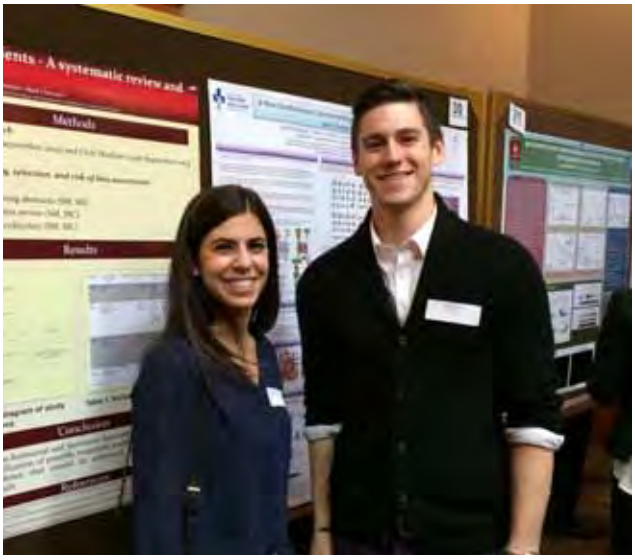
Ottawa Hospital
Research Institute
Institut de recherche
de l'Hôpital d'Ottawa

2015 RESEARCH DAY




Program and Abstracts

Wednesday, November 18, 2015
7:30 a.m. – 5:05 p.m.

St. Elias Centre
750 Ridgewood Ave.
Ottawa, ON

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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
Jennifer Ganton
Dr. Luc Sabourin
Dr. Jay Baltz
Dr. Jim Dimitroulakos
Dr. Jelena Ivanovic
Dr. William Stanford
Jane Canniff
Dr. Dean Fergusson
Dr. Ian Lorimer
Dr. Duncan Stewart
Dr. Ketul Chaudhary
Dr. Anouk Fortin
Dr. Tim Ramsay
Dr. Catherine Tsilfidis

Volunteers

Greg Canham
Melanie Genereaux
Wayne Lowe

WELCOME TO RESEARCH DAY

Duncan Stewart, MD, FRCPC

Executive Vice-President, Research, The Ottawa Hospital

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

Evelyne and Rowell Laishley Chair

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

Today, we celebrate and showcase the outstanding work of our young researchers. Their insight, passion and commitment to scientific excellence are critical to our success as one of Canada's top research hospitals, ranking 3rd overall in terms of funding from the Canadian Institutes of Health Research.

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

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

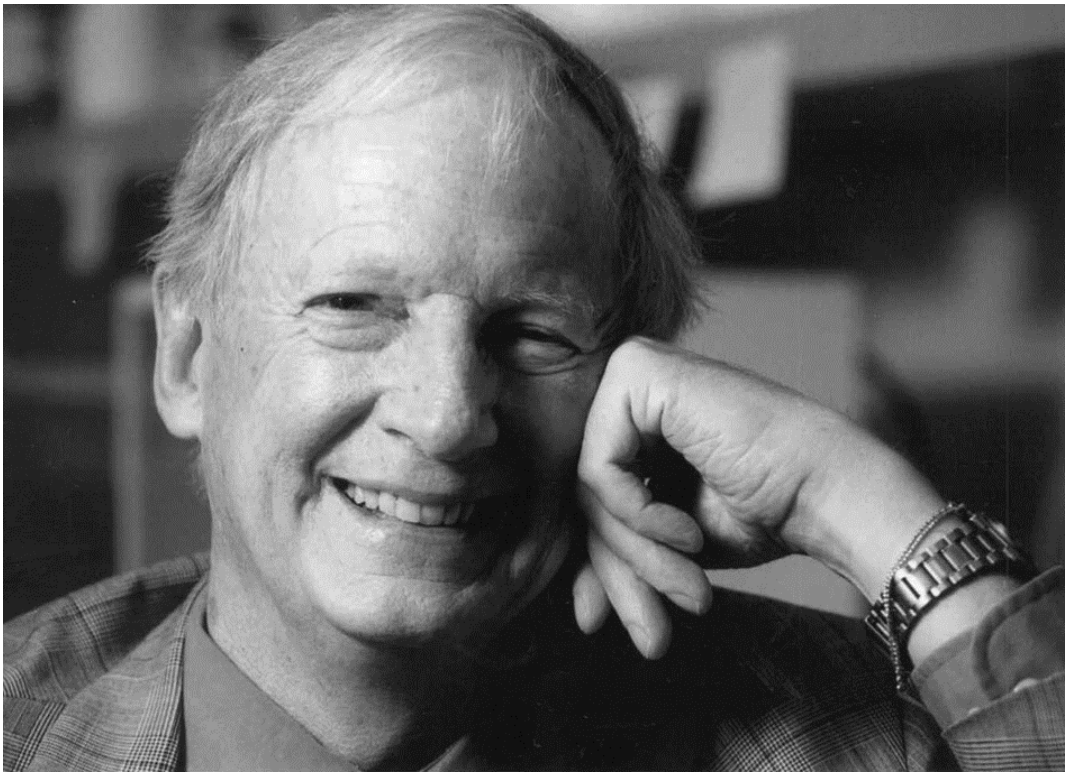
The first will be given by Dr. Peter Zandstra, a world-renowned pioneer in stem cell bioengineering. Dr. Zandstra's research has generated important insights into the fundamental mechanisms that control stem cell fate, and led to the development of robust technologies for the use of stem cells and their derivatives to treat disease. The title of his talk today is "Patterning mesoderm and blood development from human pluripotent stem cells".

