected from respective transportation Ministries across Canada. Self report collisions were documented every 4 months. Driving practices and perceived ability to drive was examined using the Driver Behaviour Questionnaire, Driving Comfort, Situational Driving Avoidance, Situational Driving Frequency, Decisional Balance Plus, Driver Behaviour Questionnaire and Perceived Driving Ability.

Results: Pair-wise comparisons were performed on all measures considered. Among the driving safety outcomes, the two groups were found to be significantly different on self-reported crashes collected in year 3 of the study ($p<.01$). While collision outcomes were not different between the two groups many of the variables pertaining to modification of driving practices and decreased perceived ability to drive were significantly associated with multiple medical conditions.

Conclusion: Our findings show that risk of crash does not appear to be increased (Ministry of Transportation data) in older drivers with at least two medical conditions of moderate severity. The observed difference on self-reported crash needs to be considered with caution and its interpretation should await replication in year 4 data analyses. However, individuals with at least two medical conditions clearly indicate that their driving practices differ from those observed in the healthier group and that their self reported ability to drive has declined.

57 For Baccalaureate Nursing Education, Do Outcomes Vary Among Different Curricula or Models of Delivery? A Rapid Review

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Background. In Nova Scotia, an educational collaboration among the three provincial universities is desired, and a multi-agency provincial review committee requested a rapid review to inform the effectiveness of nursing education models.

Objective. To conduct a rapid review to address the question: for baccalaureate and master's entry-to-practice pre-licensure nursing education, do outcomes vary among different curricula or models of delivery?

Methods. This rapid review was guided by a protocol developed before the conduct of the review. MEDLINE, CINAHL, and The Cochrane Library (1990 to July 2013) databases were searched, along with several websites as sources of grey literature. English or French language articles addressing pre-licensure nursing education, including master's, standard and accelerated baccalaureate, and transfer programs for practical nurses were included. Excluded were post-RN baccalaureate, diploma, associate nursing, registered psychiatric nursing, practical nursing-only, advanced practice, post-graduate specialization, other graduate, and post-licensure continuing education programs. Of interest were studies evaluating whole nursing programs, nursing competencies, and/or educational models of delivery. Studies were limited to those conducted in pre-defined countries. Studies were systematically screened in duplicate at two levels. Ten percent of extracted articles were verified, and all risk of bias assessments of studies were verified. Due to sparse studies for the various intervention and control combinations, quantitative syntheses were not possible. Studies are mapped according to various characteristics.

Results. No relevant systematic reviews were identified, so 41 randomized and 9 non-randomized controlled trials were included. Thirty-seven studies were conducted in the United States. Sample sizes ranged 12-250. Ninety-two percent of studies were conducted in a baccalaureate program context. Only three studies included students from both generic and accelerated baccalaureate programs, while one study was a master's entry-to-practice program.

Studies were further characterized and visually mapped according to educational content or focus, intervention types, control groups, mode of educational delivery, and outcomes assessed. No studies addressing whole programs were found. More than half of the studies addressed clinical teaching and learning interventions, such as simulation and computer-based learning. For outcomes, half of the studies evaluated performance, knowledge in 16 studies, and another 10 studies were unclear as to whether performance or knowledge. Studies assessed outcomes that were at a medium or high risk of bias.

Conclusions. Insufficient evidence exists to determine the effectiveness of various educational models of delivery and/or competency-directed interventions. No studies addressing whole nursing education programs were located. The presentation will include suggestions for areas of future research.

58 The Cochrane Corner: Increasing the Impact and Relevance of Evidence-Based Information

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Background:
The Cochrane Collaboration produces systematic reviews that synthesize results from individual studies. One challenge faced by the Collaboration is ensuring that these reviews have an impact: that they improve health outcomes, change clinical practice, and influence policy. Tools and knowledge translation strategies have been developed to meet this challenge, such as Cochrane Corners, which bring systematic reviews to stakeholders and highlight relevant aspects of the reviews.

Objective:
This presentation aims to introduce policy-makers, practitioners, and producers to Cochrane Corners. We will outline strategies for those developing Cochrane Corners for their reviews or constituents.

Methods:
The first step in developing a Cochrane Corner is determining the purpose of the Corner: will the Corner simply list reviews, or will some critical appraisal or reflection be included? The next step is to select appropriate reviews, using pre-determined criteria. The

reviews should be reported, summarized or assessed as appropriate, and the Corner disseminated to relevant stakeholders.

Results:
Cochrane Corner examples will be provided and their use for policy/practice will be described. The CIHR Institute of Gender and Health's Cochrane Corner, developed with the Campbell and Cochrane Equity Methods Group, will be used as an example of an evidence-based initiative that aims to increase awareness of sex and gender issues and to improve health outcomes.

Conclusions:
We will describe challenges and opportunities encountered in preparing the IGH Cochrane Corner, and present suggestions for strengthening the process. We hope that a more rigorous approach with review author involvement will identify areas for methodological development.

59 A strategy to increase partnerships between health care professionals and Cochrane Canada: Online peer review modules for dietitians

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Background: Cochrane Canada continues to work to create partnerships with health care professional groups to increase the awareness and use of Cochrane Reviews. Recently, Cochrane Canada and the Dietitians of Canada saw the opportunity to involve dietitians more actively in the systematic review process, and in particular, to include the unique perspective of dietitians in the peer review/referee process.

Objectives: Develop online peer review modules for dietitians to increase their confidence, expertise and comfort in knowledge synthesis; use the experience of practicing dietitians to ensure Cochrane Reviews are relevant to dietitians' needs; involve dietitians in authoring nutrition-related Cochrane Reviews and integrating them into practice or guidelines.

Methods: Twenty-one stakeholders from 15 organizations across Canada – representing a variety of clinical, public health, educational, research and community backgrounds – joined the brainstorming, planning, development, revision and pilot testing of three online peer review training modules. Managing editors from 15 Cochrane Review Groups were invited to provide nutrition-related protocol and review examples for the modules. After pilot testing, a one-day stakeholder planning and dissemination meeting was held to review the pilot testing results, exchange ideas and plan module dissemination.

Results: Evaluations from dietitians completing the modules were positive and Cochrane managing editors welcomed the new peer reviewers. Six months following the launch of the modules on October 1, 2012, thirty dietitians have completed the modules with twelve deciding to become peer reviewers/referees. Dietitians' peer review areas of interest cover 14 Cochrane Review Groups and one field.

Conclusions: Although collaborating with multiple stakeholders requires time, the iterative development of the online modules harnessed the collective strengths and unique perspectives of both researchers and clinicians. In the future, the basic online module structure could be used and examples adapted to other healthcare professions to solidify additional partnerships between Cochrane researchers and other clinicians.

60 The Influence of Changes in Vision on Driving Outcomes: Preliminary results from the Candrive Prospective Older Driver Study

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Background: The number of elderly drivers in Canada is predicted to double in the next 25 years. Vision is central to safe driving and is known to decrease with age yet, the relationship between standard visual assessment measures used by health care professionals and licensing bureaus and driving outcomes still remains unclear. In this study, we examined the relationship

between changes in visual conditions and various driving outcomes in a longitudinal study of older drivers.

Objective: We predicted that a greater decline in vision would be associated with greater crash and violation rates, change in driving habits, as well as lower driving confidence and perceived driving ability.

Methods: The CIHR study in driving in older persons (Candrive II) is a longitudinal study following a cohort of 928 active drivers over the age of 70 for up to 5 years. Comprehensive annual assessments were completed in office, which included measures of visual function such as the Snellen Visual Acuity Chart, the Pelli-Robson Contrast Sensitivity chart and Visual Fields by Confrontation. Change in visual conditions were assessed using an expanded version of the self-reported Cumulative Illness Rating Scale (CIRS). It was used to identify participants with glaucoma, cataracts and macular degeneration, as well as the severity of the condition. A deterioration in visual conditions applied those who had a decrease of 2- s.d. on visual acuity and contrast sensitivity and those who reported a worsening of their condition or developed a visual condition based on the CIRS. Driving outcomes included Ministry of Transportation reported collisions and moving violations as well as self-report of involvement in crash. Participants also completed questionnaires that assessed driving practices and drivers' perception of their ability to drive (The Driving Behaviour Questionnaire, Driving Comfort Scales, Perceived Driving Ability scales and Situational Driving Avoidance).

Results: The groups who experienced change in their visual condition versus those who did not were compared using pair-wise comparisons on all driving outcomes. The analyses indicated that a change in visual condition did not lead to a significant difference in any of the driving outcomes examined in this study.

Conclusions: While hypothesised that individuals who had a change in vision would fare worse on driving outcomes, this was not found not to be the case. We conclude that a change in vision does not necessarily translate into greater risk of crash. Driver's capacity to adapt to their conditions is a likely interpretation of the findings.

61 **Updated Clinical Practice Guidelines for Persistent Mental Health Disorders following Concussion/Mild Traumatic Brain Injury**

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Background: Early post-concussive symptoms following mTBI can include irritability, anxiety, emotional lability, depressed mood, and apathy. Subsequently, a significant proportion of individuals may develop persistent mental health disorders, with major depression and anxiety disorders observed most often. Regardless of etiology, these disorders require prompt recognition and management, given their potential to impact on functional status or impede recovery.

Objective: To present revised guidelines for the assessment and management of persistent mental health disorders following concussion/mTBI in order to provide information and direction to physicians managing patients' recovery from mTBI.

Methods: The second edition of the Guidelines for Concussion/Mild Traumatic Brain Injury and Persistent Symptoms was published by the Ontario Neurotrauma Foundation in September 2013. An updated search for new clinical practice guidelines addressing mTBI and a systematic review of the literature evaluating treatment of persistent post-concussive symptoms (PPCS) were conducted. Health care professionals representing a range of disciplines from across Canada, the United States and abroad were brought together at an expert consensus conference to review the existing guidelines and new evidence to revise the original guideline. Evaluation of the guidelines by sport medicine and military physicians, as well as other external experts and stakeholders in the field, has also provided key feedback that informed revisions for the second edition.

Results: A modified Delphi process was used to create more than 90 recommendations that address the diagnosis and management of concussion/mTBI and persistent symptoms. Guideline recommendations specific to persistent mental health disorders address diagnosis/assessment, as well as pharmacological and non-pharmacological treatment. Resources, tools and treatment algorithms, which were formally evaluated by consensus members and external reviewers, are also included in the guideline to aid in the implementation of the recommendations.

Conclusion: The current guideline update aims to provide an up-to-date framework for health care professionals who encounter patients with mTBI with the intent of either preventing persistent symptoms or minimizing the effects of PPCS, including mental health difficulties. Further research is required to improve the evidence for treatment of persistent mental health disorders following concussion/mTBI.

62 **Determining the validity of the AMA guidelines: A prospective analysis of the ADReS and rate of crash involvement in older drivers**

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Background: As people age, a number of medical conditions develop, impairing driving skills. The Canadian Driving Research Initiative for Vehicular safety in the Elderly (Candrive/OzCandrive) is a 5-year study funded by the Canadian Institutes of Health Research. The aim of this study is to develop an in-office screening tool that will allow clinicians to identify potentially at-risk older drivers. The American Medical Association (AMA), in collaboration with the National Highway Traffic Safety Association (NHTSA) developed the Assessment of Driving Related Skills (ADReS) guide. The ADReS was developed from expert opinion to facilitate physician's assessments in determining fitness to drive of their older patient's, by providing tests that examine domains, which dictate driving ability. ADReS is a test battery conducted to measure vision, cognitive and motor/somatosensory function pertinent to movements encountered when driving.

Objective: We hypothesize that participants who obtain lower scores on the ADReS components will be significantly more likely to be involved in a motor vehicle collision where the participant is deemed at-fault.

Methods: Upon entry into Candrive/Ozcandrive, 928 participants aged 70 and older from 7 Canadian cities and 257 participants aged 75 and older from Melbourne Australia, completed an in-office assessment, which included the tests outlined in the ADReS. Motor vehicle collision (MVC) data for all Candrive/Ozcandrive participants is annually obtained from the provincial licensing boards. Participants involved in at- fault (at least 50% responsible) MVCs are compared against participants who have not been involved in a collision or were involved in a "not-at fault" collision. Statistical analyses were conducted on in-office assessment data obtained in the first 2 years of Candrive/Ozcandrive. Data for the current study was analyzed using SPSS version 20.0; Pearson's chi-squared test or Fisher's exact test were used to compare categorical variables and an independent t-test was used to compare continuous variables.

Results: The analyses combining collisions of year 1 and 2, accounted for 34 participants deemed at-fault. Analyses of the ADReS scores for vision, cognition, and motor/somatosensory tests did not differ significantly between "at-fault" status and "not at-fault" status of collisions in both year 1 and 2.
Conclusion: By identifying at-fault MVCs over two years following in-clinic assessments for the Candrive/Ozcandrive sample of older adults, our findings further define the lack of association between the ADReS and collision risk in older drivers. Current findings affirm the need for a valid predictive tool that can be easily integrated in clinical practice.

Neuroscience Program

63 **DIP2, a putative fatty acyl-AMP ligase, regulates neuronal migration, axon guidance and neuronal morphology**

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A properly functioning nervous system involves the coordinated action of genetic pathways that regulate neuronal migration, axon wiring and the maintenance of neuronal morphology. We have identified mutations in a C. elegans gene that lead to defects in neuronal migration and neurite outgrowth and branching. A whole genome sequencing approach revealed that these mutations disrupt the worm orthologue of Disco-interacting protein-2 (DIP2). DIP2 are a highly conserved but poorly understood protein family containing an N-terminal DNA methyltransferase 1 (DMAP1) binding domain followed by two domains that are predicted to acts as fatty acyl-AMP ligases (FAAL). FAALs modify fatty acyl groups in combination with polyketide synthases to generate new lipid moieties. We will present our molecular and phenotypic characterization of the dip2 gene in C. elegans.

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

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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. However, new evidence has recently surfaced regarding Parkin's protective effects against oxidative stress and mitochondrial dysfunction. The field has long speculated on a non-specific anti-oxidant effect by Parkin in cells. LaVoie et al (2005) have previously shown that Parkin can directly interact with reactive radical species (such as those generated during dopamine metabolism) leading to irreversible oxidation of some of its many thiol groups as a cysteine-rich protein. Furthermore, LaVoie et al (2007) have also shown reversible oxidation of cellular Parkin under ROS generating conditions, including a rise in H2O2. Through the Amplex® Red Hydrogen Peroxide/Peroxidase Assay, we recently investigated hydrogen peroxide activity in wild type versus parkin knock-out mice. There, we observed no significant differences in purified mitochondria of the brains between wild type and knock-out animals, which we attributed to the presence of compensatory mechanisms, namely superoxide dismutase and/or catalase activity. Here, we present the generation of a parkin/MnSOD double mutant mouse model for recessive PD. Manganese superoxide dismutase located within mitochondria is the first line of defense against the generation of superoxide, and in turn hydrogen peroxide; the MnSOD gene has also been reported as a susceptibility factor for PD. Our mice are deficient in murine Parkin and haploinsufficient for MnSOD. We will examine hydrogen peroxide activity in the double mutant mice, while comparing it to Parkin knock-out animals, hoping to observe higher levels in the parkin/MnSOD double mutant mice, resulting in primary mitochondrial dysfunction. The molecular analysis of parkin/MnSOD double mutant mice will help us elucidate the cascade of events from the generation of ROS, calcium dysregulation, to mitochondrial dysfunction, and hopefully, in metabolic stress of dopamine neurons. The generation of this mouse model is the first step to creating a true model for recessive PD: a tetragenic mutant mouse (parkin-/-, DJ-1-/-, PINK1-/- and MnSOD+/-).

65 **Testing Motor and Spatial Memory Repeatedly Throughout the Lifespan of Laboratory Mouse**

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Introduction: Preclinical research in the field of neurodegeneration requires the use of a variety of behavioral tests to measure functional deficits in both motor and cognitive function during disease progression. This often necessities that the same cohort of mice is repeatedly tested on the same behavioral measures throughout their lifespan. Using this approach it is often interpreted that differences in behavior on repeat testing are due to an aging effect, however we question whether the confounding variable of repeat testing affects the behavioral outcome. This project tested how performance on the rotarod test and Morris Water Maze (MWM) changed during aging in the presence or absence of previous testing experience.

Method: Mice (C57/BL6C3 background) were tested at 4 times during their lifespan when they were young adults (8 weeks), mature adults (5 months) and aged adults (1 and 1.5 years old). The behavioral outcomes of the mice at the 1.5 year time point were compared to outcomes obtained from a group of "naïve mice" that were age and strain matched and that had no previous

behavioral testing experience.

Conclusion: Our results suggest that both aging and repeated testing can alter behavioral outcomes in rotarod and MWM. We hypothesize that the repeated testing on the rotarod reduced their performance due to an aging and floor effect since there was no significant difference in the naïve versus experienced mice with all mice performing relatively poorly. When the rotarod task was made easier, the performance of the experienced mice was better suggesting that experience may improve performance. We also found that in the MWM test there was improved performance with aging. This could be due to a learning/memory effect that allows the mice to perform better in subsequent testing trials throughout their lifespan, or could be due to the exercise of testing itself promoting motor function and cognition throughout the lifespan. Since all behavioral tests are known to be sensitive to mouse strain, age, and facility, we hope that these data will also provide a useful reference to normalize data for these behavior tests at our behavioral core facility in University of Ottawa, as well as for investigators that study neurodegenerative disorders, (i.e., Parkinson's disease, dementia).

66 **Effect of UV Sterilized PCL Biomaterial on the Proliferation and Differentiation of In Vitro Neurospheres**

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Introduction: Spinal cord injury (SCI) is the neurological damage to the spinal cord that results in reduced or loss of sensation and mobility. An innovative way of treating SCI is to promote and guide the regeneration of spinal tracts by means of a biomaterial scaffold. This biomaterial scaffold will isolate the injury site from the local environment and supply essential factors for axonal regeneration (1, 2). One promising biomaterial to form the scaffold is polycaprolactone (PCL). For translation to clinical use it is important to understand how the sterilized PCL biomaterial affects in vitro neurospheres that represent the cell population of the Central Nervous System (CNS). This project specifically focuses on analysing the direct effects of surface sterilized PCL biomaterial using UV light, on the proliferation and differentiation of in vitro neurospheres.

Hypothesis: The biodegradation of UV sterilized PCL biomaterial has no effect on the proliferation and differentiation of in vitro neurospheres.

Methodology: In the experiment, progenitor cells collected from the sub-ventricular zone (SVZ) of the brain and sub-ependymal zone (SEZ) of the spine of young adult female Sprague Dawley rats were proliferated for 7 days in normal culture media to from neurospheres. Similar sized brain and spine neurospheres were then subjected to two different conditions. The neurospheres in the biomaterial condition were proliferated in culture media that was pre-exposed to PCL biomaterial that was UV sterilized for 12.5 minutes, while neurospheres in the control condition were proliferated in culture media absent of biomaterial. Proliferation was monitored every 48 hours for a total time of 7, 14 or 21 days. Diameter of each neurosphere was measured in µm, where higher diameter showed the presence of higher number of cells and thus indicated a higher rate of proliferation. Neurospheres were then allowed to differentiate for 7 days and were assessed through immunostaining, where GFAP positive cells were identified as astrocytes and O4 positive cells were identified as oligodendrocytes. Significance of proliferation and differentiation data was assessed through Kruskal-Wallis test and ANOVA respectively.

Results: It was found that there is no statistically significant difference in proliferation between control and biomaterial conditions for both the spine and the brain neurospheres. In terms of differentiated cells, there was no statistically significant difference in the amount of astrocytes present in the control and biomaterial conditions for both the spine and the brain neurospheres proliferated for 7 days. The same was observed in oligodendrocytes.

Conclusion: UV sterilized PCL biomaterial does not significantly alter the proliferation and differentiation of spine and brain neurospheres in vitro. Therefore UV sterilized PCL biomaterial may be a potential candidate to form the scaffold for in vivo testing and perhaps for future clinical usage to treat SCI.

67 **LMO4 is critical for paraventricular hypothalamic neuronal activity to prevent hyperphagia**

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Background: The dramatic increase in the prevalence of obesity reflects a lack of progress in combating one of the most serious health problems of this century. Recent studies have improved our understanding of the appetitive network by focusing on the

paraventricular hypothalamus (PVH), a key region responsible for the homeostatic balance of food intake. Our previous studies revealed that mice with glutamatergic neuron-specific ablation of LIM domain only 4 (Lmo4), including all neurons of the PVH, the majority of neurons in the ventromedial hypothalamus (VMH) and some neurons in the dorsomedial hypothalamus (DMH), displayed metabolic defects including diabetes and obesity

Objective: To determine what LMO4 does specifically in the PVH to affect metabolic homeostasis, we ablated Lmo4 selectively in the PVH through Cre recombinase-dependent excision driven by the single-minded 1 (Sim1) promoter

Methods: Genetic Tools, Electrophysiology, Food Intake Studies in AAV-DREADD Injected Mice, Immunohistochemistry, Real-Time Quantitative RT-PCR, Glucose and Insulin Tolerance Test, Paired-feeding, Indirect Calorimetry, PTP1B Phosphatase Activity Assay
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Results: Here we show that mice with PVH-specific ablation of LIM domain only 4 (Lmo4) become rapidly obese when fed regular chow due to hyperphagia rather than to reduced energy expenditure. Brain slice recording of LMO4-deficient PVH neurons showed reduced basal cellular excitability together with reduced voltage-activated Ca2+ currents. Real-time PCR quantification revealed that LMO4 regulates the expression of Ca2+ channels (Cacna1h, Cacna1e) that underlie neuronal excitability. By increasing neuronal activity using designer receptors exclusively activated by designer drugs (DREADD) technology, we could suppress food intake of PVH-specific LMO4-deficient mice. Taken together, these results demonstrate that reduced neural activity in LMO4-deficient PVH neurons accounts for hyperphagia.

Conclusions: In this study we have identified an essential role for LMO4 in maintaining neuronal activity of the PVH to control feeding behavior. We have shown that the lack of LMO4 in PVH neurons impairs normal activity and the normal expression of voltage-activated Ca2+ channels required for neuronal activity. Consequently, reduced activity of the PVH profoundly increases body weight due to hyperphagia.

Regenerative Medicine Program

68 **Engineering miRNA-enhanced endothelial progenitor cells for therapeutic application in cardiovascular disease.**

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Background: Endothelial progenitor cells (or circulating endothelial cells, CACs) are adult stem cells (emerging from peripheral blood mononuclear cells, PBMCs) that are used in autologous cell therapy, possessing proangiogenic activity but lacking vasculogenic activity [1]. After three weeks in culture, late outgrowth EPCs (or blood outgrowth endothelial cells, BOECs) emerge from CAC populations, possessing vasculogenic activity, displaying endothelial (as opposed to haematopoietic) surface antigens, eNOS, clonal proliferation[1], and the ability to integrate into damaged regions of venous and arterial vessels[2]. A research focus is to generate BOECs in significantly less time using a technique that will not alter the DNA of isolated cells. MicroRNAs (or miRNAs) are post-transcriptional regulators of gene expression; A single miRNA can regulate an entire gene network[3]. Alterations in miRNA expression can affect CAC function, and the differentiation of BOECs[4]. MiRNA levels may be important in the regulation of CAC function, and control CAC differentiation[4]. We previously showed that there is progressive change in miRNA expression profiles as PBMCs are cultured into early and BOECs, with miRNAs correlating strongly with the angiogenic and endothelial cell nature of EPCs[5]. It has yet to be established whether manipulating miRNA expression within EPC populations isolated from peripheral blood can accelerate BOEC emergence from CACs.

Hypothesis: Expression of specific miRNAs contributes to the angiogenic potential of CACs and the emergence of BOECs when PBMCs are cultured in endothelial-differentiation medium.

Methods: A genome-wide miRNA array was performed on CACs and respective BOECs generated from adult peripheral blood collected of healthy participants (3 male, 3 female) using the Affymetrix Genechip® miRNA3.0 array, accounting for all short non-coding RNAs. In concurrence, a complete GeneChip® Human Genome U133 Plus 2.0 hybridization microarray was also performed from the same respective samples for all coding RNAs. A bioinformatic correlative pathway profiling pipeline will be used for probing variations in signalling pathways innervated between the two EPC cell types. A systematic approach will test each candidate miRNA knock-in/down for the following; Functional characterization: Gain or loss of motility in chemotactic migration ability will be assessed using a modified Boyden chamber assay to VEGF and SDF-1 in transfected CACs compared to BOECs and mock transfection. Angiogenic capillary-like network formation using a matrigel tube formation assay will be performed. Proliferation and apoptosis: Based candidate miRNAs from the miRNA array, and identified targets from our microarray data we postulate that proteins regulating the Ras/ERK/VEGF and/or PI3K/Akt/eNOS pathways are modulated upstream, affecting proliferation and apoptosis[4, 6]. Negative regulators of the VEGFR2-eNOS pathway (such as SPRED-1) have been implicated in additional pathways affecting proliferation and apopotosis (negative regulator of ERK/MAPK)[7]. An EdU cell proliferation assay (Life Technologies®) will be used for detection and quantification of synthesized DNA in miRNA transfected cells. Variations in Caspase-3 activity will be used to evaluate apoptosis following transfection with candidate miRNAs followed by a luciferase reporter assay to detect target effect of selected miRNAs. Nitric Oxide Synthesis: Western blot analysis of lysates from post-transfected EPCs will be probed for the presence of the eNOS; as well, nitric oxide production will be quantified using a DAF-FM assay to compare between miRNA transfected non-treated cell types.

69 **Regulation of Carm1 in myogenic commitment of satellite stem cells**

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The paired-box transcription factor Pax7 is required for direct transcriptional activation of the myogenic regulatory factor Myf5 and myogenic commitment of muscle satellite stem cells. Moreover, recent findings from our lab indicate Carm1, an arginine methyltransferase, as a direct regulator of Pax7. Carm1 methylates Pax7 in vivo, and de novo activation of Myf5 expression following asymmetric satellite stem cell division requires Carm1. As a key regulator of Pax7, Carm1 plays an essential role in regulating asymmetric cell division and myogenic commitment of satellite cells. Thus, the activation of Carm1 represents a key regulated step during asymmetric satellite stem cell division. In an effort to elucidate the molecular mechanisms that regulate Carm1 activity in satellite stem cells, a candidate kinase approach was employed to identify Carm1 regulatory kinases. We found that Carm1 is a substrate for p38-gamma MAP kinase. p38-gamma is highly expressed in skeletal muscle and has been shown to phosphorylate MyoD to prevent premature myogenin expression in satellite cells. The interaction between Carm1 and p38-gamma proteins was confirmed by co-immunoprecipitation analysis. Moreover, proximity ligation assays performed on cultured myofibers revealed that Carm1 and p38-gamma interactions occur at the cell membrane in activated satellite cells. p38-gamma is therefore a candidate kinase for the regulation of Carm1 in asymmetric satellite stem cell division, and its role in satellite cell function is

actively being pursued.

70 **Vascular progenitor cells for optimal revascularization of acellular lung scaffolds.**

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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 endothelial progenitor cells (EPCs) as a critical first step in engineering truly functional grafts.

METHODS: Acellular lung scaffolds were generated by isolated lung perfusions using decellularization buffer (8mM CHAPS, 1M NaCl, 25mM EDTA in 1X PBS). Decellularization of lungs was evaluated by tissue histology, total DNA measurement and immuno-blotting. For revascularization, EPCs were generated from rat bone marrow derived mononuclear cells. EPCs cultured from rat bone marrow were characterized for EPC markers including acetylated low-density lipoprotein (Ac-LDL) uptake, lectin binding and expression of CD31 and CD45.

Results: Perfusion decellularization produced acellular lung scaffolds with maintained extracellular structure. Perfusion decellularization with CHAPS buffer consistently demonstrated complete absence of cells, below detectable DNA remnants and intracellular protein (GAPDH), with more optimal vascular perfusion. EPCs were successfully derived from rat bone-marrow mononuclear cells. On Day-7, rat-EPCs demonstrated Ac-LDL uptake and lectin binding as well as these cells expressed CD31. Further, these cells were positive for CD45. On the other side, Day-16 EPCs demonstrated higher Ac-LDL uptake, lectin binding and CD31 expression. Moreover, these cells did not express CD45.

Conclusions: We successfully prepared acellular lung scaffolds and identified distinct cell populations, early EPCs (Day-7) and late outgrowth EPCs (Day-16), which can be utilized to generate truly functional grafts.

71 **Molecular rescue of satellite stem cell deficiency in Duchenne Muscular Dystrophy**

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Background

The dystrophin-glycoprotein complex (DGC) is a network of proteins linking intracellular scaffolding to the extracellular matrix and provides structural support to stabilize the plasma membrane of skeletal muscle fibers. This mechanism is not only required to maintain the fiber membrane integrity, but also transduces signals from the extracellular matrix. Deletions or mutations in dystrophin (Dmd) or genes coding for other proteins in the DGC lead to various forms of muscular dystrophy. Studies analyzing the function of the DGC have been focused in the context of muscle fibers. However, its signaling roles in muscle stem cells remain unknown. Although therapeutic rescue of fiber necrosis has been a major focus for treatment of muscular dystrophy, the possibilities surrounding the modulation of stem cells have only recently begun to be explored. Expressions of DGC components dystrophin and dystroglycan are clearly detectable in satellite cells by mRNA-sequencing and immunostaining. The prevailing dogma is that the deficiency of these genes occur in differentiated myofibers. Our laboratory has identified a deficit in the self-renewal of satellite stem cells lacking key components of the dystro-glycan complex (DGC) (unpublished data). Disruption of the dystroglycan (DAG1) gene specifically in satellite cells results in the loss of asymmetric satellite stem cell self-renewal, and leads to a delayed regeneration after injury. The loss of asymmetric divisions can be a tractable marker that can be used to identify therapeutic compounds that will rescue the satellite stem cell function in a mouse model of Duchenne muscular dystrophy (mdx).

Objective

The objective of this study is to screen for a therapeutic compound that will, by attenuating symmetric cell division, expand asymmetric cell division to increase the regenerative capacity within skeletal muscle cell in dystrophic mdx mice.

Methods

We will develop a screening protocol to identify therapeutic compounds that will rescue the observed phenotype in satellite stem cells lacking dystrophin from mdx mice. Positive targets will then be analyzed with in-vitro and in-vivo regeneration studies. To perform this screen in a high-throughput fashion, we will prospectively isolate single FDB fibers of mdx mice and assay for asymmetric stem cell divisions using a novel in-niche satellite cell screen developed in our laboratory. This screen will be performed against various libraries of well-characterized compounds, such as the OICR kinase inhibitor library, OICR toolkit library, TOCRIS kinase inhibitor library, and the NIH clinical collection library. Positive results will provide additional information on the molecular pathways dysregulated in the asymmetric divisions

72 **Modeling Hutchinson-Gilford Progeria Syndrome Using Induced-Pluripotent Stem Cells**

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(Background) Hutchinson-Gilford Progeria Syndrome (HGPS) patients exhibit traits of premature aging. The leading cause of death in HGPS individuals is due to deterioration in their vascular tissues. This disease is characterized as a laminopathy-based disease, where a heterozygous mutation in the LMNA gene cause the accumulation of mutant lamin A protein termed Progerin, leading to downstream effects such as alterations in gene expression and heterochromatin ormation. The molecular mechanism of altered gene expression is unknown.

(Aim) HGPS-induced pluripotent stem cells (iPSCs) were used to model the disease to dissect the molecular mechanism underlying this genetic disorder. By characterizing HGPS iPSCs, we aim to examine whether reprogrammed cells have the capacity to differentiate normally, whether reprogramming can reset altered gene expression in HGPS cells, and how mutation in LMNA gene in HGPS cells can affect chromatin formation upon differentiation into vascular smooth muscle cells (VSMCs).

(Method) Reprogramming: Cells derived from Progeria patients were reprogrammed into iPSCs through retroviral infection using four transcription factors: Oct4, Klf4, Sox-2, and c-Myc. Cell potency of each reprogrammed cell line is characterized using alkaline phosphatase staining and immunofluorescence staining of cell surface pluripotent markers. iPSC colonies were induced to form embryonic bodies (EBs), which are then plated to allow growth and differentiation into the three germ layers. Their differentiation capabilities were characterized through immunofluorescence staining using differentiation marker antibodies. Differentiation of iPSCs to VSMCs: EBs were plated on gelatin-coated plates, EB outgrowth were trypsinized and cells were replated on Matrigel with growth supplement. Immunostaining on VSMC markers was performed on these cultures. Phospho-H2A.X localization was characterized using western blot as well as Duo-link assay.

(Results / Conclusions) iPSCs derived from patients diagnosed with HGPS exhibited traits of pluripotency, and could differentiate into cells characteristic of the three germ layers. Reprogramming can reset perturbed gene expression in iPSCs caused by mutation in the LMNA gene, reverse HP1 binding distribution back to the normal state. However, the localization pattern of HP1 changes upon differentiation upon heterochromatin formation. VSMCs differentiated from HGPS iPSCs exhibited similar marker expression as coronary artery VSMCs. However, an up-regulation in phospho-H2A.X marker was observed at the DNA replication fork in HGPS VSMCs, suggesting that there is an increase in DNA damage in HGPS VSMCs. In general, a disease model to investigate the progression of HGPS was developed from the study.

73 **Acute effects of hyperoxia on endogenous lung mesenchymal stromal cells in rat pups**

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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. Understanding how alveoli normally develop, and how this is disrupted in BPD, is crucial for the development of new effective therapies. Mesenchymal stromal cells (MSCs), which have regenerative properties, are associated with the development of BPD if found in the tracheal aspirates of preterms.

Hypothesis:
Hyperoxia exposure perturbs endogenous lung MSCs in an oxygen-induced rat model of BPD.

Methods:
Sprague-Dawley 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 Ficoll-purification. MSCs were characterized according to the International Society for Cellular Therapy criteria. mRNA analysis was performed for periostin, fibronectin, collagen, elastin, a-smooth muscle actin (aSMA) and transforming growth factor ß1 (TGFß1). MSC-conditioned media was used for wound healing and vessel formation assays.

Results:
Hyperoxia-exposed lungs yielded 30% fewer MSCs compared to controls. Hyperoxia-exposed MSCs had higher colony forming potential, had significantly lower mRNA levels of collagen, elastin and aSMA and periostin. Conditioned media from hyperoxia-exposed MSCs decreased in vitro wound healing, but not vessel formation.

Conclusions:
Hyperoxia exposure not only decreased MSC numbers, but also altered their function and expression of matrix proteins essential for alveolar development. These insights will help to understand the effectiveness of exogenous MSC-therapy in BPD.

74 **Identification of integrin-linked kinase as a regulator of oligodendrocyte precursor cell migration**

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Background
Oligodendrocyte precursor cells (OPCs) migrate throughout the central nervous system (CNS) prior to receiving cues to differentiate. In demyelinating diseases such as Multiple Sclerosis, OPC migration is essential in order for remyelination to occur in damaged areas of the CNS. However, in damaged areas OPCs are often found stalled outside of the lesion. Thus, it is of great interest to explore the molecular mechanism of OPC migration in order to further our understanding of how to promote migration into lesions within the CNS. The ß-integrin transmembrane receptor is important for OPC migration, at least in response to the extracellular matrix protein laminin-2. From this, integrin-linked kinase (ILK), the downstream intracellular binding partner of ß-integrin, may also be crucial for this process.

Objective
The goal of this work was to investigate the role of integrin-linked kinase (ILK) in OPC migration.

Methods
We created a novel in vitro migration assay, which uses primary mouse OPCs. Oligospheres (spherical clumps of OPCs) are produced through the process of OPC purification from a mixed glial culture. In order to quantify migration, OPCs are allowed to migrate out of the oligospheres for defined periods of time and are then processed for microscopy. Through the addition of TAT-Cre recombinase, this method becomes applicable to conditional gene knockout studies. We completed this assay following the deletion of ILK in the primary OPCs.