The second lecture will be given by Dr. Harvey Max Chochinov. Dr. Chochinov is one of the world's leading experts in palliative care - defining best practices for helping people near the end of life. His talk, titled "Dignity, Personhood & the Culture of Medicine" is incredibly timely given the Supreme Court's recent ruling that Canadians have a right to physician-assisted suicide.

I would also like to draw your attention to the 5th Annual IMPACT Award. Standing for "Identification of Marketable Products, Applications and Commercializable Technologies," the IMPACT Award is designed to encourage our researchers to consider how their work could lead to innovations and to identify technologies, products or services that stem from their work. The IMPACT Award is part of our larger effort to create a culture that is proactive in translating research into benefits for Canadians. You will find the finalists' posters in the lobby.

On behalf of everyone at the Ottawa Hospital Research Institute, 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 tables.

DR. J. DAVID GRIMES LECTURE



Dr. J. David Grimes, MD, FRSPC

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

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

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

DR. J. DAVID GRIMES LECTURE

"Patterning mesoderm and blood development from human pluripotent stem cells"

Dr. Peter Zandstra

Dr. Peter Zandstra is a Professor at the University of Toronto and holds the Canada Research Chair in Stem Cell Bioengineering. He is also the Chief Scientific Officer of the Centre for the Commercialization of Regenerative Medicine and Co-Director (with Dr. Janet Rossant) of the Ontario Institute of Regenerative Medicine. Research in Dr. Zandstra's laboratory is focused on the generation of functional tissue from adult and pluripotent stem cells. His group's quantitative, bioengineering-based approach strives to gain new insight into the fundamental mechanisms that control stem cell fate and to develop robust technologies for the use of stem cells and their derivatives to treat disease.

KEYNOTE LECTURE

"Dignity, Personhood & the Culture of Medicine"

Dr. Harvey Max Chochinov

Dr. Harvey Max Chochinov is a Distinguished Professor of Psychiatry at the University of Manitoba and Director of the Manitoba Palliative Care Research Unit, CancerCare Manitoba. His seminal publications addressing psychosocial dimensions of palliation have helped define core-competencies and standards of end-of-life care. He holds the Canada Research Chair in Palliative Care, is an Officer of the Order of Canada and is a recipient of the Order of Manitoba. He is the Chair for the Canadian Virtual Hospice, a Fellow of the Royal Society of Canada and a Fellow of the Canadian Academy of Health Sciences. His most recent book, *Dignity Therapy: Final Words for Final Days*, is published by Oxford University Press and was the 2012 winner of the American Publisher's Association Prose Award for Clinical Medicine.

DR. GOODMAN COHEN SUMMER STUDENT AWARDS

Every year, the Ottawa Hospital Research Institute holds the summer student seminar series, which gives students the opportunity to present their research to other students. This year, more than 50 students participated from throughout the Institute, ranging from high-school students to newly graduated Bachelor's students. Awards are given for the best presentations, based on both peer and coordinator evaluations. The students then submit a written paper and the top students are awarded the Dr. Goodman Cohen Summer Student Award. This year they competed in two categories: Senior (returning students) and Junior (new students).

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

Winners of the Dr. Goodman Cohen Summer Student Award

Senior Award

Olivia Cook (supervised by Venkatesh Thiruganasambandamoorthy)
"Reasons for Referrals and Hospital Admissions among Emergency Department Syncope Patients"

Junior Award

Lubina Nayak (supervised by Rodney Breau)
"Continence and Complications in Patients with Neobladder Diversions"