Results
Reductions in migration distance were observed in ILK-null OPCs at the 4 hour time point on laminin-2 substrate. Reductions in migration distance were also seen on fibronectin substrate at both 10 and 24 hour time points. Interestingly, the severity of this phenotype appears to be dependent on the substrate.

Conclusions
Overall, ILK-null OPCs demonstrate an observable migratory defect at some, but not all, time points studied for a given substrate and it appears as though the migration deficits may be substrate-dependent. Future work will test the migration of ILK-null OPCs on poly-D-lysine, a neutral substrate. These findings will be beneficial in the development of novel therapeutics aimed at promoting the migration of OPCs into damaged areas of the CNS.

75 **Aberrant postnatal muscle development in mouse models of Spinal Muscular Atrophy**

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Background: Spinal muscular atrophy (SMA) is one of the leading genetic killers in infants under two years of age. This neuromuscular disease is characterized by selective loss of motor neuron in the spinal cord and muscular atrophy due to survival motor neuron (SMN) protein depletion. Most research to date has focused on understanding why motor neurons are preferentially affected, and muscular atrophy has been considered as a secondary consequence of the motor neuron degeneration. Emerging evidence is demonstrating that muscle cell-autonomous defects are also implicated in the overall clinical picture of SMA.

Objective: Investigate the effect of reduced levels of SMN in skeletal muscle of SMA mice, more particularly in myogenesis.

Methods: To achieve this goal, we assessed the mRNA expression of myogenic regulatory factors, the satellite cell pool size and extended our analysis to programmed cell death in two mouse models of SMA.

Results: We found that myogenic regulatory factors are deregulated in both mouse models, showing distinct mRNA profiles at the phenotype stage. We also noted that the SMA muscle satellite cell pool size was significantly different in the SMA model mice than in their control littermates.

Conclusion: Taken together, these results strongly reinforce the idea that muscular defects other than atrophy exist in SMA. However, the specific role of SMN in skeletal muscle remains unclear. Future research will emphasize on filling the gap between the various roles of SMN and the reported muscle defects in this study, which will provide a better understanding of SMA pathology.

76 Uncovering novel transcriptional networks of human early lineage specification

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Background :
Despite the interest in applications of human pluripotent stem cells (hPSCs) for cell-based therapy and drug screening, the nature of initial events occurring in hPSCs, specifically in human embryonic stem cells (hESCs) as a model of early human embryogenesis, and the transcriptional networks involved in pluripotency maintenance, lineage selection and commitment to differentiation have remained largely unexplored. The hypothesis is that construction of transcriptional networks of pluripotency and early lineage commitment in hESCs using an integrative systems biology approach will allow the identification of novel factors regulating these events.

Objectives and Methods:
The overall objective is to identify and test novel transcriptional networks regulating human pluripotency and early lineage specification. The specific objectives are: 1) Develop and validate a robust culture system permissive to downstream applications. 2) Develop a reliable approach to genetically modify hESCs. 3) Develop functional cell-based assays to evaluate phenotypes of genetically modified hESCs. 4) Construct a first draft of a pluripotency transcriptional network and of a commitment transcriptional network and identify putative nodes. 5) Perform Chip-Seq on the initial predicted nodes identified in objective 4. 6) Generate a set of knockdown and overexpressor hESC lines and evaluate the phenotypic effect of modifying levels of the identified nodes. 7) Perform genome-wide expression analysis on the under-expressors and over-expressors. 8) Integrate both datasets from objectives 5 and 7 and form novel predictive networks. 9) Test and validate the novel predictive networks experimentally.

Results and Conclusions:
We show here the successful development and validation of a standardized cell culture system for hESCs based on a chemically defined medium, as well as functional cell-based assays using flow cytometry and high-content imaging to quantitatively assess retention and loss of pluripotency or differentiation at the single cell level. A first draft of a directed transcription network covering pluripotency and early commitment in hESCs has been constructed based on our previous studies in the mouse model and published literature. From this we have selected nodes and have developed a hESC-specific transfection and selection protocol and validated the successful knockdown of these nodes at the transcript as well as protein level. We are following up these results with analysis of the genetic overexpression of the same nodes and then will proceed with data integration to draft novel transcriptional networks defining early human lineage specification. Supported by CIHR and the Fonds de la Recherche en Santé du Québec.

77 Fzd7 receptor represses satellite cell proliferation

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Background:
A large number of injuries and diseases, such as muscular dystrophies, affect muscle integrity and function. The activation, proliferation and differentiation of satellite cells is essential for muscle regeneration. Importantly, a small population of satellite stem cells is able to proliferate without differentiate in order to replenish satellite cell population. A ligand of the wingless family, Wnt7a, has been shown to be critically involved into the symmetric expansion of satellite stem cells and fiber hypertrophy through its interaction with the receptor Frizzled-7 (Fzd7). However, the exact role of Fzd7 receptor in muscle regeneration is still unclear.

Objective:
Characterize the effect of Fzd7 on satellite cell function and muscle regeneration

Methods:
Myf5-cre ROSA-YFP mice were crossed with Fzd7 knockout (Fzd7-KO) mice. These mice allow the identification of satellite stem cell (Pax7+Myf5-) population from committed satellite cells (Pax7+Myf5+). Primary myoblast cell culture and fiber culture were used to assess satellite cell behavior in vitro. Western blot and qPCR were also used to analyze different signaling pathways affected by Fzd7. Dystrophic mice (mdx) crossed with Fzd7-KO mice were also used to assess the effect of Fzd7 in a model of muscle degeneration/regeneration.

Results:
In vitro proliferation assay indicated that Fzd7-KO primary myoblasts proliferate much faster compared to Fzd7 heterozygous (Fzd7-Het) cells (3 times faster after 5 days). This observation was confirmed by Ki67 staining (55% positive for Fzd7-KO vs 30% positive for Fzd7-Het). Fiber culture in vitro revealed that satellite stem cells (Pax7+Myf5-) proliferation is similar in Fzd7-Het and KO, however the absence of Fzd7 favors their asymmetric division. On the other hand the proliferation of committed satellite cells

(Pax7+Myf5+) is increased in Fzd7-KO. Consistently, qPCR results indicate that Myf5 expression is increased in Fzd7-KO (3-fold), while MyoD and myogenin expressions are decreased (0.5-fold and 0.1-fold respectively). Similar results were observed in mdx-Fzd7-KO primary myoblasts. These results suggest that Fzd7-KO primary myoblasts are forced into a proliferative state. Preliminary data from western blot indicated that Akt and ß-catenin pathways are both overstimulated in Fzd7-KO myoblasts and muscles.

Conclusions:
These results suggest that Fzd7 acts as a repressor of Myf-5 expression and myoblast proliferation only in committed satellite cells. Signaling mechanisms leading to this repression are currently under investigation.

78 **Characterization of bone marrow-derived cells isolated from rats with hypoxia-induced pulmonary hypertension**

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Background:
Pulmonary Hypertension (PH) is characterized by endothelial cell damage/dysfunction leading to increased pulmonary vascular resistance, pathological right ventricle hypertrophy, and a poor prognosis due to right heart failure. Bone marrow-derived endothelial progenitor cells (EPCs) have been shown to contribute to the vascular repair of damaged endothelial cells in animal models of PH and PH patients. Due to their unique regenerative capacities, the efficacy of autologous EPC therapies for PH patients has been the subject of recent clinical trails. However, compared to healthy individuals, EPCs isolated from PH patients may be fewer in number, have a diminished ability to migrate/adhere to areas of vascular injury, and show a reduced ability to form vascular networks in vitro. In cell-based clinical trials for PH, the patient's own EPCs are expanded ex vivo, genetically enhanced and returned; therefore it is of considerable interest to further characterize EPC dysfunction in PH for future trials.

Objective:
To characterize and compare the regenerative capacities of bone marrow-derived cells (BMCs) isolated from rats with hypoxia-induced PH and normoxic controls.

Methods:
Male Sprague Dawley rats (190.3±6.8 g) were exposed to 1 week of hypoxia (9% 02) (n=4) or normoxia (20.9% 02) (n=4). Right ventricle systolic pressure (RVSP) and right ventricle hypertrophy (RVH) measurements were conducted to confirm development of PH. BMCs were isolated from femurs and tibias of hypoxic and normoxic rats and cultured in standard endothelial growth media. After 7 days in culture, immunofluorescence staining was performed to detect the expression of putative EPC surface marker CD31 and lectin binding. Transwell migration and matrigel tube-formation assays were performed to determine migration and angiogeneic potential.

Results:
Hypoxic rats exhibited significant PH confirmed by increased RVSP and RVH compared to normoxic controls (50±2 vs. 30±3 mmHg, $p<0.001$; 0.46±0.01 vs. 0.28±0.02, $p< 0.001$, respectively). Following 7 days in culture, BMCs from hypoxic rats showed decreased lectin binding (27% vs. 44%) and decreased expression of the endothelial cell marker CD31 compared to BMCs from normoxic rats (5% vs. 38%). Furthermore, BMCs from hypoxic rats did not show any differences in migration towards known chemoattractants VEGF and/or SDF-1, but showed increased potential to form capillary-like networks on matrigel.

Conclusions:
These preliminary findings suggest that hypoxia-induced PH can impact BMC differentiation and regenerative function, which may have broader implications regarding the use of autologous cell therapies for patients with PH.

79 **Gene Selection for the Analysis and Reconstruction of Stem Cell Differentiation Trees**

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Background
Surface marker genes are frequently used to differentiate between different cell types in stem cell differentiation. However this is only possible when the structure of the differentiation tree and its component cell stages are known. Marker genes are usually not controllers or regulators of stem cell differentiation but are chosen by historical convenience or human preference and thus they do not determine the tree topology.

Objective
Our objective is to first analyze gene expression patterns in 38 cell types of hematopoietic stem cell differentiation, then formulate a gene selection method that will allow us to reconstruct the differentiation tree from gene expression data, and finally predict missing states in the tree and fit in newly defined states through supervised machine learning.

Methods
Standard clustering techniques such as hierarchical clustering, minimum spanning tree and maximum parsimony analysis were applied to the gene expression data of 38 cell states in hematopoietic stem cell differentiation, and their performance was evaluated using a simple error measuring method. Gene expression maps were generated by measuring differences in gene expression between consecutive cells in a lineage as well as between each pair of cells in a lineage. Genes were then labeled as either activated, deactivated, stable or fluctuating in the lineage. Finally using a weighted Euclidean distance metric we formulate a set of 1332 linear constraints that we enforce on the cells in order to transform the differentiation tree to a biased minimum spanning tree. By minimizing the sum of weights for the 22215 genes we solve a linear program that assigns a weight of zero to as many genes as possible.

Results
We first show that traditional methods such as hierarchical clustering, minimum spanning tree, and maximum parsimony analysis fail to reconstruct the differentiation tree from marker gene expression data and even from the expression data of all genes. We were then able to select genes that show differential expression in distinct lineages of the tree. From these selected genes we arrive at several maps of gene expression in hematopoietic stem cell differentiation. By solving a linear program with a set of linear constraints we were able to select 152 out of 22215 genes that allow us to re-construct the tree as a minimum spanning tree using a weighted Euclidean distance metric.

Conclusion
Our work forms a solid base for future work on predicting missing states in a tree through supervised machine learning.

80 **Shifting the paradigm for the comparative analysis of genome-wide transcription factor binding during hematopoiesis and leukemogenesis**

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Transcription factors are the main drivers of cell fate determination during differentiation and development. They work through the activation of cell-type specific gene regulatory networks (GRNs) while simultaneously counteracting the programs of alternative cell types. If a transcription factor is ectopically expressed in the wrong cellular environment, it can aberrantly activate a number of genes leading to cellular re-programming and oncogenic transformation. Therefore comparing transcription factors binding across multiple healthy and diseased cell types is a critical step to decipher the mechanism of cell fate determination and oncogenic transformation. While the study of GRNs has been recently facilitated by genome-wide approaches for transcription factor binding (i.e. ChIP-sequencing) and gene expression (i.e. RNA-sequencing), extracting biologically meaningful information is rendered difficult by the size, complexity and variability of these high-throughput datasets.

The development of a new paradigm will be presented for the comparative analysis of genome-wide transcription factor binding across multiple cell types of the hematopoietic lineage, including both healthy and leukemic cells. As a model system for the development of this paradigm, the transcription factor TAL1 (also known as SCL) was used. TAL1 is a master regulator of the hematopoietic lineage. The cell lines, which were investigated, include three healthy differentiated lines with TAL1 normally expressed, one leukemic T-ALL cell line with TAL1 aberrantly expressed, three T-ALL patients with TAL1 aberrantly expressed, and two stem/progenitor cell lines. Determining the variation in enriched binding regions of TAL1 is required in order to understand more about the function of TAL1 in T-ALL.

The first phase of enrichment region investigation will be presented here. The first phase analysis involved the discovery of transcription factor motifs in enrichment regions that are shared between specific cell lines, and those found uniquely in each. Variants in binding structure indicate a method of specific identification of T-ALL cells.

Future phases of analysis will involve gene association and functional ontology to add a second layer to the motif-based composite structure variation in transcription factor binding between cell lines. This will permit for greater specificity concerning co factor variation between different cell types. Understanding the variation in binding characteristics of key transcription factors can provide T-ALL specific variants in binding cofactors. The existence of critical T-ALL specific variants may provide a window for the development of clinical treatments of T-ALL.

81 **Differentiation of Induced Pluripotent Stem Cells into Endothelial Progenitor Cells: A Superior Product for Cell therapy and Recellularization?**

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Introduction: Pulmonary arterial hypertension (PAH) is characterized by loss of functional pulmonary vasculature and increased in pressure, and triggered by injury and degeneration of endothelial cells lining distal lung arterioles. Previous studies have documented promising results using transplantation of endothelial progenitor cells (EPCs) into experimental models of PAH. Generation of therapeutically-relevant quantities of EPCs to treat PAH may be circumvented through the use of pluripotent stem cells capable of indefinite self-renewal and multilineage differentiation. We hypothesized that EPCs derived from induced pluripotent stem cells (iPSCs) will have progenitor-like properties which may be superior to adult progenitor cells, enabling them to repair and revascularize the damaged pulmonary bed.
Methods: We studied three different iPSCs lines from distinct parental origins (fibroblast, 090-iPSC; peripheral blood derived late out-growth EPCs, PB-iPSC; and aphaeresis derived late outgrowth EPCs, EPC-iPSC) using three different published protocols from the Srivastava, Nakatsuji and Scadden laboratories.

Result: Preliminary testing showed suboptimal cell recovery (~10%) and selection efficiency using the Srivastava's protocol. Cobble-stone looking cells resembling EPCs and fibroblast-like cells were observed in Nakatsuji's and Scadden's protocols within 3 days in culture. Selection for CD34+ cells with MAC microbeads yielded an efficiency of 35% and 15% for the Scadden and Nakatsuji protocol, respectively, with the Scadden protocol yielding ~ 50% more CD34+ cells compared to the Nakatsuji's protocol. Following selection, adherent cells, actively proliferated. Initially, these appeared spindle-like with cellular extensions towards neighbouring cells before adopting cobble-stone morphology within 4-5 days in culture. Overall, based on visual assessment, 85% of selected CD34+ cells derived from 090-iPSC were actively proliferating compared to 70% of PB-iPSC and 30% of EPC-iPSC.

Conclusion: Of the three protocols assessed, the Scadden protocol appears the most robust given the consistently high yield of CD34+ EPC-like cells across all cell lines. The 090-iPSC cell line is also likely to provide a better source material for generating clinically-relevant quantities of cells as it possesses the greatest differentiation efficiency.

82 **Myoblast expansion defects leads to muscle growth delay and subsequent compensatory adaptation in adult Atrx cKO skeletal muscle**

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Background
The growth of muscle tissue and its regeneration from injury is crucially dependent on a self-renewing population of muscle progenitor cells called satellite cells. Activated satellite cells give rise to a rapidly expanding population of myoblasts that increase muscle mass by differentiating into new or adding into pre-existing muscle fibers. Patients with mutations in the chromatin remodeling gene ATRX are clinically characterised by severe cognitive disabilities and muscular hypotonia, thus dramatically compromising their independent locomotory ability.

Materials and methods
We generated a skeletal muscle specific knockout mouse model by interbreeding Myf5-Cre mice with mice harbouring the Atrx floxed conditional knockout allele (herein referred to as Atrx cKO). Body mass of Atrx cKO and control littermates were measured along with general morphometric analysis of select hindlimb muscles at 3-weeks, 5-weeks, and greater than 8-weeks of age. Methods of analysis included single muscle fiber preparations from the extensor digitorum longus (EDL) and soleus muscles as well as RNA expression analysis from the EDL and soleus muscles. Primary myoblasts were also cultured from dissociated muscle tissue from the hindlimbs and analyzed via immuno-fluorescent microscopy. Neuromuscular acuity and endurance was assessed in Atrx cKO and control littermates by the roto-rod apparatus. Muscle strength was assessed by utilizing a digital grip strength measuring apparatus.

Results
Atrx cKO mice presented with telltale characteristics of weaker musculature, exemplified by spinal kyphosis and reduced body mass at 3-weeks of age. Satellite cell derived myoblasts from Atrx cKO mice were incapable of rapid expansion in culture but were fully capable of terminally differentiating. Atrx cKO myoblasts displayed delayed cell cycle progression through mid-late S-phase and rampant signs of genomic instability characterised by fragmented nuclei, ?-H2AX foci, and telomeric aberrations. Despite inefficient myoblast proliferative capacity, Atrx cKO animals were able to re-establish normal body mass by adulthood. Muscular fitness and function in Atrx cKO mice was also age dependent, as younger 3-week old Atrx cKO mice had a reduced capacity to stay on the roto-rod and poorer gripping strength. However, differences in grip strength of adult Atrx cKO mice were almost indistinguishable from their control littermates. Data regarding pathways mediating the hypertrophic compensatory adaptation in

Atrx cKO mice will also be presented.