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

Amelia Aitken
Almohanad Alkayyal
Khalid Al-Zahrani
Leonard Angka
Marc Thomas Avey
Andrew Aw
Justine Baron
Katherine Baxter
Laura Boland
Marie-Claude Bourgeois-Daigneault
Caroline Brun
Adriana Carbonel
Natasha Ching-Yun Chang
Shazia Hira Chaudhry
Carolina Cieniak
Jennifer Collins
Jillian Conway
Juthaporn Cowan
Sarah Cummings
Charlotte Dafoe
Kristin Danko
Colin Davis
Genevieve de Caen
Marc-Olivier Deguise
Nicolas Dumont
Heidi Dutton
Daniel El Kodsi
Mehdi Eshraghi
Zach Ferraro
Stacey Fisher
Megan Fitzpatrick
Nicole Forbes
Alexander Gont
Daniel Guindon
Tavis Hayes
Mirabelle Ho
Amy Hsu
Hua Huang
Danton Ivanochko
Lisa Marie Julian
Brian Keller
Faizan Khan
Sarwat Khan
Samantha Kornfeld
Ramya Krishnan
Melanie Lacaria
Manoj Lalu
Caroline Lefebvre
Jung Jin Lim
Patricia Lima
Marissa Lithopoulos
Chao-Chia Lu
Anisha Lynch-Godrei
Taylor McClatchie
Ines Midzic
Sedah Mo
Leslie Nash
Jennifer Petkovic
Larissa Pikor
Benjamin Pryce
Charis Putinski
Nischal Ranganath
John Saber
Bratati Saha
Teslin Sandstrom
Aimee Sarti
Mohammed Selman
Mehdi Shafa
Melissa Snyder
Roger Stanev
Colin Suen
Maxwell Sunohara
Mohamad Taha
Lee-Hwa Tai
Jean-Francois Thibodeau
Jacqueline Tokarew
Bruno Trindade
Anne Tsampalieros
Hideaki Tsuyoshi
Yu Xin Wang
Sarah Wassmer
Nour Yhahfoufi
Yifan Yuan
Boyang Zhang
Meiying Zhang

OHRI RESEARCH DAY PROGRAM

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

8:15 AM **OPENING REMARKS** (Duncan Stewart, Fraser Scott)

8:30 AM **VIRUS AND CELL-BASED THERAPEUTICS** (50 Minutes)
(Talks: 9 minutes plus 3 minutes discussion)
Moderators: **Alex Gont** and **Katherine Clark-Knowles**

- **Colin Davis** (Jean-Simon Diallo Group) Comparative study of colon cancer subclones uncovers new elements associated with cellular antiviral response
- **Dominic Roy** (John Bell Group) Insect Cell Carriers For Systemic Delivery of Oncolytic Viruses
- **Lee-Hwa Tai** (Rebecca Auer Group) Phosphodiesterase-5 inhibition augments postoperative natural killer cell antitumor immunity by reducing myeloid-derived suppressor cell function
- **Nischal Ranganath** (Jonathan Angel Group) Latently HIV-1 infected cells have defects in type I IFN response that can be exploited by VSVdelta51 and MG1 viruses

9:20 AM **SIGNALING CHANGE** (50 Minutes)
(Talks: 9 minutes plus 3 minutes discussion)
Moderators: **Vian Peshdary** and **Riya (Binil) Raghupathy**

- **Roshan Sriram** (Luc Sabourin Group) Loss of Periostin/OSF-2 in ErbB2/Neu-driven tumors results in Androgen Receptor-positive molecular apocrine-like tumors with reduced Notch1 activity
- **Marc Avey** (David Moher Group) An Analysis of Reporting According to the ARRIVE (Animal Research: Reporting of In Vivo Experiments) Guidelines for Pre-Clinical Studies of Mesenchymal Stromal Cells for the Treatment of Acute Lung Injury
- **Samantha Richard** (Jay Baltz Group) Glycine-Dependent Cell Volume Control in Mouse Oocytes is activated due to Release of Inhibition Mediated by the Intact Antral Follicle Independent of the Release of Meiotic Arrest
- **Kendra Hodgkinson** (Barbara Vanderhyden Group) The potential of GREB1 as both a novel therapeutic target and as a diagnostic biomarker for ovarian cancer

10:10 AM **REFRESHMENT BREAK** (15 minutes) Supported by BioCanRx

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

11:25 AM **DR. J. DAVID GRIMES LECTURE** (35 minutes plus 10 minutes discussion)
Patterning Mesoderm and Blood Development from Human Pluripotent Stem Cells
Peter W. Zandstra, Professor, University of Toronto Institute of Biomaterials and Biomedical Engineering
Moderator: **Michael Rudnicki**

12:10 PM **BUFFET LUNCH** (60 minutes) Supported by DRS Construction

1:10 PM **MUSCLE REGENERATIVE MEDICINE** (50 Minutes)
(Talks: 9 minutes plus 3 minutes discussion)
Moderators: **Natasha Chang** and **Kiran Nakka**

- **Megan Fitzpatrick** (Michael Schlossmacher Group) Early-Onset Motor Deficits and Reduced Skeletal Muscle Force in an Alpha-Synuclein Mouse Model of Parkinson's Disease
- **Leslie Nash** (Robin Parks Group) SMN in Exosomes: A Potential Biomarker for Spinal Muscular Atrophy
- **Samantha Kornfeld** (Rashmi Kothary Group) Investigation of microRNA miR-145-5p as a novel MS therapeutic target through its regulation of critical myelination regulator MYRF
- **Nicolas Dumont** (Michael Rudnicki Group) Muscle Stem Cell Dysfunction in Duchenne Muscular Dystrophy