Conclusions
Inefficient expansion of activated satellite cells in Atrx cKO mice results in delayed muscle development up to 3-weeks of age, when myonuclear accretion reaches its plateau. Reduced muscle mass at 3-weeks of age also correlated with poorer performance in physical tasks that

83 Poster withdrawn

84 Overexpression of miR-145 negatively impacts oligodendrocyte differentiation by inhibiting cytoskeletal organization

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Background:
A key problem in progressive multiple sclerosis (MS) is the diminished capacity for myelin repair. Although oligodendrocyte progenitor cells (OPCs) can be observed at the lesion site, their ability to differentiate appears inhibited. MicroRNAs are vital regulators of oligodendrocyte (OL) differentiation, and have been observed to be misregulated in MS lesions compared to healthy white matter. Thus, aberrant microRNA expression in MS lesions may disrupt the ability of incoming OPCs to differentiate.

Objectives:
A specific microRNA known as miR-145 is downregulated as OPCs progress to OLs in healthy individuals but is upregulated in chronic progressive MS lesions. Putative targets of miR-145 are significantly enriched for factors which promote actin cytoskeleton organization and myelination. In this study, we investigated how misregulation of miR-145 affects OL differentiation in vitro.

Methods:
An immortalized OL cell line was transduced with an inducible lentivirus to create stable lines that overexpress miR-145 after treatment with tetracycline. Stemloop qRT-PCR was used to quantify changes in miR-145 expression between uninduced and induced cells. These stables lines were characterized while proliferating (P), and early (day 3 DD3) and late (day 6, DD6) during differentiation. Immunofluorescence was used to quantify morphological differences while qRT-PCR arrays were used to quantify changes in microRNA target expression levels between uninduced and induced cells.

Results:
Two stable lines were created: ON-145-1 and ON-145-2, which upon induction overexpress miR-145 ~30-fold and ~12-fold, respectively. When proliferating, no significant morphological differences nor target expression differences could be detected between uninduced and induced cells. In contrast, during early and late differentiation, both induced cell lines showed significant morphological defects characterized by a reduction in both primary and secondary branching. Interestingly, defects in ON-145-2 appeared less severe than in ON-145-1, suggesting a dose-dependent relationship between miR-145 overexpression and loss of branching ability. We also observed significant decreases in expression levels of multiple miR-145 targets required for promoting actin organization, such as Pak4, Tmod3, and Ctnnd1, and for myelination, such as Serinc5.

Conclusions:
These results suggest that overexpression of miR-145 during OL differentiation may disrupt actin organization required for successful process extension and subsequent myelinating ability. Thus, the increase in miR-145 in MS lesions may be a significant contributing factor to the loss of myelin repair in progressive MS lesions.

85 The Role of Wnt7a in Neuroplasticity and Post-stroke Recovery

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Previous studies in our lab determined that the secreted signalling protein Wnt7a and its receptor Frizzled7 (Fzd7) are up-regulated in satellite stem cells during muscle regeneration and that overexpression of Wnt7a enhances muscle regeneration through symmetric expansion of the stem cell pool.

More recently, Wnt signalling has also been shown to regulate the symmetry of NSC division – a process that is up-regulated following induced stroke in mice. Furthermore, Wnt7a is a known regulator of synaptic differentiation and axonal remodeling in the adult brain, and it has been shown to stimulate NSC proliferation and self-renewal, resulting in an increase in the number and function of excitatory neurons and synapses.

Taken together, these previous studies suggest a role for Wnt7a as a key regulator of NSC division and neuroplasticity. In this

study, in vitro studies confirm a role for Wnt7a in the regulation of NSC proliferation and self-renewal.

Future directions aim to investigate the potential of Wnt7a as a therapeutic treatment for post-stroke recovery by assessing NSC expansion and neurogenesis in vivo by immunohistochemistry following intracranial injection of Wnt7a and intraperitoneal injection of bromodeoxyuridine (BrDU) in mice, and by assessing the effect of Wnt7a on recovery following induced stroke in mice.

86 **Retinal Interneuron Function and Survival is Dependent on Cell-Extrinsic Atrx Activity**

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Background: Retinal degenerative diseases involve the progressive loss of photoreceptor cells of the retina and are the leading cause of blindness in the developed world. Therapeutic strategies that aim to replace or bypass the lost photoreceptors 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 leading to effective visual perception. 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 amacrine and horizontal cells in mice lacking the chromatin remodeling protein Atrx

Methods: We use conditional knockout approaches to selectively remove Atrx from different retinal cell populations in vivo, including the production of transgenic mice and the surgical delivery of genetic excision tools targeted to specific cell types. Phenotypic analysis of the Atrx-deleted 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 genetic and immunohistochemical analysis of the mutant mice reveals dysregulation of bipolar cell marker genes, misexpression of bipolar subtype-specific proteins, and alterations in neuronal morphology that may underlie defects in retinal synaptic communication.

Conclusions: The loss of amacrine and horizontal cells from Atrx-deleted retinas appears to occur through a cell non-autonomous mechanism. Electrophysiological assessment, cell type-selective gene inactivation in the retina and subtype-specific marker gene expression analysis 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.

87 **Enhanced engraftment of human induced pluripotent stem cells using a hydrogel encapsulation approach**

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Cell based therapeutic strategies are limited by low cell viability, low organ retention, and low engraftment at target sites. Inadequate extracellular matrix cues and subsequent apoptotic cell death play a vital role in these cell therapy limitations. Further development of cell delivery systems is crucial in applying cell-based therapies. There are currently no studies investigating cell delivery systems in the lung. Recently, there is increasing attention towards the clinical applications of human induced pluripotent stem cells (hiPSCs). hiPSCs can specifically derived from patients and overcome the social and ethical concerns posed when using embryonic stem cells. We hypothesize that hiPSC hydrogel encapsulation provides enhanced cell survival, retention, and engraftment in the lung. hiPSCs were stained with CellBrite Cytoplasmic Membrane Labeling Dye and encapsulated in either agarose or an agarose/vitronectin mixture. Capsules were subjected to immunostaining with Anti Vitronectin, to confirm the presence of encapsulated vitronectin; Hoechst, a nuclear stain; Nanog, a transcription factor unique to stem cells; Calcein AM, a live cell stain; and Phalloidin, an actin microfilament stain to determine the fate of hiPSCs post encapsulation. Current findings

demonstrate that vitronectin can be incoporated in agarose capsules. Moreover, the encapsulation procedure is efficient, the hiPSC phenotype is not altered, and the cell viability is unaffected. Furthermore, in vivo intratracheal injection of agarose capsules less than 100µm showed no adverse effects in mice. This result supports that the agarose encapsulation system will be useful in conducting further in vivo studies in the lung.

88 **Effects of Ionizing Radiation on Mesenchymal Stem Cells of Normal and Leukemic Bone Marrow Hematopoietic Niches**

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Background:
Total body irradiation followed by allogeneic hematopoietic stem cell transplantation (HSCT) has emerged as a promising strategy to treat chemo-refractory acute myeloid leukemia (AML). Unfortunately, radiation-based transplantation is often insufficient to eradicate AML and more than 70% of patients relapse and succumb to their disease. While HSCT supplies healthy donor-derived hematopoietic stem cells, the bone marrow hematopoietic niche remains patient-derived and undergoes severe physiological stress upon irradiation. The major cellular component of the hematopoietic niche is the mesenchymal stromal cell. Our previous studies have demonstrated that MSCs from patients with AML are abnormal and exhibit reduced capacity to support normal hematopoiesis. We hypothesize that MSC function must revert to normal following anti-leukemia therapy in order to prevent relapsed disease. The extent to which MSC function is normalized following radiation treatment remains unknown but may be central to the success of radiation-based transplantation strategies. In this study, we have compared functional properties of MSCs from patients with AML and normal controls and tested the effects of high dose radiation on MSC function.

Methods:
Normal and AML-derived MSCs were treated with X-ray doses consistent with TBI protocols. Cells were compared based on CD marker expression, differentiation, cell proliferation and viability. Induction of DNA damage following radiation treatment was assessed using immunostaining for ?H2A.X histone.

Results:
Flow cytometric analysis showed normal surface marker expression profile for leukemic and irradiated cells. However, MSCs from AML patients had increased adipogenic and decreased osteogenic capacity in comparison to normal controls. Interestingly, irradiation of healthy MSCs resulted in the same abnormal differentiation profile as observed in AML cells. AML-MSCs had decreased proliferation, as measured by BrdU incorporation and CFU-F assay, and increased necrotic cell death. Radiation treatment substantially decreased both normal and AML-MSC cell proliferation rates and completely inhibited colony formation. Surprisingly, no changes in cell viability were observed upon irradiation of either cell type. Finally, immunocytochemistry analysis demonstrated substantial generation of ?-H2A.X histone following irradiation in both normal and AML cells suggesting extensive DNA damage and induction of DNA repair mechanisms.

Conclusions:
Taken together, MSCs from AML patients exhibited increased adipogenesis, decreased osteogenesis and reduced proliferation indicative of physiologically abnormal and/or stressed bone marrow. Radiation treatment induced similar changes in normal MSCs and therefore, the ability of high dose radiation to normalize MSC function is dubious.

89 **A disruption in the autophagic process underlies the sensory neuropathy in dystonia musculorum mice**

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Background:
A newly identified lethal form of hereditary sensory and autonomic neuropathy in humans (designated HSAN-VI) is caused by a homozygous mutation in the dystonin gene (DST). Dystonin is a cytoskeletal linker protein involved in microtubule-based motor protein transport and loss of function leads to sensory neuron degeneration and severe ataxia in dystonia musculorum (dt) mice. Both microtubules and the dynein motor protein are essential to the autophagic pathway.

Objective:
Assess the influence dystonin imparts on autophagy in the murine nervous system.

Methods:
Using dt and wild type mice, evaluate autophagic flux in cortical neurons and dorsal root ganglion sensory neurons through immunofluorescence, electron microscopy, and western blot analysis.

Results:
LC3-II, a marker of autophagy, was significantly increased in pre- and phenotypic stage sensory neurons from multiple dt alleles. Electron microscopy revealed authentic autophagosomes and explained the increased LC3-II protein levels. In addition, we observed significant decreases in the autophagosome motor protein dynein-intermediate chain 1 (dynein-IC1). A difference in autophagic flux was detected between dt and wild type primary sensory neurons. In accordance with this, dt sensory neurons also displayed impaired protein turnover of the autophagosome substrate p62 and of poly-ubiquitinated proteins. We then addressed which dystonin isoform was responsible for the autophagic defects. dt sensory neurons exogenously expressing dystonin-a2 under the the prion protein promoter were observed to have an autophagic flux profile similar to wild type, as well as fewer markers of autophagy.

Conclusions:
Taken together, our studies show that dysfunctional autophagy underlies dt pathogenesis and suggests that the dystonin-a2 isoform is critical for this process to occur normally within sensory neurons.

90 Targeting of adenovirus to different tissues types

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Background: Adenovirus (Ad) is one of the most commonly used gene delivery platform for human clinical trials. However, Ad predominately accumulates in the liver.

Objective: In terms of gene therapy it would be ideal if the viral vector specifically infected the damage tissue. Altering Ads' native targeting protein, fiber, may decrease non-specific viral infection and redirect infection to a desired tissue.

Methods: Three viral constructs were investigated to determine if biodistribution could be varied: wildtype (wt), shortened fiber or shortened fiber containing poly-lysine motifs. Three injection methods were also examined: intra-peritoneal (IP), intra-venous (IV) and intra-muscular - tibialis anterior (TA). Tissues were analyzed by chemiluminescent enzymatic detection or ß-galactosidase staining.

Results: TA injections showed promising results with homogenized liver and TA samples as both showed a good level of viral infection. However, even at the highest viral dose, virus was not detectable in the spinal cord. IP injections displayed infection in the liver and in the diaphragm yet, systemic spread into the muscle of spinal cord was not detected. Lastly, for IV injections virus was only detectable in the liver.

Conclusions: Overall, our results show that, regardless of the injection method or alteration of fibre, Ad vectors localize predominantly to the liver.

91 Expression profiling of differentially vulnerable motor neurons in spinal muscular atrophy

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Spinal Muscular Atrophy (SMA) is a neuromuscular disorder manifesting as muscle weakness and corresponding loss of lower motor neurons from the spinal cord. This disease is caused by mutations and deletions within the SMN1 gene. In SMA, degeneration of neuromuscular junctions representing occur very early, before symptom onset. Furthermore, not all motor units appear equally affected. Work from both mouse models and human patients has revealed that whilst there is a high degree of denervation in some muscles, others remain innervated even at late stages of disease. In this study we aim to investigate what makes these different pools of motor neurons differentially vulnerable to pathology. We have optimized methods for retrograde tracing of motor neurons, laser capture of the motor neuron cell body and RNA seq analysis of the transcriptome. We have recently used RNAseq to transcriptionally profile differentially vulnerable motor neuron populations from P10 pre-symptomatic Smn2B/- mice and P10 wild-type mice. We are currently undertaking bioinformatic analysis to identify transcripts and pathways which are altered when Smn levels are reduced and which might account for the onset of the degenerative process. This novel approach will not only reveal what makes specific pools of motor neurons more vulnerable to a loss of Smn, but also reveal the primary mechanisms regulating neuronal pathology. This study therefore has great potential to further our understanding of motor neuron biology, SMN function and SMA pathogenesis and move us closer to finding an effective treatment for this devastating disease.

92 The role of Integrin-linked kinase in oligodendrocyte development

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Background:
Interactions between the extracellular matrix (ECM) and the ß1 integrin signaling pathway in oligodendrocytes (OLs) are key for the production of myelin. A major downstream effector of ß1 integrin signaling is integrin-linked kinase (ILK), which is involved in stabilizing focal adhesions and transducing ECM signals. ILK is expressed during OL development in culture, along with its obligate binding partner alpha-parvin.

Objective:
The current study is aimed at determining the importance of ILK in the development of primary OLs.

Methods:
In order to address this aim, we have devised a cell culture system in which we can conditionally ablate ILK from OLs using the cell-permeable TAT-Cre recombinase. Addition of TAT-Cre to cultured ILKfl/fl OLs results in the excision of the Ilk kinase domain, in conjunction with EGFP expression.

Results:
Upon ILK knockout, there is a delay in the expression of developmental stage-specific markers MAG and MBP. In addition, MAG+ ILK-null OLs are deficient in their ability to extend processes, and produce myelin-like membrane. While some ILK-null OLs are able to produce such membranes, the overall area of these structures is reduced as compared to controls. When co-cultured with dorsal root ganglion neurons (DRGNs), the morphological impact of losing ILK is seemingly less severe. At DIV3 of co-culture, there is no effect of ILK loss on the capacity of OLs to contact and overlap with DRGN neurites. However, at DIV6, ILK-null OLs have a reduced capacity to contact neighbouring DRGN neurites and wrap them with MAG+ membrane. In addition, ILK-null OLs are deficient in their ability to produce myelin-like leaflets when cultured with DRGNs. The reduced production of these structures appears to be the driving force underlying the diminished wrapping of DRGNs.

Conclusions:
In summary, ILK is important for various aspects of OL development. Its loss results in delayed development, both in terms of morphology and expression of differentiation markers. When cultured with DRGNs, the morphological phenotype persists but is less severe as compared to when cultured alone. Future work is aimed at investigating signaling alterations in ILK-null OLs that may explain said defects.

93 Proteomics@ohri: Biological mass spectrometry services at OHRI

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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.

94 Caspase-dependent pathways governing cardiac hypertrophy

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Background:
Cardiac hypertrophy occurs when the heart size increases to maintain cardiac output at times of stress. This is initially an adaptive response; however, chronic stress on the heart is a maladaptive process that inevitably leads to end-stage heart failure. Interestingly, this pathological process is also 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.

Objectives:
Here, we investigated the requirement of apoptotic caspase pathways in mediating cardiomyocyte hypertrophy. We aimed to determine: 1) if an increase in caspase activity occurs during the early stages of cardiomyocyte hypertrophic induction. 2) if caspase activation is required for the development of cardiomyocyte hypertrophy. 3) if caspase activation is sufficient to induce cardiac hypertrophy. 4) the caspase-dependent signalling pathways that promote the hypertrophic phenotype by investigating caspase cleavage substrates.

Methods:
Primary cardiomyocytes were isolated from rat pups followed by various treatments. Cardiomyocytes were treated with hypertrophic agonists phenylephrine, isoproterenol, angiotensin II and endothelin 1; caspase inhibitors followed by hypertrophy agonist treatment; a small molecule activator of caspase 3 (PAC-1) to determine if caspase 3 activation is sufficient to induce hypertrophy. Immunofluorescence analysis allowed the effects on cardiomyocytes to be observed. Infusion of hypertrophy agonists by use of osmotic mini-pumps allowed for analysis within the intact myocardium.

Results:
Cardiomyocytes treated with hypertrophy agonists displayed rapid and transient activation of the intrinsic cell death pathway, characterized by elevated caspase 9 followed by caspase 3 activity. 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, a temporal caspase-dependent decline in full length HDAC3 protein levels suggests HDAC3 as a potential hypertrophic caspase cleavage target.

Conclusions:
Overall, these results suggest that caspase-dependent signalling is necessary and sufficient to promote cardiomyocyte hypertrophy. These results also demonstrate that cell death signal pathways behave as active remodelling agents in cardiomyocytes independent of inducing an apoptosis response. This work will contribute to establishing a role for caspases in cardiac hypertrophy and to overall enhance the understanding of this physiological process.

95 **New techniques for short-read sequencing data analysis**

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Background:
Next-generation sequencing (NGS) technologies have revolutionized molecular biology with their unprecedented capabilities for genome-wide measurements of various kinds of biological events.

Objective:
Our research focuses on the development of new and improved algorithms for NGS data analysis with the broader goal of a quantitative characterization of NGS signals into various components such as true binding, control, GC bias, mappability, etc.

Methods & Results:
Here we first report a collection of results from our recently completed investigations dealing with (1) a mappability-sensitive cross-correlation (MaSC) algorithm for accurate estimation of mean fragment length of short-read sequencing data, (2) an adaptive-bandwidth kernel density estimator for accurate reconstruction of the genome-wide signal profiles of NGS datasets, and (3) a set of read-distribution analyses that provides conclusive evidence that a "mock"-ChIP control is significantly better than input DNA control for characterizing background noise in ChIP-Seq experiments. Finally, we describe our ongoing investigation involving a multiscale analysis framework for NGS datasets.

Conclusions: Our contributions described here have resulted in improved techniques for NGS data analysis.

96 **Effects of Phosphorylation on the Stability of the Human Survival of Motor Neuron Protein.**

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Background
Spinal muscular atrophy (SMA) is a devastating neurodegenerative disorder affecting one in every 6,000 live births. The disease is caused by genetic mutations within the survival of motor neuron 1 (SMN1) gene, causing a loss of function phenotype, leading to motor neuron degeneration and muscle atrophy. While there is currently no cure for SMA, a variety of therapeutic approaches are currently being investigated. One proposed method for treating SMA involves administering functional copies of SMN into the

desired tissues via gene therapy. In order to maximize the therapeutic benefits of this approach, our research has led us to examine the stability profile of the SMN protein. Other groups have shown that SMN can be phosphorylated by kinases such as PKA (cAMP dependant kinase) and this event increases the half life and functionality of the protein. Several possible PKA phosphorylation sites have been identified in the SMN protein, although which site(s) is involved in protein stability has not been determined.

Objective
To find and characterize phosphorylation sites within SMN that are important for stability and to create a phosphomimetic SMN mutant for therapeutic use.

Methods
Human 293 cells were transfected with plasmid constructs encoding either wild-type (WT), empty vector control (pCI-neo) or FLAG-SMN. Protein synthesis was interrupted using 50µM cyclohexamide 24 hours post transfection and the levels of SMN were analyzed over a 24h time-course using western blot. The effects of PKA agonists were also tested by treating 293 cells with 10µM of forskolin 30 minutes prior to administration of cyclohexamide, and analyzing SMN stability as described above. Candidate phosphorylation sites were also tested using plasmid constructs encoding GST tagged SMN (GST-SMN) or one of five GST-SMN point mutants (S187A, T85A, S187A/T85A, S4A/S5A/S8A or S4A/S5A/S8A/T85A/S187A). 24 hour cyclohexamide time-courses were carried out using these constructs as described above.

Results
We have currently begun to characterize the stability profile of wild type SMN and found a slight decrease in stability in transfected FLAG-SMN over wild type. The administration of forskolin successfully reduced the rate of SMN decay in 293 cells. Analysis of the stability profiles of each GST-SMN mutant is currently underway.

Conclusions
While a definitive phosphorylation sites have yet to be identified, we have shown that up-regulation of PKA causes an increase in SMN stability, indicating that phosphorylated SMN could potentially confer resistance to proteasome degradation. Furthermore, the enhanced stability of a phosphomimetic version of SMN may be attractive for therapeutic use.

97 **Screening of small molecules inhibiting adenovirus replication**

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Background:
The human adenovirus (Ad) causes minor illness in most patients, but can lead to severe disease and death in immunocompromised individuals, young children and geriatric populations. Within the first few hours of infection, the wildtype adenoviral DNA dissociates from the virus-encoded DNA condensing protein VII and associates with cellular histone H3.3. Assembly of the viral genome into physiologically-spaced nucleosomes is important for efficient expression of virus-encoded genes. Thus, one approach to treating Ad-induced disease may be to inhibit proteins involved in transitioning the viral DNA to a transcriptionally active state. Controlling epigenetic mechanisms to prevent Ad genome decondensation and chromatinization may inhibit virus gene expression, replication and spread.

Objective:
To identify small molecule inhibitors of cellular proteins involved in epigenetic regulation of Ad gene expression and replication in early infection.

Methods:
We have constructed an Ad vector containing a red fluorescent protein (RFP) gene under “late” regulation. Thus, RFP will only be expressed after DNA replication. We can follow viral replication by monitoring RFP expression using live-cell fluorescence microscopy. We will use this virus to test chemical libraries for compounds that inhibit Ad gene expression and replication. We have also constructed a control virus that contains RFP under regulation of the ubiquitously expressed CMV promoter. In this virus, Ad-CMV/RFP expression is independent of virus replication.

Results:
An adenovirus construct encoding an RFP gene within the “late” transcription region of the Ad genome (Ad-Late/RFP) was produced. In preliminary studies, RFP from the Ad-Late/RFP was only produced in cells permissive for viral replication, and the level of RFP expression correlates to the level of viral DNA replication. Furthermore, the histone deacetylase inhibitor Trichostatin A (TSA) reduced RFP expression from Ad-Late/RFP in a dose-dependent manner, while affecting expression from Ad-CMV/RFP minimally.

Conclusion:
An adenoviral construct with RFP under “late” regulation has been produced, which provides the necessary stringency for use in

the small molecule screen. Thus, Ad-Late/RFP can be used to test for small molecules that affect Ad replication, which may identify new compounds to combat Ad induced disease.

98 **Circulating Extracellular MicroRNA-26a as a Biomarker of Disease Activity in Clinical and Experimental Pulmonary Arterial Hypertension**

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Background:
Pulmonary arterial hypertension (PAH) is a progressive and fatal disease characterized by arteriolar remodeling and obliteration of the lung vasculature, ultimately causing right ventricular failure and death. Although the prognosis for PAH is poor, early diagnosis and treatment can increase patient quality of life. Unfortunately, prompt diagnosis remains a challenge as the early stages of PAH are asymptomatic, and there is no specific marker for this disease. MicroRNAs (miRNAs) are key regulators of gene expression that have recently been shown to circulate in blood in a stable extracellular format. Changes in specific circulating miRNAs have been associated with disease, and hold promise as non-invasive biomarkers.

Objective:
To identify circulating miRNAs with utility as biomarkers of disease activity in PAH.

Methods and Results:
RT-qPCR arrays were used to measure 1066 different miRNAs in plasma from 4 treatment-naive patients with idiopathic (I) PAH and 3 healthy controls. Twenty-five miRNAs differed between the IPAH and control groups within this discovery cohort (-2.8 to 3.4 fold change, $p<0.05$). The down-regulation of 4 miRNAs with novel links to PAH was confirmed in a separate validation cohort of 14 PAH patients (6 IPAH and 8 associated PAH) and 13 healthy controls. Among these, miR-26a exhibited significant diagnostic potential with an area under the receiver-operator characteristic curve of 0.85 ($p<0.05$), and also correlated with a functional index of PAH severity (i.e., 6 min walk distance, $r = 0.64$, $p<0.01$). MiR-26a was similarly found to be reduced in plasma from rats with monocrotaline (MCT)-induced PAH, which exhibited characteristic elevations in right ventricular systolic pressure and right ventricular hypertrophy compared to controls after 3 weeks. Moreover, a reduction in miR-26a was observed in MCT-rat lung tissue, and specifically in the right, but not left ventricle of the heart. In vitro degradation assays demonstrate that miR-26a circulates in blood in a nuclease-resistant form, which becomes susceptible to endogenous plasma RNase activity following treatment with Proteinase K but not detergent (Triton X-100), indicative of a mechanism of transport and stabilization mediated primarily by protein complexation rather than vesicular encapsulation.

Conclusion:
This study establishes novel links between circulating miRNAs and PAH, and supports their potential utility as biomarkers of underlying disease activity in affected tissues.

99 **Study of epigenetic modifications and transcriptional regulation of gene expression in muscle satellite cells from healthy and dystrophic mice**

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Background:
Duchenne Muscular Dystrophy (DMD) is a recessive X-linked disease that affects children, is incurable and is caused by mutations in dystrophin, a gene normally expressed in all myogenic cells including stem cells. Dystrophin-deficient (mdx) muscles have increased HDAC expression and higher deacetylase activity that is associated with epigenetic changes. Importantly, treatment of mdx mice with deacetylase inhibitors has proven to be efficient in preventing muscle degeneration.

Objectives:
We hypothesize that aberrant HDAC activity in mdx mice affects global gene expression patterns in muscle stem cells and progenitors, perturbing their function. We study HDAC binding and histone acetylation in muscle stem and progenitor cells from wild type and mdx mice.

Methods:
HDAC genome-wide binding is analyzed by Chromatin immunoprecipitation sequencing (ChIP-seq). Histone acetylation marks will also be studied, namely H3K9Ac (associated with active transcriptional start sites) and H3K27Ac (associated with enhancers of expressed genes). In order to perform ChIP-seq in satellite cells, the protocol has been adapted for small numbers of cells. A whole genome amplification step, required prior to the library generation for high-throughput sequencing, has been optimized.

Results:
We first determined by RT-qPCR the expression level of 12 different HDACs (Hdac1-11 and Sirt1) in satellite cells, proliferating myoblasts and in cells induced to differentiate into myotubes from WT and mdx mice. This analysis brought our attention to HDAC6/7/10, for two reasons: A) these HDACs are expressed at a higher level in satellite cells compared to committed progenitors; B) these HDACs are up-regulated in mdx cells compared to WT cells. Therefore, HDAC6/7/8 binding to the genome will be analyzed by ChIP-seq. We then asked if the difference in HDAC expression between WT and mdx cells have an impact on their differentiation speed. To this end, we induced differentiation of WT and mdx cells, and monitored the expression of three differentiation markers (Mef2c, Myh1 and Myl1). We determined that mdx cells differentiate faster than WT cells.

Conclusions:
The difference in HDAC expression and in differentiation speed may depend on aberrant epigenetic regulation of gene expression in dystrophic cells. This work will determine the acetylation status, chromatin architecture and expression profile of satellite cells. In addition, the comparison between wild type and mdx mice will determine the importance of HDAC activity in the pathogenesis of DMD. Potentially, such insights could lead to the development of muscle stem cell-based therapies for DMD.

100 Cell-free conditioned media from endothelial progenitor cells in the treatment of pulmonary arterial hypertension

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Background:
Endothelial progenitor cells (EPCs) have been shown to be effective in the prevention of preclinical models of. Interestingly, our group has demonstrated that human early EPCs delivered to monocrotaline (MCT)-treated immunodeficient rats could prevent pulmonary arterial hypertension, despite the lack of cell persistence at 24 hours after delivery. Therefore, we hypothesize that the therapeutic effects of EPCs in PAH are mediated by a paracrine mechanism.

Methods/Results:
Human EPCs were derived by cryopreserved leukapheresis product from healthy human donors. Day 6 EPCs or cell-free EPC-conditioned media (EPC-CM) were delivered intravenously to wild-type (WT) immune competent Fisher CDF or athymic nude rats 3 days after induction of PAH using MCT. 21 days after MCT treatment, xenotransplantation of human EPCs into WT rats did not prevent PAH, and there was no effect of EPC-CM. However, delivery of human EPCs in the nude athymic rat model of PAH, resulted in a significant, partial improvements in pulmonary hemodynamics measured by right ventricular systolic pressure (71.85±2.40 vs. 80.76±1.30, respectively; $p < 0.05$) and pulmonary artery acceleration time (20.00±2.00 vs. 14.38±0.73; $p < 0.05$) compared to MCT alone but without a significant improvement RV remodeling.

Conclusions:
In this pilot study, we demonstrate that xenotransplantation of human early EPCs may be effective in reducing experimental PAH only when delivered to an immunodeficient rat strain, suggesting that immune mismatch has a detrimental impact on efficacy. The relatively modest benefit seen in the nude rat model may be due to the use of cryopreserved rather than fresh mononuclear cells.

101 Lack of Sirt1 catalytic activity leads to exacerbated pulmonary hypertension in response to chronic hypoxia in mice

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Background:
Shortness of breath and fatigue, followed by syncope and peripheral edema and ending with right heart failure and death are symptoms of Pulmonary Hypertension (PH). Caused by occlusion and remodelling of the pulmonary vasculature, PH is characterized by an increase in pulmonary vascular resistance leading to right ventricle hypertrophy and eventually failure. PH patient five year survival rate is about 45% according to recent registries and current treatments only delay its progression; there is no cure. Sirtuin (Sirt)-1 is an NAD+-dependent deacetylase that has been strongly implicated in maintaining endothelium homeostasis in systemic vessels, but little is known about its role in the lung vasculature.

Objective:
Investigate the role of Sirt1 in the response of lung vasculature to chronic hypoxia-induced PH

Methods:

Sirt1-/-, sirt1Y/Y (H355Y point mutation lacking catalytic activity) and their wild type littermates were exposed to chronic hypoxia (CH: 9-10% O2) for up to three weeks followed by hemodynamic and hematocrit (percent RBCs of total blood volume) assessments and lung tissue collection.

Results:
Exposure of sirt1Y/Y mice to CH for 3 weeks resulted in a marked increase in right ventricle systolic pressure (RVSP) compared to their WT littermates exposed to the same conditions (41.5±1.8 sirt1Y/Y vs. 29.7±0.8 WT; n=27 both, p< 0.001) with significantly greater RV remodeling, measured by the RV/LV+S weight ratio (0.56±0.02 sirt1Y/Y vs. 0.43±0.01 WT, n=27 both; p< 0.001). Interestingly, there was a profound increase in hematocrit compared to wild type animals in CH (71±1.5% sirt1Y/Y vs. 63±1.2% WT, n=17 both; p<0.001). Similarly, sirt1-/- mice showed exaggerated RVSP, RV/LV+S and heamtocrit in responses to CH. Timeline analysis of PH progression indicated that sirt1Y/Y mice develop significantly elevated RVSP and RV hypertrophy within the first week of the hypoxic exposure compared to WT mice, which progressively increased over the 3 week CH exposure period. Histological and molecular assessment of lung tissue indicated exacerbated pulmonary smooth muscle cell hyperplasia in the mutant mice, and two-fold increase in Sirt1 protein expression in WT CH mice compared to normal WT mice.

Conclusions:
Our data implicates Sirt1 as a crucial player in modulating the hypoxic response pathway, where its absence leads to an exaggerated response and a more severe PH phenotype. This provides us with an excellent model system to explore the mechanism and relevance of Sirt1 downstream targets in the adaptation of the lung circulation to CH and pathogenesis of PH.

102 Characterizing the cellular role of PHF6: Identification of genomic targets within the nucleus and nucleolus

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Background:
PHF6 mutations cause Börjeson-Forssman-Lehmann syndrome (BFLS), a developmental disorder. PHF6 mutations occur throughout the gene and we and others have also identified inactivating PHF6 mutations in patients with T-cell acute lymphoblastic leukemia (T-ALL) and acute myeloid leukemia (AML). The PHF6 protein has both nuclear and nucleolar localization, and contains two atypical PHD zinc fingers. We recently published an interaction between PHF6 and the NuRD chromatin remodeling complex, and an interaction with the PAF1 transcriptional elongation complex has also been reported. Both NuRD and PAF1 are known to regulate rRNA synthesis, although the exact nucleolar role of PHF6 is unknown.

Objective:
To identify PHF6 genomic targets and to determine the basis for the nucleolar localization of PHF6 in a mammalian cell model.

Methods:
We developed stable HEK 293T cell lines for PHF6 overexpression and shRNA-mediated knockdown. Cell growth properties were assessed by WST-1 assays and BrdU pulse-chase. Gene expression changes were identified using DNA microarrays and validated by qRT-PCR. To assess nucleolar localization, cells were treated with 5,6 dichloro-1-ß-D-ribofuranosylbenzimidazole (DRB), actinomycin D (ActD), or various nucleases (DNase I, RNase A), followed by immunocytochemistry with markers for each subnucleolar compartment (FC, fibrillar centre; DFC, dense fibrillar component; GC, granular component). PHF6 binding throughout the rDNA gene was assessed by ChIP-qPCR.

Results:
PHF6 loss did not affect cell proliferation, cell cycle progression, or DNA damage accumulation. Gene expression analyses of PHF6 knockdown cell lines revealed 24 genes with an expression level change of 2-fold or greater versus control. In the overexpression cell line, 285 genes had a 2-fold or greater change. DRB and ActD treatments of HEK 293T cells revealed partial co-localization of PHF6 to FC and DFC, but not GC. Consistent with this localization, ChIP-qPCR demonstrated PHF6 binding to rDNA repeats. Interestingly, PHF6 localization to the nucleolus was found to be RNA-dependent rather than DNA-dependent.

Conclusions:
Several candidate target genes (e.g. HES5, SPARC) were found to correlate with PHF6 gain or loss in HEK 293T cells, but await further characterization in a primary cell model. RNA-dependent localization of PHF6 to FC and DFC, and its binding to rDNA, suggest a possible role for PHF6 in the transcriptional regulation and/or early processing of rRNA. Future directions are aimed at confirming if PHF6 gain or loss correlates with rRNA expression level changes or rDNA epigenetic changes. The mechanism of RNA-mediated PHF6 targeting to the nucleolus also needs to be further clarified.

103 StemCore Laboratories Genomics Facility

Katayoun Sheikhelelslamy[1], **Caroline Vergette**[1], Atieh Jalali-Pakdaman[1], Pearl Campbell[1].

1. StemCore Labs, Ottawa Hospital Research Institute

StemCore Laboratories (www.stemcore.ca) is OHRI's high-throughput genomics facility located at the General Campus of the Ottawa Hospital Research Institute. Our mandate is to provide access to research-enabling technologies which are beyond the scope of individual laboratory operations, thereby facilitating biological and medical research through the use of high-end technology and state of the art equipment. StemCore is continuously developing a world-class genomics services infrastructure, and is capable of facilitating large-scale scientific research and biotechnology projects. StemCore seeks out projects that are challenging, cutting-edge, extend the boundaries of biological knowledge, and will positively impact the state of human health.

104 Screening for molecular regulators of muscle stem cells in their niche

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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 pathway as a novel modulator of asymmetric satellite stem cell divisions. Ex vivo perturbation of Aurora kinase A and B activity by TC-A2317 hydrochloride 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 by TC-A2317 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.

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 neuromuscular disorders.