2:00 PM **KEYNOTE LECTURE** (35 minutes plus 10 minutes discussion)
Dignity, Personhood & the Culture of Medicine
Harvey Max Chochinov, Distinguished Professor of Psychiatry, University of Manitoba; Director, Manitoba Palliative Care Research Unit, CancerCare Manitoba
Moderator: **Dean Fergusson**

2:45 PM **REFRESHMENT BREAK** (15 minutes) Supported by Bio-Rad

3:00 PM **POSTER VIEWING / JUDGING OF POSTERS PRESENTED BY POSTDOCTORAL FELLOWS, CLINICAL FELLOWS, RESEARCH ASSOCIATES, RESIDENTS and MEDICAL STUDENTS** (60 minutes)

4:00 PM **VASCULAR BIOLOGY** (50 Minutes)
(Talks: 9 minutes plus 3 minutes discussion)
Moderators: **Mohamad Taha** and **Naomi Read**

- **Ketul Chaudhary** (Duncan Stewart Group) Genetic Basis for Sex-Related Hyper-Responsiveness to Severe Pulmonary Arterial Hypertension Induced by SU5416 in a Substrain of Sprague Dawley Rats
- **Jean-Francois Thibodeau** (Christopher Kennedy Group) Vascular-specific deletion of the EP4 receptor promotes hypertension-induced kidney injury
- **Sylvain Fraineau** (Marjorie Brand Group) Activation of major signaling pathways in human endothelial progenitors through de-repression of bivalent genes
- **Amy Hsu** (Douglas Manuel Group) Health care transitions among people with dementia at the end of life

4:50 PM **POSTER / ORAL PRESENTATION AWARDS AND CLOSING REMARKS**
Moderators: Duncan Stewart and Fraser Scott
IMPACT Award supported by BLG (Borden Ladner Gervais) and CCRM (Centre for Commercialization of Regenerative Medicine).
Oral Presentation Award supported by CDRD (Centre for Drug Research and Development).

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

ORAL PRESENTATIONS

Oral Presentation Award supported by the Centre for Drug Research and Development (CDRD).

Virus and Cell-based Therapeutics (8:30 to 9:20)

Moderators: Alex Gont and Katherine Clark-Knowles

1-1 **Comparative study of colon cancer subclones uncovers new elements associated with cellular antiviral response**

Colin Davis[1,2], Rachel McPhedran[1,2], Parisa Mazrooei[3,4], John Bell[1,2], Nicole Forbes[1,2], Fabrice Le Boeuf[1,2], Mathieu Lupien[3,4], Jean-Simon Diallo[1,2]

1. Centre for Innovative Cancer Research, Ottawa Hospital Research Institute, Ottawa, ON, Canada.
2. Faculty of Medicine, University of Ottawa, Ottawa, ON, Canada.
3. Ontario Cancer Institute, Princess Margaret Cancer Centre/University Health Network, Toronto, ON, Canada.
4. Department of Medical Biophysics, University of Toronto, Toronto, ON, Canada.

Background: Tumour heterogeneity is a key hurdle for the effective treatment of cancer using oncolytic viruses (OVs). Better understanding of the pathways involved in delineating tumour cell resistance and hypersensitivity to OVs is critical in order to guide the development of new therapeutic strategies to enhance OVs; for example using small molecule viral sensitizers (VSes). The commonly used mouse colon cancer cell line CT26.WT and its subclone CT26.LacZ cells display marked differences in their response to OVs in vitro and in vivo, providing a model system to investigate pathways that lead to hypersensitivity to infection and that could be exploited to increase the frequency of durable cures using OVs.

Objective: Determine the sources of CT26.lacZ sensitivity and CT26.WT resistance to OV treatment.

Methods and Results: We performed a top-down analysis using large-scale genetic (microarray) and epigenetic (ChIP-seq) comparisons of hypersensitive CT26.LacZ to their OV resistant parental cell line CT26.WT. Both the microarray and ChIP-seq transcription factor-binding site analyses converge on the finding that genes downstream of the response to the antiviral cytokine interferon-beta fail to be activated in the hypersensitive CT26.LacZ cell line. Follow-up In vitro studies confirmed that while the hypersensitive cells do produce interferon-beta, they do not respond to its presence and fail to become protected against viral infection. As CT26.LacZ have been transduced to express the LacZ gene using a retrovirus, inverse PCR was performed and identified 4 previously unknown integration sites of the LacZ gene, with 2 being in intronic regions of transcribed genes. These genes have not been previously shown to be involved in eliciting the interferon response; however, their expression profiles differ markedly between the CT26.LacZ and CT26.WT cells.

Conclusions: We conclude that the hypersensitive CT26.LacZ cells display a genetic, epigenetic, and phenotypic profile consistent with defects in the response to interferon beta but not its production. Ongoing studies will focus on assessing the impact of modulating the expression of the 2 candidate genes identified through knockdown and over-expression in order to determine their contribution to the hypersensitive phenotype.