105 Adenovirus-mediated fusion-associated small transmembrane protein expression promotes apoptosis and sensitivity to chemotherapeutic drugs

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Background:
Adenoviruses (Ad) have been used in many preclinical and clinical studies for delivery and expression of anti-cancer genes or as an oncolytic vector. However, Ad does not spread well throughout the tumour mass. The p14 fusion-associated small transmembrane (FAST) protein can mediate cell-cell fusion and apoptosis of fused cells, leading to enhanced membrane permeability. Its

expression has been shown to enhance the efficacy of oncolytic viruses such as vesicular stomatitis virus and vaccinia virus.

Objective:
To determine if Ad-mediated FAST protein expression will promote virus spread and enhance cell death in cancer cells.

Methods:
293 human embryonic kidney and A549 human lung adenocarcinoma cells were infected by early region 1(E1)-deleted adenoviral constructs expressing the p14 FAST protein. Immunoblotting confirmed FAST protein expression. Infected cells were observed for fusion using fluorescence microscopy and Giemsa staining. Fluorescence based live/dead assays and MTS assays were conducted to assess cell viability. Cell death was confirmed through immunoblots probing for cleaved caspase 3 while cell membrane permeability was determined using LDH release assays.

Results:
Ad-mediated FAST protein expression caused extensive cell fusion in replication permissive 293 cells and at high multiplicity of infection, in A549 cells. Ad-mediated FAST protein expression also reduced cellular metabolic activity and increased membrane permeability. Infected A549 cells showed increased cell death which was further enhanced upon administration of the membrane impermeable chemotherapeutic drug bleomycin.

Conclusion:
FAST protein expression can enhance uptake and action of chemotherapeutic drugs and improve Ad spread among tumour cells through cell-cell fusion.

106 The Smn-independent beneficial effects of Trichostatin A on an intermediate mouse model of SMA

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Background: Trichostatin A (TSA) is a histone deacetylase inhibitor with beneficial effects in SMA mouse models that carry the human SMN2 transgene. Whether TSA specifically targets the upregulation of the SMN2 gene or whether other genes respond to TSA and in turn provide neuroprotection in SMA mice is unclear. We have taken advantage of the Smn2B/- mouse model, which does not harbor the human SMN2 transgene, to test the hypothesis that TSA has its beneficial effects through a non-Smn mediated pathway.

Results: Daily intraperitoneal injection (IP) of TSA (10 mg/kg body weight) or vehicle DMSO from postnatal day (P) 12 to P25 was performed in the Smn2B/- mice and littermate controls. Treatment with TSA increased the median lifespan, attenuated the weight loss, and improved motor behaviour in the Smn2B/- mice. Motor neurons in the spinal cord of Smn2B/- mice were protected from degeneration after TSA treatment. Both the size and maturity of neuromuscular junctions were significantly increased in TSA treated Smn2B/- mice. qPCR analysis revealed no changes in the level of Smn transcripts in TSA-treated SMA mice compared to control mice. Furthermore, western blot analysis revealed no significant upregulation in Smn protein levels in the brain, spinal cord or hindlimb muscles of the TSA treated Smn2B/- mice. These results were corroborated with our in-vitro studies where Smn levels did not increase after up to 500nM of TSA treatment for a 24hr period in mouse embryonic fibroblast and myoblast obtained from the Smn2B/- mice. Therefore, we suggest that the beneficial effects on the SMA phenotype are likely Smn-independent.

Conclusions: Serial TSA treatment significantly extends lifespan, attenuates weight loss and improves motor function in Smn2B/- mice. TSA treatment protects spinal cord motor neuron number, and promotes maturity of NMJs in Smn2B/- mice. TSA did not increase the level of Smn transcripts or Smn protein in various tissues of Smn2B/- mice. The beneficial effect of TSA is therefore likely through a Smn-independent manner. Identification of these pathways will be of therapeutic value for the treatment of SMA.

107 Enhanced Expression of Endothelial Nitric Oxide Synthase in Late Outgrowth Endothelial Progenitor Cells using Non-viral Minicircle DNA

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Background:
Human late outgrowth endothelial progenitor cells (EPCs) exhibit high proliferation capacity, contribute to neovascularization and participate in re-endothelialization of damaged or denuded surfaces. Endothelial nitric oxide synthase (eNOS) catalyzes the production of nitric oxide, is involved in regulation of vessel tone and angiogenesis in inflammation and ischemic cardiovascular diseases. As such, it is an ideal gene for restoring endothelial functional activity. However, the current applications of plasmid

based gene therapy are limited by inefficient transgene expression and the adverse responses to bacterial motifs. Minicircle vectors are double stranded DNA of reduced size and are devoid of bacterial sequences.

Objective:
Here we have demonstrated using minicircle eNOS vectors driven by two different constitutive promoters (CMV and EF1a) enhanced expression in late outgrowth EPC transfection.

Methods and Results:
Human late outgrowth EPCs were derived from peripheral blood monocytes obtained by leukapheresis which exhibit similar antigen profiles (CD31, VEGFR2 and vWF) with mature endothelial cells. Plasmid-based eNOS (PVAX-eNOS), eNOS minicircle DNA under control of CMV promoter (pMini-CMV-eNOS) and eNOS minicircle DNA under control of EF1a promoter (pMini-EF1a-eNOS) were used for late EPC transfection. Production of minicircle DNA encoding eNOS was studied by western blot. Nitric oxide and superoxide were then analyzed by 4-Amino-5-Methylamino-2',7'-Difluorofluorescein (DAF-FM) and dihydroethidium (DHE). Cell migration and angiogenesis after transfection were also determined. We have demonstrated that pMini-CMV-eNOS showed up to 2 times more eNOS expression compared to conventional plasmid (PVAX-eNOS) and pMini-EF1a-eNOS in EPCs at 24 hours after transfection. At 72 hours, pMini-CMV-eNOS showed significantly higher (about 3 times more) and more sustained eNOS expression than conventional plasmid eNOS.

Conclusions:
This result suggests that minicircle DNA is an efficacious gene vector for eNOS expression in late EPCs and may be useful for enhancing EPCs functionality.

Vision Program

108 Norrie Disease Protein: A Novel Mediator of Early Tumorigenesis in Hedgehog Pathway-Induced Medulloblastoma

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Background: Medulloblastoma, a tumour of the cerebellum, is the most common malignant brain tumour in children. A significant proportion of medulloblastoma patients remain incurable, and current therapies often result in severe side effects, indicating a strong need for novel treatments. One third of all human medulloblastoma exhibits a gene expression signature of Sonic Hedgehog (Shh) signaling. These Shh-medulloblastomas have responded to Hedgehog (Hh) pathway inhibitors in clinical trials; however, tumours develop resistance to these compounds, highlighting the need to identify additional therapeutic targets.

Objective: We recently identified a novel target of the Hh pathway in neural progenitors, Norrie Disease pseudoglioma (Ndp), which encodes a secreted protein best known for its role in angiogenesis. We studied the role of Ndp in the cerebellum and in Shh-medulloblastoma.

Methods: To examine the role of Ndp in vivo, standard histololgical and immunostaining techniques were used in combination with Ptch+/- and NdpKO mutant mouse lines. Expression profiling was performed with the Illumina Mouse WG-6 BeadChip.

Results: We determined that Ndp is expressed in Shh-medulloblastoma precursor cells and in actual Shh-medulloblastomas that develop in mice heterozygous for the tumour suppressor Patched (Ptch+/-), which models human Shh-medulloblastoma. NDP is also upregulated in human Shh-medulloblastoma relative to all other medulloblastoma subgroups. To investigate the requirement for Ndp expression in medulloblastoma, we compared tumour frequency and latency as a function of Ndp expression in the medulloblastoma-prone Ptch+/- mouse by generating NdpKO;Ptch+/- (DKO) mutants. Unexpectedly, loss of Ndp caused extreme acceleration of medulloblastoma in the Ptch+/- model, dramatically increasing incidence and decreasing latency. This accelerated medulloblastoma in DKOs was associated with earlier onset, higher frequency and increased vascular invasion of pre-neoplastic lesions in the cerebellum, suggesting that Ndp functions during medulloblastoma initiation and/or early progression. Loss of Ndp also disrupts cerebellar blood brain barrier integrity and leads to enhanced vessel leakiness in pre-neoplastic lesions. Whole genome expression profiling revealed that DKO and Ptch+/- tumours have clearly separable gene signatures, particularly with respect to extracellular matrix genes.

Conclusions: We have uncovered a novel Hh target gene with a strong tumour suppressive role in Shh-medulloblastoma. Our data show that Ndp normally inhibits early stages of tumorigenesis, and implicates a possible role for Ndp in modulation of the early tumour microenvironment.

109 Sortilin Regulates Sonic Hedgehog Trafficking and Release

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BACKGROUND: Sonic Hedgehog is a secreted morphogen, involved in patterning numerous tissues during development. Secreted Shh can act short and long range, affecting tissues dozens of cell bodies away. In the optic nerve, the range of Shh signaling is even greater, as Shh produced by the Retinal Ganglion Cells (RGCs) is trafficked via the regulated secretory pathway down RGC axons, though the mechanisms involved in this transport remain largely unknown. Sortilin (Sort), a multifunctional sorting receptor specializing in targeting ligands to the regulated secretory pathway, was identified in an affinity purification screen as a novel interacting candidate for Shh.

OBJECTIVE: 1. Determine the impact of Sort perturbation on Shh trafficking in primary neurons. 2. Determine the mechanism of the interaction in vitro. 3. Examine the functional consequences of the interaction in vivo.

METHODS: 1. Shh distribution was examined via confocal microscopy in primary cortical neurons subjected to Sort gain and loss of function (GOF and LOF). For GOF, Shh was coexpressed with wild type Sort or dominant negative, truncated Sort (tSort); for LOF , Shh was coexpressed with short hairpins targeting Sort mRNA (with Scrambled control), or in CNs from Sort nullizygous embryos (with wild type littermate controls). 2. A neuronal cell line, PC12's, were stably transfected with GOF or LOF constructs, and Shh secretion was measured in stimulated or unstimulated conditions via ELISA. Aspects of Shh trafficking were examined in protein stability assays using western blotting. 3. Shh::GFP (LOF) mice were crossed to Sort LOF mice, and phenotypic differences were examined in midline structures via H&E, immunofluorescence, and in situ hybridization.

RESULTS: Sort GOF decreased Shh in the axons and in synaptic vesicles, while tSort abolished Shh signal in the axon. Sort LOF increased Shh in the axons and in synaptic vesicles. Rescue of the LOF condition (expression of Sort in null cortical neurons) reduced Shh signal in the axons and synaptic vesicles, reversing the Sort null phenotype. Sort LOF in vivo appears to affect Shh release, resulting in a partial rescue of the cyclopic phenotype in Shh LOF conditions.

CONCLUSIONS: Sort appears to be a negative regulator of Shh trafficking to the regulated secretory pathway, affecting release in a biologically relevant context.

110 Investigating the interaction between Agrin and Sonic Hedgehog

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Background: Sonic hedgehog (Shh) is a secreted morphogen that regulates patterning and cell fate in several tissues though activation of a signaling cascade that alters target gene expression. The mechanisms underlying Shh trafficking has been characterized in many tissues, excluding the optic nerve, where the molecular basis of its release and transport remain unknown. Through a GST-based affinity screen, we identified Agrin as a novel Shh interacting protein, and confirmed the interaction via reciprocal co-immunoprecipitation. Agrin is a heparan sulphate proteoglycan that is required for neuromuscular junction formation, where it is secreted from the nerve terminal to cluster acetylcholine receptors (AChRs) on muscle. This role of Agrin in directing the development of the NMJ has been well documented, although its role in modulating Hedgehog signaling is not known. Objective: To investigate the nature of the Agrin-Shh interaction and its impact on the secretion and biological function of each respective protein. Methods: To determine the impact of Agrin on Shh secretion, media from cells co-transfected with Agrin and Shh was tested for Hh levels by ELISA and for Hh activity by measuring luciferase activity in Hh-responsive LIGHT2 cells. To determine the impact of Agrin on Shh response, NIH3T3 fibroblasts expressing both Agrin and a Gli-reporter were tested for Hh activity after Smo agonist and recombinant Shh treatment. Results: Co-expression of Agrin and Shh in fibroblast cells increased the Shh concentration in the cell culture medium as measured via ELISA. Furthermore, conditioned media harvested from cells co-expressing Agrin and Shh stimulated a higher fold activation of the Shh signaling pathway as compared to cells transfected with Shh alone. In terms of Hh response, cells transfected with the full-length variant of Agrin reduced Hh response following both Smo agonist and recombinant Shh treatment, as compared to controls. Preliminary data measuring the impact of the interaction on Agrin-induced AChR clustering demonstrated no functional impact. Conclusion: At the secreting cell, soluble Agrin appears to increase the release of biologically active Shh from a cell. Conversely, at the receiving cell, full length Agrin attenuates Shh response following pathway stimulation with either Smo agonist or recombinant Shh. Thus, it can be concluded that Agrin and Shh interact, and that this interaction at least appears to modulate Shh response at both the secreting and receiving cell.

111 The role of Gli2 in neural progenitor proliferation

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Background: The Sonic hedgehog (Hh) signaling pathway is a critical regulator of growth and patterning in many tissues. In the developing retina, a model for the central nervous system development, Hh signaling from neurons targets the adjacent neural progenitor cells. Studies of conditional Hh inactivation in-vivo and gain-of-function in-vitro have demonstrated that Hh is required to maintain progenitor proliferation and control cell fate.

How this important signaling pathway affects these different developmental processes is largely unknown, but is likely to involve the downstream mediators of the Hh signaling pathway, the Gli transcription factors. In the retina, Hh target gene expression and proliferation require Gli2, but not Gli1. Interestingly, other systems show that the relationship between Hh activation and Gli2 levels is not direct, suggesting other pathways synergize with Hh to promote Gli2 expression, but which pathways remain unknown.

We hypothesize that transcriptional and posttranscriptional regulation of Gli2 is essential for neural progenitor self-renewal and differentiation.

Objective: Determine the levels of Gli2 protein and message over the course of retinal development and investigate how interactions key developmental pathways affect Gli2 function and stability in neural progenitor cells.

Methods: Cultured neonatal retinae were subjected to western blot or qRT-PCR. To over express transgenes, explants were transfected using electroporation.

Results: During retinal development, Gli2 message and protein is robustly detected in the proliferating neuroblast layer at early stages of development and loss of Gli2 mRNA and protein correlates with loss of retinal progenitor cells, suggesting that down regulating Gli2 transcription may be part of the neuronal differentiation program.

Interestingly, ectopically expressed Gli2:GFP fusion proteins in retinal explants only exert a transient effect on proliferation and are down regulated after several days, which corresponds to the timing of cell cycle exit and neurogenesis. In addition, ectopic Gli2 expression in explants resulted in only minimal Hh target gene induction, suggesting that additional regulators are required.

Inhibition of Notch, a pathway important for RPC maintenance, in the presence of activated Hh signaling results in loss of Gli2 protein. Moreover, constitutive activation of Notch signaling up regulated Gli2 activity and protein levels, but not message.

Conclusion: Based on results thus far, restricting Gli2 activity might be a mechanism utilized by RPCs to activate the differentiation program and Notch may act as a permissive factor allowing RPCs to respond to Hh signaling by regulating Gli2 protein levels.

112 **The Role of Norrie Disease Pseudoglioma (Ndp) in cerebellar development and its relationship with the sonic hedgehog pathway**

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Norrie Disease Pseudoglima (Ndp) is an X-linked cysteine-rich secreted protein that is best known for its role in regulating vascular development in the retina and inner ear. Norrie disease is caused by various mutations in the NDP gene. In humans, the resulting phenotypes are congenital blindness, sensorineural deafness and in some cases, cognitive impairment. We recently showed that in the developing retina, Ndp is a direct target of Sonic hedgehog (Shh) signaling in retinal progenitor cells (RPCs), and is required for Shh-mediated RPC proliferation. Similar to the retina, a neural progenitor called the granule neuron progenitor (GNP) undergoes Shh-mediated proliferation in the developing cerebellum. Given that Ndp expression has been reported in the cerebellum, our study aims to investigate the possible functional and modulatory roles that Ndp plays in this region of the brain, and its relationship to the Shh pathway. We have examined the spatial and temporal expression profile of Ndp in relation to Shh pathway components, as well as the role of Ndp in GNP proliferation and survival. This investigation was carried out in vivo in transgenic mice and in vitro using primary GNP cell cultures. Our data shows that during postnatal cerebellum development, Ndp is expressed in neural progenitor cells located in the external granule layer, during a period of Shh mediated proliferative expansion. Investigating the relationship between Hh and Ndp in cerebellar development has the potential to identify novel therapeutic targets for the treatment of medulloblastomas.

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113 **The Cellular and Molecular Mechanisms of XIAP Neuroprotection in Photoreceptor Cells**

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Abstract withheld.

Other

114 Effects of Folic Acid Supplementation on Placental Health & Function

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Background:
Though white flour products have been fortified with folic acid (FA) in North America, daily FA supplementation throughout pregnancy is commonly recommended to decrease the rate of neural tube defects in newborns. However, it is estimated that many pregnant North American women taking supplements in addition to their diet, have FA intakes that exceeds the tolerable upper intake level (UL). Human and animal studies have shown that excessive FA intake may be associated with increased insulin resistance, respiratory impairment, mammary tumorigenesis, and impede embryonic development. However, effects of excessive FA on the fetoplacental unit are unclear. It is hypothesized that exceeding the UL for FA may negatively impact placental health and function, putting the developing fetus at increased risk for adverse health outcomes.

Methodology:
1. A subsample of 269 2nd trimester, 3-day food records of pregnant women from The Integrated Research Network of Perinatology in Quebec and Eastern Ontario was analyzed for FA intake. 2. HTR8 trophoblast cell line was exposed to increasing concentrations of FA (2 - 4000 ng/mL) over a 48-hour incubation period (N=3). Dose-response experiments were performed at both 3% and 21% oxygen. Cell counts were performed and cell viability was assessed using trypan blue exclusion. HTR8 cell proliferation was also assessed using BrdU incorporation.

Results:
1. Majority of pregnant women studied were found to be at risk of inadequate intake of dietary folate equivalents. All pregnant women taking prenatal supplementation containing 1 mg FA are at risk of excess FA consumption. 2. No differences in total cell viability or cell proliferation were observed across the increasing doses of FA in the HTR8 cell line. Oxygen tension also had no effect on these parameters. Measurements of cellular invasion and hCG production are currently ongoing.

Conclusion:
Majority of pregnant Canadian women are at risk of inadequate folate intake from diet alone and require a supplement to reach recommended intake levels. High concentrations of FA do not seem to affect placental cell health as measured by cellular viability. Investigations into the potential effects of these concentrations of FA on placental cell function are ongoing. Additional research is needed to confirm that FA intake past the currently established UL for pregnancy does not harm the feto-placental unit.

115 Understanding Chronic Pain in the Emergency Departments: What are the Characteristics of Patients who Seek Treatment in the Emergency Department?

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Background
Chronic pain (CP) complaints account for 11-14% of emergency department (ED) visits, with 7% of patients visiting frequently. At The Ottawa Hospital (TOH) in 2012-2013, 12.9% of all visits were associated with CP. Managing CP in acute care settings is costly, generally ineffective, and contributes to ED overcrowding. To develop solutions, we need to understand what leads patients with CP to the ED.

Objective
Our study aims to define the demographic and medical characteristics of patients who use TOH ED for their chronic or recurrent pain, understand the factors leading to ED visits, and investigate patients' interests in alternatives.

Methods
This is a cross-sectional study using mixed-method combining surveys and one-on-one interviews. We report our survey data. Participants were medically stable adults who presented to the ED for chronic or recurrent pain during one of the 30 randomly selected shifts in July or August 2013. Medical residents conducted the interview and administered questionnaires measuring pain

intensity and interference, beliefs about pain, psychological distress, and signs of substance dependence. The interview focused on patients' understanding of their conditions, the process that led to the ED visits, coping skills, and interest in alternatives.

Results
Fifty-nine patients (M age = 46.43, SD = 16.82) agreed to participate. The majority were women (64.4%), Caucasian (79.9%), had a family physician (85%), and were on disability (32.2%). The most prevalent medical comorbidities were Asthma (33.9%), Arthritis (27.1%), Hypertension (27.1%), and Diabetes (20.3%). The primary reasons for the ED visits were an inability to cope with pain, worry about the cause of pain, and recommendation by family physicians. Participants reported high rates of pain intensity and they endorsed a high degree of helplessness, tendency to ruminate, and to focus on their pain. More than half of the participants (57.6%) had moderate or severe depression, 42.4% had anxiety, 33.9% had evidence of PTSD and 32.2% had evidence of substance-dependence. Participants were interested in more effective medications for their pain (50.8%), having a health professional explain their pain, and in a Pain Clinic referral.

Conclusion
Patients come to the ED primarily because they feel they are unable to cope with their pain. We also found a high incidence of medical and mental health problems among patients presenting to the ED for CP. To address such combination of medical and mental health issues, we are developing an interdisciplinary program to address chronic pain in the ED at TOH.

116 A Smartphone Approach for the 2 and 6 minute Walk Tests

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The 2 and 6 minute walk tests (2 or 6MWT) are used by rehabilitation professionals as a measure of exercise capacity (i.e., the maximum distance walked in 2 or 6 minutes). Our research has produced a new 2 or 6MWT BlackBerry Smartphone application that can be used to run the 2 or 6MWT and also provide new information about how the person moves during the test.

The BlackBerry is worn on a belt at the lower back to record phone sensor data while walking. This data is used to identify foot strike, calculate the total distance walked and step timing, as well as measure acceleration and range of pelvic motion. This provides new information on symmetry, walking changes over time, and poor walking patterns that is not currently available from a typical 2 or 6MWT, and could help with clinical decision-making.

117 Understanding the Burden of Chronic Pain at The Ottawa Hospital Emergency Department

Dr. Catherine Smyth[1], **Amanda Carson**[2], Jennifer Nelli[1,3], Steven Tremblay[1,3], Rebecca Small[4], Myka Caluyong[2], Aaron Zambrana[2], Yaad Shergill[2,5], Dr. Patricia Poulin[1]

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5. The Canadian Memorial Chiropractic College

Background:
Chronic pain (CP) patients account for 11-14% of all emergency department (ED) visits. Managing CP in acute care settings incurs high costs without significant progress made towards long-term solutions. The use of EDs for non-urgent medical conditions contributes to overcrowding and diverts resources away from patients in need of immediate attention. This study focuses on understanding the role of CP at The Ottawa Hospital (TOH) EDs to inform the development of an out-patient chronic pain management program.

Objectives:
The purpose of this study was to 1) estimate the proportion of CP-related visits to TOH ED; 2) to estimate the proportion of patients with CP amongst frequent ED visitors; and 3) to describe the demographic and medical characteristics of patients with CP who frequently use ED services.

Methods:
We conducted two retrospective reviews. First, we reviewed a random sample of 1000 visits from the 2012-2013 FY to establish the proportion of ED visits related to CP. Second, we reviewed the records of all patients who had visited TOH ED 12 times or more during the same period to ascertain the proportion of patients with CP and to collect medical and demographic characteristics.

Results:
We found that in 2012-2013, 12.9% of visits from the random sample were related to CP. Among the 255 patients who presented twelve or more times to the ED, 36.5% had CP and visited the ED on average 19.59 (SD = 11.6) times. Frequent visitors with CP were middle aged (M = 47.5; SD = 16.5), and primarily women (59%). CP-repeated admissions were related to back pain (21.5%), abdominal pain (21.5%), musculoskeletal pain (11.8%), chest pain (9.7%), and headaches (5.3%). In terms of co-morbidities noted in

charts, 80.6% of frequent ED visitors with CP had other medical problems, the most prevalent being hypertension (26.9%), gastro-intestinal disease (24.7%) and diabetes (18.3%). 41.9% of ED patients with CP showed evidence of prior or co-occurring mental-health problems such as depression, anxiety, substance dependence or personality disorders. The number of medical and mental-health related co-morbidities was positively correlated with the number of ED visits ($r = .234$, $p < .05$).

Conclusion:
There is a high prevalence of CP related visits at TOH EDs. The medical and demographic information assessed in this study may be used to inform the development of future innovative CP management for TOH patients who live with chronic pain.

118 **Should magnetic resonance imaging for tumours of the musculoskeletal system be performed in a sarcoma-designated health care centre?**

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Background:
Reporting of magnetic resonance imaging (MRI) findings are crucial in the management of primary musculoskeletal (MSK) tumours; reports dictate patterns of referral, guide treatment decisions and aid in pre-operative planning.

Objective:
1) to evaluate the concordance of MRIs of musculoskeletal lesions reported outside of a high volume sarcoma centre with expert second opinion reports, 2) to evaluate the accuracy of reporting technical details, descriptive and interpretive characteristics

Methods:
A retrospective review identified 521 consecutive surgical referrals to a multi-disciplinary sarcoma centre from July 2007 to June 2011. Two hundred and fifty one patients (117 females; 134 males) presented with an MRI performed in a referring institution. An adjudication panel of 4 MSK-trained radiologists and one orthopedic oncologist evaluated all diagnostic-imaging studies independently. Four categories were assessed: 1) technical details, 2) descriptive characteristics, 3) interpretative characteristics, and 4) global impression; the latter two areas were graded on a 1-5 scale. Interpreters were blinded to reporting institution and pathology diagnosis. Disagreement was resolved through consensus opinion, and percent agreement was recorded (93%). Exclusion criteria included MRIs lacking a report, imaging of a previously resected tumour bed, known metastatic lesions as well as incidental findings.

Results:
83 of 251 MRI reports (33%) showed discordance between the initial report and secondary interpretation. The discrepancies observed were inadequate tumour descriptors in up to 38%; one in three reports did not discuss proximity to neurovascular structures or presence of heterogeneity when it was indicated. Presence and characterization of edema, an important characteristic for surgical planning, was missing in 24% of reports. Discordance in differential diagnoses occurred in 30% of reports, and inappropriate recommendations were made in 15%. Technical sequencing errors occurred in 5 to 43% of studies, which limited the quality of interpretation or required repeat scans for contrast enhancement. Major discriminators, defined as having the capacity to alter clinical care, were identified by the clinician in 69% of inaccurate MRI reports.

Conclusions:
This study identified a significant proportion of discordance in MRI interpretation between referring centres and sarcoma-designated units. Our findings suggest that more accurate reporting for suspected musculoskeletal neoplasia may be achieved by disseminated guidelines and synoptic reporting, or by collaboration with a centre with expertise in musculoskeletal neoplasia.

119 **Transtibial Amputee Gait and the Effect of a Weighted Pack.**

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Background:
Backpack load carriage is essential for many hobbies and professions including, but not limited to, hiking and being a member of the military. However, biomechanical knowledge involving load carriage is minimal for the amputee population. People with lower extremity amputations may walk with asymmetries and as a result have greater knee joint forces on their intact limb, potentially contributing to osteoarthritis or other overuse injuries. Since backpack loads are expected to increase joint forces, the effect of this additional load requires investigation.

Methods:
Ten male, K4 level, traumatic, transtibial amputees walked across three different surfaces (level ground and ramp ascent/descent) with (WP) and without (WOP) a 24.5 kg backpack load. Three-dimensional motion analysis data, ground reaction forces (level

ground), and FScan plantar pressures were collected for 5 trials per pack condition. Motion data was analysed for all surfaces, knee forces and impulses were analysed for level ground, and impulses from in-shoe plantar pressures were analysed for ramp ascent and descent.

Results:
The weighted backpack produced significantly greater knee force and knee impulse components ($p<0.001$), with the largest differences found for axial forces before normalization. Interestingly, the people who had larger intact limb loads also had greater knee loads between backpack conditions. While descending the ramp plantar pressure analysis displayed significantly greater impulses when descending the ramp with a backpack load, which may be an indicator for potentially greater knee loading when descending inclines. Other key findings include greater difficulty on the ramp down surface, and ankle-foot system deformation due to the weight of the backpack. Further research with kinetics for surfaces other than level ground with backpack loads is required.

Conclusions:
This research enhances our understanding of weighted amputee gait. The additional load produced approximately 25% greater forces and impulses at the knee. For the people who rely more on their intact limb, the loading effect is enhanced. With increased weight both forces on the intact limb, and deformation of the prosthetic ankle-foot could become an issue. These outcomes support the need for appropriate prosthetic component development and selection, prosthetic fitting, and gait training to enhance limb loading symmetry during load carriage activities. The high-load population could reduce the risk of long-term wear and injuries by reducing intact limb loads. Knee load outcome measures could determine if clinical adjustments or more gait training are required. Lastly gait training should involve ramp descent as it proved to be the most difficult surface.

120 Virtual reality exercise therapy in stroke rehabilitation improves outcomes – a randomized study

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Background:
Innovative methods of delivering stroke rehabilitation would contribute to the provision of optimal treatment to stroke survivors. Impaired balance has been identified as a key component of mobility problems post stroke. Exercise training using virtual reality (VR) — interactive simulations created with computer hardware and software — has been shown to improve balance in adults with traumatic brain injury and hemiparetic stroke survivors. However, rigorous randomized studies regarding its efficacy, safety and applicability are lacking.

Objective:
To determine whether VR therapy, as an adjunct treatment, 1) improves balance, weight bearing on the affected side and exercise performance in stroke rehabilitation inpatients, and 2) is safe and feasible.

Methods:
Blinded randomized controlled trial (RCT) studying 59 stroke survivors newly admitted to the Élisabeth Bruyère Hospital inpatient stroke rehabilitation unit over 3 years. Participants had had an ischemic or hemorrhagic stroke in the cortical or subcortical region, had resultant balance and gait deficits, and could stand independently for >1 minute. The treatment group (n=28) received standard stroke rehabilitation therapy + a program of VR exercises that challenged balance (e.g., soccer goaltending, snowboarding) performed while standing. The control group (n=31) received standard stroke rehabilitation therapy + exposure to identical VR environments that provided visual stimulus while seated; there was no challenge to balance. The VR intervention consisted of 10 to 12 30-minute daily sessions. Clinical outcome measures were assessed before and at the end of training. Measures included the Berg Balance Scale (and, if =48, Community Balance and Mobility Test), Functional Independence Measure (FIM), Chedoke-McMaster Stroke Assessment, Two-Minute Walk Test (TMWT) and Timed Up & Go Test (TUG). Instrumented balance assessments and assessment of gaming improvement were done at the beginning of each exercise session.

Results:
Average FIM scores on admission were significantly higher for study participants than for nonparticipants (84.2±15.0 vs. 71.8±16.6, $p < 0.001$). Both the treatment group and the control group improved significantly on all outcome measures, with the treatment group improving more on the TMWT ($p=0.02$), TUG ($p=0.029$) and Chedoke-McMaster – Leg ($p=0.006$). Patient interest and satisfaction were high, and there were no adverse events.

Conclusions:
A VR intervention for inpatient stroke rehabilitation is safe and feasible and improves mobility-related outcomes. The inclusion criterion of standing unaided for >1 minute limited participant selection to higher-functioning stroke survivors. An RCT in

nonambulatory stroke inpatients is planned. The challenge now is to extend this intervention to more community rehabilitation facilities.

121 Too much evidence? Sweet solutions for newborn pain management

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Background:
Extensive evidence exists of analgesic effects of sweet solutions for newborn pain management. Fifteen years ago, a Cochrane systematic review of 10 randomized controlled trials (RCTs) of sucrose for pain management in newborns clearly demonstrated the analgesic efficacy of sucrose. Three years later the International Evidence-Based Group for Neonatal Pain included sucrose in their recommendations. Despite the evidence, RCTs of sweet solutions compared to placebo or no treatment continue to be published.

Objective:
To systematically review published studies evaluating sweet solutions for pain reduction in infants to ascertain when there was sufficient evidence of analgesic effectiveness.

Methods:
Databases searched included Medline, Embase, Cumulative Index to Nursing and Allied Health Literature database, and PsycINFO, using the terms pain*, infant*, neonat*, newborn*, sucrose, glucose, and alternative sugars. Cumulative meta-analyses using outcome measures of crying duration (in seconds) and standardized mean differences (SMD) in composite pain scores were conducted. Analyses were conducted using STATA Version 11 and verified using Comprehensive Meta-Analysis Version 2.0.

Results:
200 studies were identified, of which 179 (90%) had placebo/no-treatment groups. Cumulative meta-analysis for crying time included 22 trials and showed that from the first included RCT in 1995, the mean reduction in cry time for sweet solutions compared to placebo was – 14.14 seconds (95% Confidence Interval (CI), -17.23, -11.05) At no stage did the mean crying time or the 95% CI cross 0.00. Cumulative meta-analysis of the 22 RCTs shows a 22.40 second reduction (95% CI, -37.98, - 18.82). Cumulative meta-analysis for SMD for pain scores included 45 trials and showed that from the second included RCT in 1999, the SMD in pain scores for sweet solutions compared to placebo during painful procedures was – 1.01 (95% CI, -1.94, -0.07) Cumulative meta-analysis of the 45 RCTs conducted up to 2013 shows a -0.99 point-reduction in SMD pain scores (95% CI, -1.21, -0.76).

Conclusions:
A state of equipoise relating to analgesic effects of sweet solutions during single episodes of painful procedures in newborn infants has not existed since the publication of the first Cochrane Systematic Review in 1998. This is confirmed by our cumulative meta-analyses showing evidence of analgesic effects based on crying time, from 1995, and on pain scores, from 1999. It is unethical to conduct RCTs of sweet solutions with a placebo/non-treatment group. Sucrose or glucose should be considered standard of care for neonatal procedural pain trials.

122 Centre of pressure and total force analyses for amputees walking with a backpack load over four surfaces

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Background
People in professional, military, and recreational settings often carry a weighted backpack; however, the additional weight can result in injuries such as stress fractures, knee pain, and lower back pain. While previous research has shown that backpack loads affect ground level walking in able-bodied population, no biomechanical research is available for transtibial amputees. People with disabilities may use different walking strategies to accommodate to the extra weight.

Objective
The study objective was to examine backpack load effects on transtibial amputee gait while walking over level ground, uneven

ground, and slopes.

Methods
Nine subjects with unilateral transtibial traumatic amputations (K-4 functional level) were recruited through The Ottawa Hospital Rehabilitation Centre and the Canadian Forces Health Service. F Scan plantar pressure sensors were fitted to the participant's insole. Plantar pressure distributions were collected at 50 Hz while participants walked at a self-selected pace with no backpack (NP) and with a 24.5 kg backpack (WP) over four surfaces (level ground, uneven ground, incline, and decline). Centre of pressure (COP) and total force were analyzed.

Results
Few COP parameter changes were found when walking with a backpack, compared to no backpack, and the differences between load conditions were small for this high functioning amputee population. The backpack load resulted in increased total force for all walking surfaces. However, when normalized by total weight, total force was similar between backpack conditions. Double support time was greater when walking with a backpack, which is considered a compensatory strategy to enhance stability and/or reduce loading forces.

Conclusions
The backpack load was not a limiting factor for these real-world activities. High functioning individuals with transtibial amputations were able to accommodate to a standard backpack load and to maintain a similar COP progression compared to able-bodied results from the literature, even when walking on surfaces other than level ground.