1-2 **Insect Cell Carriers For Systemic Delivery of Oncolytic Viruses**

Dominic Roy[1,2], Anthony Power.[1], Marie-Claude Bourgeois-Daigneault[1], Theresa Falls[1], Lisa Ferreira[1], Andrew Stern[1], Christiano Tanese de Souza[1], Andrea McCart[3], David Stojdl[2,4,] Brian Lichty[5], Harold Atkins[1], Rebecca Auer[1], John Bell[1,2], Fabrice Le Boeuf[1]

1. Centre for Cancer Therapeutics, Ottawa Hospital Research Institute.
2. Department of Biochemistry, Microbiology and Immunology, University of Ottawa.
3. Division of Experimental Therapeutics, Toronto General Research Institute.
4. Apoptosis Research Center, Children's Hospital of Eastern Ontario.
5. Department of Pathology and Molecular Medicine, McMaster University.

Background: Systemic delivery of oncolytic viruses (OVs) remains a challenge due to the host immune system, which has evolved numerous mechanisms to neutralize and clear pathogens such as viruses from the bloodstream. Cell-mediated delivery of OVs represents a unique approach in order to overcome some of the barriers associated with systemic delivery. However, there remain significant obstacles to using any mammalian cell type as cell carriers for systemic OV delivery. Adherent solid tumor cells and mesenchymal stem cells are unable to traverse capillary beds and generally arrest within the vessels of the first organ they encounter, while leukocyte-based carriers are able to re-circulate but still exhibit receptor-mediated homing to lymphoid organs and bone marrow. Thus interactions between mammalian carrier cells and off-target host tissues interfere with systemic tumor

targeting Given VSV, an OV of clinical interest, establishes a persistent, non-cytolytic infection in insect cells, we thought that this might simplify delivery and also enable further manipulation of the cell carriers to improve delivery and therapeutic activity. Furthermore, we hypothesize that using a cell carrier platform genetically distinct from a mammalian system would limit homing of the cell carriers to off-target tissues.

Objective: To investigate the potential of insect cells to act as novel cellular vehicles for systemic delivery of OVs.
Methods: Insect cell carriers persistently infected with VSV were tested for their ability to deliver virus to tumor cells both in vitro and in vivo. We also compared the circulatory kinetics and biodistribution of systemically administered insect cell carriers and mammalian cell carriers.
In vitro studies were also performed to investigate magnetic targeting of insect cell carriers.

Results: Insect cell carriers were able to deliver their virus cargo to tumor cells both in vitro and in vivo. Systemically administered insect cell carriers also exhibited improved biodistribution and circulatory kinetics as compared to mammalian cell carriers. Finally, in vitro studies suggest insect cells loaded with iron oxide nanoparticles could be targeted to tumor cells with magnets.

Conclusions: Systemically administered insect cell carriers were well tolerated, shielded virus from immune recognition and neutralization, circulated at stable levels with little off-target tissue homing, and effectively delivered OVs to tumors in immunocompetent animals.

1-3 **Phosphodiesterase-5 inhibition augments postoperative natural killer cell antitumor immunity by reducing myeloid-derived suppressor cell function**

Lee-Hwa Tai[1], Almohanad Alkayyal[1,2], Christiano Tanese de Souza[1], Amanda Lynn Leslie[1], Shalini Sahi[1], Sean Bennett[3], Jiqing Zhang[1,4], John Bell[1], and Rebecca Auer[1,2,3]

1. Centre for Innovative Cancer Research, Ottawa Hospital Research Institute
2. Department of Biochemistry, Microbiology and Immunology, University of Ottawa
3. Department of Surgery, University of Ottawa
4. Department of Cellular and Molecular Medicine, University of Ottawa

Background and Objective: Cancer surgery while necessary for primary tumor removal, has been shown to induce immune suppression and promote metastases in preclinical models and human cancer surgery patients. We have previously shown that cancer surgery induces natural killer (NK) cell dysfunction and promotes postoperative metastases formation. Activating the immune system and reversing immune suppression have emerged as promising ways to treat cancer and they can be safely employed in the perioperative period. In this study, we investigated the role of Myeloid Derived Suppressor Cells (MDSC) as the mediators of postoperative NK cell impairment and subsequent metastases and evaluated the ability to target MDSC with a phosphodiesterase-5 (PDE-5) inhibitor in the clinically relevant postoperative period.

Methods: The suppression of NK cell cytotoxic activity by MDSC from control and surgically stressed mice was compared both in vitro and in vivo. Further, NK cell function and lung tumor burden was assessed following perioperative PDE-5 administration. In human cancer patients, MDSC expansion and function were assessed prior to and following surgical resection.