123 **Cartilage Disease Modelling Using Induced Pluripotent Stem Cells**

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Pluripotent stem cells (PSCs) are a powerful tool for studying human development and disease in vitro due to their ability to self-renew and differentiate into all cell types of the adult body. Human embryonic stem cells (hESCs) and induced pluripotent stem cells (iPSCs) are PSCs but from different cell sources – hESCs are derived from the inner cell mass of the late stage blastocyst and iPSCs are derived from reprogrammed somatic cells that become hESC-like. iPSCs derived from disease patients allows modeling of disease progression since iPSCs can be differentiated towards affected tissue types. Campomelic Dysplasia (CD) is a skeletal malformation disease caused by heterozygous mutations affecting Sox9, a key transcriptional regulator of chondrogenesis. Neonatal death in humans is common due to a narrow thoracic cage and defective tracheobronchial cartilage resulting in respiratory distress but severity of the disease varies and some patients live on into adulthood. To better understand the molecular mechanisms governing this disease, we have derived and characterized iPSCs from two CD patients carrying different mutations in Sox9 (referred to as CD short and CD long) along with two healthy patient controls (referred to as WT). CD and WT iPSCs along with a human embryonic stem cell line (H9) were differentiated towards chondroprogenitors using a two-step process. Cells were first differentiated towards a mesendoderm population and subsequently differentiated towards chondroprogenitors through micromass culture. During mesendoderm differentiation, WT iPSCs and H9 hESCs exhibited increased expression of mesendoderm and epithelial-to-mesenchymal (EMT) genes, loss of epithelial morphology, and acquisition of a mesenchymal morphology. CD short iPSCs differentiated similarly to WT iPSCs and H9s. However, differentiated CD long iPSCs showed reduced expression of mesendoderm and EMT genes compared to WT and H9 hESCs and maintenance of an epithelial morphology. During chondroprogenitor differentiation, CD long iPSCs developed chondrogenic nodules later than WT iPSCs, H9s, and CD short iPSCs resulting in lower glycosaminoglycan deposition and lower expression of cartilage matrix genes and SOX9 cofactor genes compared to WT, H9s, and CD short iPSCs. Thus far, CD long iPSCs exhibit a delayed EMT response and mesendoderm differentiation which affects their capacity to differentiate towards chondroprogenitors. We have shown that iPSCs can be derived from CD patients, can be differentiated towards chondroprogenitors, and different mutations in Sox9 can lead to different phenotypic outcomes in vitro. Further, the assays we have developed can be applied to other diseases where cartilage tissue or chondrogenesis is affected.

124 **Stability of certain cognitive measures in older drivers over 1 year**

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Background: Older drivers still represent a large proportion of drivers with a valid driver's license. Multiple chronic conditions common to older drivers may affect their cognitive function and driving ability. The stability of cognitive measures widely used to assess older drivers' functional ability has been assessed over 2-week intervals.

Objective: The objective of this study is to identify the long-term stability of certain cognitive measures over the span of one year within the Candrive II sample, and to verify if a learning effect takes place over time.

Methods: A series of sensory, physical and cognitive measures were administered twice by the same researcher to 693 study participants during 2.5-4 hour clinic appointments one year apart. To determine those of stable health, exclusion criteria was created using data from; the Expanded Cumulative Illness Rating Scale, Older American Resources and Services survey, Mini-Mental State Examination (MMSE) and the Timed Up and Go (TUG) test. For those participants with stable health, the relative and absolute reliability of the trail making test A and B, months in reverse order, digits span (forwards and backwards), and the Montreal Cognitive Assessment (MoCA) were statistically analyzed.

Results: Across year one and year two assessments 85 of 663 (13%) participants were determined to be in stable health. Participants' age ranged from 70 to 89, with the majority of the participants being male (64.6%). All but four cognitive tests were found to have moderate reliability (ICC 0.52-0.69). Despite a moderate correlation coefficient, a significant (p=0.013) systematic improvement was observed for the trails making A test (M¬year1 = 35.0(±10.4), Myear2 = 37.8(±13.4)). A systematic effect was also observed for the road sign recognition test, however, no significant difference (p=0.584) was observed between years (M¬year1 = 11.09(±1.26), Myear2 = 11.00(±1.32)). Both SEM% and CV% were very similar for each cognitive measure. Only the MVPT and the months in reverse order (timed) assessments had measurement variability (SEM% and CV%) under 10%. The Digit span total scores and the road sign recognition tests had values between 10 and 20% with the remaining six assessments having variability measurements above 20%.

Conclusions: Our results indicate that in a healthy population, certain cognitive measures remain stable over one year with no learning effect. These results support the potential use of these measures in a screening tool to help safety professionals identify older drivers who may be unsafe to drive.

125 Correlation of diffusion tensor imaging (DTI) parameters with ASIA clinical injury motor scores in patients with traumatic spinal cord injury.

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Background: Diffusion tensor imaging (DTI) has been used to assess acute spinal cord injury (SCI), however, correlations with clinical symptoms is limited.

Objective: The purpose of the following study was to investigate the correlations between spinal cord DTI parameters and clinical presentation following acute SCI.

Methods: Three patients with cervical ASCI (male:female=2:1, mean age=28±10.5) and eight healthy controls (male:female=3:2, mean age=25±4.7) were evaluated. Axial diffusion weighted SS-EPI images were obtained using a 1.5T Siemens MR scanner. A region of interest (ROI) was used to evaluate the spinal cord at the injury level and on five axial sections (25mm) cranial and caudal to the injury level. Student t-test was used to compare regional fractional anisotropy (FA) and mean diffusivity (MD) and these values were assessed for correlation with ASIA motor scores.

Results: Two patients had complete motor and sensory loss below the level of their SCI and one patient had a central cord syndrome with hand weakness and dysesthesia bilaterally. In all patients, cranial DTI metrics did not differ from controls, however, caudal FA (p=0.001) and MD (p=0.004) values were significantly different when compared to controls. DTI measurements were not significantly different between the patients with complete SCI and the patient with central cord syndrome.

Conclusion: Our study demonstrated that DTI parameters could identify patients with spinal cord injury. Current measures cannot differentiate between complete SCI and SCI resulting in central cord syndrome. Future studies will evaluate other DTI measures that may refine diagnosis and improve prognosis with respect to patients with SCI.

126 **Learning to segment with dynamic programming framework**

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A vital part of medical images analysis is image segmentation. However no segmentation method can be applied fully autonomously with definite results in all kind of images. Especially in the presence of strong clutter many state of art methods fail. Here, we present supervised image segmentation in the dynamic programming (DP) framework to segment image interactively. The proposed segmentation technique learns a cost function from a labelled training object. The dynamic programming is applied to optimize the cost function which is the weighted combination of different features learned from labeled object boundary. Our experiments illustrate the proposed interactive segmentation method ?nds precise object boundaries based on very small set of training sample even in the presence of strong clutter and weak object boundary.

127 **Flow Cytometry and Fluorescence Activated Cell Sorting (FACS) Facility**

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Flow cytometry is the science of examining physical and chemical properties of live cells or other biological particles as they pass in a fluid stream through a measuring apparatus. This technique uses laser light scattering and/or fluorophore detection to measure cell populations. Some flow cytometers are equipped to separate and collect cells of interest in a technique called Fluorescence Activated Cell Sorting (FACS).

Our Flow Cytometry and Cell Sorting Facility is jointly operated by the Ottawa Health Research Institue (OHRI) and StemCore Laboratories. We provide academic and corporate clients with access to flow cytometry analysis as well as high-speed cell sorting services. We also offer comprehensive training and education as well as expert consultation services to enable our users to utilize this technology to enhance the scope and quality of their research.

Our Beckman Coulter MoFlo instrument can be used for high speed cell analysis and sorting (up to 20,000 cells/second). This recently upgraded system contains five excitation lasers, two light scatter detectors, and ten fluorescence detectors, allowing for the analysis of up to nine fluorescence colours at once. Specifically, we are equipped with a UV laser, as well as a 405 nm (violet), 488nm (blue), 561nm (green), and 640nm (red) laser. The two light scatter detectors are forward scatter (cell size) and side scatter (cell granularity). Additionally, the ten fluorescence detectors are completely customizable to the experiment at hand.

The following services are available with the current configuration of the MoFlo:

- Immunophenotyping/immunofluorescence by monoclonal antibodies.
- Total/Absolute population counts.
- Simultaneous analysis of surface and intracellular fluorescent labelling.
- Monitoring gene transfer and expression using fluorescent proteins.
- Simultaneous detection and sorting of GFP and various red fluorescent proteins.
- FRET measurements using GFP and RFP or DSRed.
- DNA content, cell viability, and cell cycle analysis using PI, Hoechst, BrDU, etc.
- Hoechst 33342 side population (SP).
- Cell viability with exposure to drugs, toxins, etc.
- Apoptosis.
- Telomere length measurement.
- Neutrophil functions (phagocytosis and oxidative burst).
- NK cytolytic activity.
- MLR / CTL cytotoxicity.
- Caspase activity.
- Mitochondrial function (DiO, JC-1)
- Intracellular Calcium Flux.
- Q-Prep whole blood lysis.
- Customizable fluorescence assays.

In addition, up to four subpopulations can be sorted at high-speed into bulk collection tubes or multi-well plates for single cell clonal expansion. We will be presenting results from a few of the above-mentioned techniques that have been performed in our facility. Specifically, FACS sorting of satellite cells from mice, SP sorting, and cell cycle analysis results will be presented.

128 Anatomical repair of the distal biceps tendon cannot be consistently performed through a classic single incision suture anchor technique

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Background: Occurring most commonly among young men, distal biceps tendon ruptures have been repaired using a number of surgical methods. The current study was done to assess the result of the single incision technique using suture anchors to provide a consistent anatomic repair to the ulnar aspect of the radial tuberosity.

Objective: The objective of this study is to determine the location of surgical anchors in relation to the redial tuberosity following single incision repair. Then determine elbow flexion and forearm supination strength following a minimum of one year recovery of the injured and non-injured arm to determine the functional outcome of the technique in relation to the location of the suture anchor

Methods: A retrospective review of acute distal biceps tendon repairs using the single incision technique was completed. Computer Tomography (CT) scans were obtained to investigate tuberosity dimensions and the position of the suture anchors. Using a isokinetic dynamometer, flexion and supination strength were obtained. Disabilities of the Arm, Shoulder and Hand (DASH) scores were collected.

Results: Computer tomography of 27 participants showed that suture anchor placement were on average 50 degrees radial to the apex of the radial tuberosity. This deviation did not affect flexion strength which was found to be 97 – 106% that of the contra-lateral arm. Supination strength (80-86%) and work performed (66-75%) was however significantly ($p<0.05$) weaker on the repaired side. DASH scores reported excellent recovery with an average score of 10 ± 7.

Conclusions: Anatomical suture placement on the ulnar aspect of the radial tuberosity could not be reliably achieved using through the single incision technique. Clinically this may have importance as the deviation in anchor placement reduced the mechanical advantage of the biceps to supinate the forearm.

129 Café Scientifique – Community KT through Public Parleys

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Background: The “Café Scientifique” grant program is an innovative initiative funded by the Canadian Institutes of Health Research (CIHR). The goal of a Café Scientific is to bring together researchers with members of the public to spark a discussion about some of the most interesting research currently underway in Canada.

Objectives: The Canadian Cochrane Centre (CCC) applied to the Canadian Institutes of Health Research’s (CIHR) Café Scientifique funding program to hold a public discussion titled, “Health information in the age of the internet. Why Google your health questions when you can Cochrane them?” The goal of this particular Café Scientifique was to inform the local public about a valuable online resource in which they could research reliable health treatment evidence: The Cochrane Library. Given that one in three adults search health information online, it is crucial that they understand which sites are reputable and have the best evidence on which to base their health decisions.

Results: The CCC’s application was successful and ranked sixth out of 82 approved applications (94 submissions). The Café was a tremendous success. There were 73 attendees and all feedback received was positive. The Café was held just after working hours in a pub which provided an ideal time of day and atmosphere. Many participants inquired as to when we would be holding our next event. The Canadian Cochrane Centre’s website received a significant increase in visitors after the Café with 186 visits on 5 March 2013, 619 visits the day after the Café (6 March), returning to the norm with 174 visits the following day. Additionally, there were 2036 page views on 6 March, up from the regular 500+. Furthermore, The Cochrane Library saw an increased number of Canadian visitors after the Café Scientifique: 4 March, 611; 5 March, 1662; 6 March, 3169; 7 March 1186; and 8 March 729.

Conclusions: The Café Scientifique program provided a relaxed atmosphere in which the public could feel comfortable engaging with scientists/researchers/physicians with whom they often do not have the opportunity to speak. Café Scientifiques are a great way to engage the public in learning about The Cochrane Collaboration, The Cochrane Library and Cochrane Review evidence. Given that many of today’s interactions take place online, this face-to-face event was a unique opportunity to bring the public and researchers together.

130 Comprehensive Health Care Provider Education in Long Term Care: Improving Stroke Care

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Background
Over 50,000 Canadians sustain a stroke each year and for every 100 stroke survivors, 10 are so significantly disabled that they require long-term care (LTC). The Champlain Regional Stroke Network's (CRSN) goals are fewer strokes, better outcomes through dissemination of best practices to reduce practice variation and to decrease the knowledge to practice gap. Quality of health care has been shown to improve when research findings on best practices are incorporated into every day approaches to care.

There is no one best way of transferring health care evidence to practice, although using an interactive approach has been found to be more effective than didactic methods. Currently there is little research regarding knowledge transfer and improved practice in the care of older adults.

Objectives
The objective of this study was to evaluate the effectiveness of regional, interactive knowledge transfer workshops on health care providers' perceptions of their abilities in the delivery of post-stroke care.

Methods
A full day, seven-hour, workshop was offered by CRSN on three separate occasions. The workshops were taught by a team of content experts using resources from the Heart and Stroke Foundation of Ontario based on scientific evidence of best practices for personal support workers in LTC. An 11-item questionnaire was used to gather participants' perceptions on their abilities. To increase the sensitivity of the questionnaire, an "ipost-post-testi design" was used after workshop completion, in which participants were asked to reflect on and rate both their current and prior level of abilities in delivering post- stroke care and provide comments on the workshop. Information collected was anonymous.

Results
Health care workers (61) in LTC attended the workshops. On average, participants perceived their abilities in post-stroke care prior to the workshop at 3.35 / 5. Their rating of their perceived abilities increased to 4.4 / 5 after the workshop.

Health care providers' comments indicated that participants recognized the value of the interactive workshop as:

- An opportunity to ask questions of content experts
- An effective way to acquire helpful information they could implement in their practice
- A means to obtain information on essential equipment for safe practice

Conclusions
Participation in a knowledge transfer workshop that encouraged uptake of best practices in post-stroke care resulted in improvement in participants' confidence and perceptions of their knowledge and abilities to manage post-stroke care effectively.

A weakness of this study is that effectiveness is measured by a questionnaire of perceptions of learning. Ideally, future studies will measure practice change outcomes.

131 Development of a human activity recognition system using inertial measurement unit sensors on a smartphone

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Background
Monitoring the mobility of an individual through the use of a modern smartphone can have a profound impact on rehabilitation in the community.

Objective
The objective of the research is to develop and evaluate a third-generation Wearable Mobility Monitoring System (WMMS) that implements inertial measurement units to assess the categorization of activities and determine user changes-of-state in a daily living environment. A custom suite of MATLAB software tools are developed to assess the quality of the previous WMMS iteration and aid in the construction and evaluation of the third-generation WMMS algorithm.

Methods
Experimental evaluation of the third-generation WMMS algorithm is performed on fifteen able-bodied subjects with data from a BlackBerry Z10 smartphone. The MATLAB software allows for offline data manipulation which replicates real-time feature

generation and activity state prediction. The predicted user activities include sitting, standing, lying, walking, climbing stairs, and performing small movements. A video event can be triggered on change-of-state, allowing the device to record a video clip which can be used to further assess the mobility state or evaluate the subject's surroundings.

Orientation filters such as the gravity vector and applied linear acceleration components are investigated. A rotation matrix is developed to orient the smartphone in any three-dimensional position to improve accelerometer-based activity identification. The quaternion-based rotation matrix is constructed from an axis-angle pair, produced via algebraic manipulations of acceleration components in the device's body-fixed frame of reference.

Results
Algorithm detail subsets are examined as an evaluation control in the development and useful measurement of activities with modern mobility monitoring systems. Applying a rotation matrix to user data provides a consistent accelerometer orientation between people, thereby reducing smartphone placement variability that can adversely affect activity classification and user change-of-state.

Conclusions
The WMMS can be used to gauge in smart real-time mobility monitoring for extensive rehabilitation outside of a clinical setting.

132 **Improving Treatment of Chronic Pain in the Community through a Preceptorship-Based Continuing Medical Education Program: Patient Characteristics and Physicians' Interest.**

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Background
Chronic pain (CP) affects 20% of Canadian adults. Family physicians (FP) are often uncomfortable treating patients with complex CP. Access to pain clinics is limited with waiting periods often reaching over a year.

Objective
PainConnect is an innovative program including Preceptorship-based continuing medical education for FP. From January 2011 to March 2012, five pain specialists visited 122 FP in the Champlain region. This study explores patients' characteristics and FPs' interest in the preceptorship.

Method
We conducted a retrospective chart review of all PainConnect patients. This included the collection of demographic, medical characteristics, and clinical assessment tools such as the Brief Pain Inventory (BPI), the Patient Health Questionnaire-9 (depression) (PHQ-9), and the Opioid Risk Tool (ORT).

Results
There were 343 charts reviewed. Patients were predominantly Caucasian (77.3%), women (56.3%) and had a mean age of 52.1 years (SD = 14.3). Patients had been in pain for an average of 10.7 years (SD = 9.8), reported a mean Pain Severity Score of 7.1 (SD = 1.5), and a mean Pain Interference Score of 7.3 (SD = 1.8). Close to a third (30.3%) of patients reported not knowing the cause of their pain and that it just began. A minority of patients (9.0%) reported being hospitalized for their pain. The majority of patients (70.3%) were currently being treated with opiates. In terms of psychological factors, 48.7% had been diagnosed with depression, 11.7% had a form of anxiety, and 55.1% endorsed having sleep difficulties nearly every night. Almost a fifth (17.2%) of patients had scores on the Opioid Risk Tool indicating high risk of opioid abuse, 11.1% had problems with street drugs, and 7.9% had problems with opioid abuse.

Conclusions
Most patients referred to the PainConnect program reported severe pain causing a high degree of disability. The severity and complexity of the patients' presentation in this project suggests that additional training and support from pain specialists for FPs is needed, and that in some cases other models of care (e.g., shared care with an interdisciplinary program) might be indicated. We believe that ongoing medical education for FPs would ultimately result in better patient care, lower wait list periods, better management of opioid prescriptions, and a better quality of life for patients.

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:

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- Masters
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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

The scale should be applied as follows:

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%.

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