Results: MDSC expansion was observed following surgery in mouse melanoma and breast tumor models of surgical stress and this correlates with NK suppression postoperatively. In particular, surgical stress induced increased expression of arginase 1 and IL4R-alpha on MDSC. Co-cultures of NK cells with MDSC from control and surgical stressed mice resulted in their functional suppression with surgically stressed MDSC, but not with control MDSC. Adoptive transfer of MDSC from surgically stressed donor mice into recipient mice resulted in impaired in vivo clearance of NK-sensitive RMA-S tumor cells and increase in lung tumor burden. Further, surgically stressed mice depleted of MDSC, demonstrated enhanced ability to clear RMA-S tumor cells and had fewer lung tumour metastases. Using the clinically relevant PDE-5 inhibitor (sildenafil) to target MDSC suppressive pathways, we demonstrated a reduction in arginase 1 and IL4R-alpha expression levels on MDSC, recovery of NK cell function, followed by reduction of lung tumor metastases. In cancer surgery patients, MDSC expansion and suppression of NK cells in co-culture with autologous MDSC was observed in the early postoperative period.

Conclusions: MDSC are increased in both number and suppressive activity of NK cells following surgery. MDSC represent a hurdle to successful treatment of postoperative metastatic disease, but this effect is reversible using PDE-5 inhibitors. These results provide the preclinical rationale to initiate a clinical trial of perioperative PDE-5 inhibition.

1-4 **Latently HIV-1 infected cells have defects in type I IFN response that can be exploited by VSV?51 and MG1 viruses**

Nischal Ranganath[1], Teslin S. Sandstrom[1], Sandra C. Côté[1, 2], Jonathan B. Angel[1, 2, 3]

1. Biochemistry, Microbiology, & Immunology, University of Ottawa

2. Infectious Disease, Ottawa Hospital Research Institute
3. Division of Infectious Diseases, The Ottawa Hospital

BACKGROUND: Latent HIV reservoirs represent a major barrier to HIV eradication, thereby necessitating novel approaches to target and eliminate the reservoir. The antiviral type I IFN (IFN-I) response represents an essential defense against establishment of HIV-1 infection. Consequently, HIV has evolved multiple immune evasive strategies to dysregulate IFN-I signalling, including the downregulation of various antiviral IFN-stimulated genes (ISGs). We therefore investigated if similar defects in IFN-I response are also present in latently HIV infected cells. In cancer therapeutics, impaired IFN-I responses have been exploited to develop a class of oncolytic viruses (OV), including the recombinant Vesicular Stomatitis Virus (VSV?51) and Maraba virus (MG1), to selectively eliminate cancer cells. If IFN-I defects are present in the latent reservoir, we hypothesize that VSV?51 and MG1 can selectively target and kill latently HIV-infected cells.

OBJECTIVES: We investigated IFN-I signalling and responsiveness, as well as the capacity of OV to selectively target and eliminate latently HIV-infected cells.

METHODS: Utilizing the latently HIV-infected U1 cell line and parental HIV-uninfected U937 cells, surface expression of IFNa receptor 1 (IFNAR1) was quantified. IFN-I responsiveness was then assessed by treating cells with exogenous IFNa for 24 hours and measuring several ISGs including PKR, ISG15, and MHC-I by flow cytometry. To investigate the ability of MG1 to flow cytometry. PI, MTT, and Alamar Blue assays were used to assess cell viability.

RESULTS: IFNAR1 and MHC-I expression at basal levels and in response to IFNa was significantly lower in latently HIV-1 infected U1 cells compared to U937 cells. Additionally, despite similar basal PKR and ISG15 expression, the ISGs were more potently induced in HIV-uninfected U937 cells than in U1 cells following exogenous IFNa treatment. In parallel, U1 cells were significantly more susceptible to VSV?51 and MG1 infection and killing than U937 controls in both a dose- and time-dependent manner.

CONCLUSION: The latently HIV-infected U1 cells, in contrast to healthy U937 cells, exhibit significant defects in antiviral IFN-I response pathways, as determined by IFNAR1 expression and impaired ISG induction. This impaired IFN-I responsiveness was associated with increased infection and killing by VSV?51 and MG1 viruses. Therefore, targeting impaired IFN-I signalling may represent a novel and effective approach to selectively target and eliminate the latent HIV reservoir, bringing us closer to the objective of HIV eradication.

Signaling Change (9:20 to 10:10)

Moderators: Vian Peshdary and Riya (Binil) Raghupathy

2-1 **Loss of Periostin/OSF-2 in ErbB2/Neu-driven tumors results in Androgen Receptor-positive molecular apocrine-like tumors with reduced Notch1 activity**

Roshan Sriram[1], Vivian Lo[1], Benjamin Pryce[1], Lilia Antonova[1], Alan Mears[3], Manijeh Daneshmand[2], Bruce McKay[4], Simon J. Conway[5], William J. Muller[6] and Luc A. Sabourin[1,2]

1. Department of Cellular and Molecular Medicine, Faculty of Medicine, University of Ottawa, Ottawa, Ontario, Canada
2. Ottawa Hospital Research Institute, Cancer Therapeutics, Ottawa, Ontario, Canada
3. Children's Hospital of Eastern Ontario – Research Institute, Ottawa, Ontario, Canada
4. Department of Biology and Institute of Biochemistry, Carleton University, Ottawa, Ontario, Canada
5. Developmental Biology and Neonatal Medicine Program, HB Wells Center for Pediatric Research, Indiana University School of Medicine, Indianapolis, USA
6. Department of Biochemistry and Goodman Cancer Research Center, McGill University, Montreal, Quebec, Canada

Introduction
Periostin (Postn) is a secreted cell adhesion protein that activates signaling pathways to promote cancer cell survival, angiogenesis, invasion, and metastasis. Interestingly, Postn is frequently overexpressed in numerous human cancers, including breast, lung, colon, pancreatic, and ovarian cancer.

Methods
Using transgenic mice expressing the Neu oncogene in the mammary epithelium crossed into Postn-deficient animals, we have assessed the effect of Postn gene deletion on Neu-driven mammary tumorigenesis.

Results
Although Postn is exclusively expressed in the stromal fibroblasts of the mammary gland, Postn deletion does not affect mammary

gland outgrowth during development or pregnancy. Furthermore, we find that loss of Postn in the mammary epithelium does not alter breast tumor initiation or growth in mouse mammary tumour virus (MMTV)-Neu expressing mice but results in an apocrine-like tumor phenotype. Surprisingly, we find that tumors derived from Postn-null animals express low levels of Notch protein and Hey1 mRNA but increased expression of androgen receptor (AR) and AR target genes. We show that tumor cells derived from wildtype animals do not proliferate when transplanted in a Postn-null environment but that this growth defect is rescued by the overexpression of active Notch or the AR target gene prolactin-induced protein (PIP/GCDFP-15).

Conclusion
Together our data suggest that loss of Postn in an ErbB2/Neu/HER2 overexpression model results in apocrine-like tumors that activate an AR-dependent pathway. This may have important implications for the treatment of breast cancers involving the therapeutic targeting of periostin or Notch signaling.

2-2 **An Analysis of Reporting According to the ARRIVE (Animal Research: Reporting of In Vivo Experiments) Guidelines for Pre-Clinical Studies of Mesenchymal Stromal Cells for the Treatment of Acute Lung Injury**

Marc T Avey[1,2], David Moher[1,3], Dean Fergusson[1], Gilly Griffin[1], Jeremy M Grimshaw[1,4], Brian Hutton[1,3], Manoj M. Lalu[1,5], Malcolm Macleod[6], John Marshall[7], Shirley HJ Mei[8], Michael Rudnicki[8], Duncan J Stewart[8,9], Katrina Sullivan[1], Alexis F Turgeon[10,11], Lauralyn McIntyre[1,12]

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5. Department of Anesthesiology, The Ottawa Hospital, Ottawa, ON, Canada.
6. Division of Clinical Neurosciences, University of Edinburgh, Edinburgh, UK.
7. Department of Surgery (Critical Care), University of Toronto, Ottawa, ON, Canada.
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Introduction: Incomplete reporting of preclinical research studies may mask methodological weaknesses which might temper attempts to translate findings to the clinical arena.

Objectives: We aimed to assess the completeness of reporting of studies of mesenchymal stromal cells (MSCs) for the treatment of acute lung injury in animal models using the Animal Research: Reporting of In Vivo Experiments(ARRIVE) guidelines, and National Institutes of Health's (NIH) Principles and Guidelines for Reporting Preclinical Research. We also assessed whether there was a relationship between the completeness of reporting and journal impact factor, and whether articles published after the ARRIVE guidelines were associated with improved reporting.

Methods: As part of a systematic review we previously identified 47 preclinical studies that tested MSCs for the treatment of acute lung injury. We operationalized the ARRIVE guidelines into 109 discrete reporting items and extracted a total of 5,123 data elements from 47 studies. We evaluated the completeness of reporting based on ARRIVE sections and sub-sections, and corresponding sets of items from the NIH's Principles and Guidelines. For journal impact factor and publication date relative to ARRIVE we assessed the relationships with Wilcoxon signed-rank tests.

Results: Overall, studies reported 47% of all items (median 51 range 37 – 64). Across all studies, the methods sections reported less than half (45%) of the 87 items evaluated, and the results sections less than a third (29%). The reporting by ARRIVE sub-sections ranged from 0% (Adverse Events) to 100% (Objectives) across studies. The NIH's 'core' reporting items were infrequently reported: replicates 37%, statistics 33%, randomization 16%, blinding 31%, sample-size estimation 2%, and inclusion and exclusion criteria 4%. Reporting of details of biological materials used was less than a third (28%) for details ofexperimental animals, but more than two thirds (73%) for details of stem cells used were reported. There was no association between impact factor of the journal and completeness of reporting, as the average percentages of items reported in low impact journals (median 51 impact factor <=4) and high impact journals (median 49 impact factor >4) were similar (p=0.67).Factoring in a 1-year time allowance after publication of ARRIVE, there was a significantincrease in the median number of items reported in articles that were published after the ARRIVE guidelines (p=0.01; before 47.5, after 53).

Conclusion: Incomplete reporting is a major issue in preclinical studies evaluating MSCs to treat acute lung injury.

2-3 **Glycine-Dependent Cell Volume Control in Mouse Oocytes is activated due to Release of Inhibition Mediated by the Intact Antral Follicle Independent of the Release of Meiotic Arrest**

Samantha Richard[1,2,3], Dr. Jay Baltz[1,2,3]

1. Chronic Disease Program, Ottawa Hospital Research Institute
2. Obstetrics and Gynecology, University of Ottawa
3. Cellular and Molecular Medicine, University of Ottawa

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

Objective:
Determine how GLYT1 quiescence is maintained in the antral follicle.

Hypotheses:
1) An inhibitory factor from granulosa cells acts either directly or through signaling mechanisms to suppress the GLYT1 transporter.
2) This inhibitory mechanism requires gap junctional communication but is independent of the signaling mechanism involved in the maintenance of meiotic arrest in follicles.

Methods:
To test hypothesis 1, I simultaneously cultured denuded oocytes, cumulus-oocyte-complexes, and antral follicles for at least 4 hours and then examined GLYT1 activity in oocytes from each culture system using a [3H]-glycine uptake experiment.
To test hypothesis 2, I cultured antral follicles with or without gap junction inhibitors at early time points (0-10 hours) and then measured GLYT1 activity using a [3H]-glycine uptake experiment. I also cultured cumulus-oocyte-complexes in the presence of known components of the signaling mechanism involved in the maintenance of meiotic arrest and then measured GLYT1 activity.

Results:
GLYT1 activation occurred in cultured denuded oocytes and cumulus-oocyte-complexes but was maintained in a quiescent state within cultured intact antral follicles. A prolonged delay in GLYT1 activation occurred in antral follicles cultured with gap junctions inhibitors while meiotic resumption occurred with only a minimal delay. The addition of inhibitory components of the meiotic arrest pathway did not maintain GLYT1 quiescence in cultured cumulus-oocyte-complexes, although meiotic arrest was maintained.

Conclusions:
These experiments suggest that at least mural granulosa cells are required for the maintenance of GLYT1 quiescence. Maintenance of GLYT1 quiescence seems to occur through an independent mechanism to maintenance of meiotic arrest with an inhibitory factor possibly present within follicular fluid and not requiring gap junctional communication. Understanding the mechanisms involved in this method of volume regulation is important in providing optimal conditions for oocyte/embryo culture to avoid failure of infertility treatments, and damage that could result in dysregulation in fetal development, and disease in the offspring.

2-4 **The potential of GREB1 as both a novel therapeutic target and as a diagnostic biomarker for ovarian cancer**

Kendra Hodgkinson[1,2], Bojana Djordjevic MD[3], Barbara C. Vanderhyden[1,2,4]

1. Department of Cellular and Molecular Medicine, University of Ottawa
2. Cancer Therapeutics Program, Ottawa Hospital Research Institute
3. Department of Pathology and Laboratory Medicine, The Ottawa Hospital, University of Ottawa
4. Department of Obstetrics and Gynecology, University of Ottawa

Background: Estrogenic hormone replacement therapy increases the risk of developing ovarian cancer, and 17-ß estradiol (E2) accelerates tumour initiation and progression in mouse models, but little is known about the mechanisms underlying these observations. Growth regulation by estrogen in breast cancer 1 (GREB1) is an estrogen receptor alpha (ESR1)-upregulated protein which we propose mediates some of these estrogenic effects. GREB1 is required for hormone-driven proliferation of several breast and prostate cancer cell lines, and is a transcriptional cofactor with ESR1, but may have additional functions.

Objective: The role of GREB1 in ovarian cancer is unknown, and most studies have examined its actions in vitro only. We therefore

examined GREB1 expression and function in ovarian cancer cell lines and mouse models.

Methods: Lentiviral constructs were used to overexpress or knock down GREB1 in several ovarian cancer cell lines to determine the effects on proliferation, migration, and tumour growth in mice. To examine GREB1 expression in human ovarian cancer, we measured mRNA levels by QPCR in frozen tumours obtained from the Ottawa Ovarian Cancer Tissue Bank and protein levels by IHC on a tissue microarray (TMA) containing 4 ovarian cancer subtypes (20 cases each; Cooperative Human Tissue Network).

Results: GREB1 was upregulated by E2 in both mouse and cell line models of ovarian cancer. Ovarian cancer cell proliferation was decreased by GREB1 knockdown and increased by overexpression. Cell migration was also increased by GREB1 overexpression. GREB1 knockdown in cells injected into mice slowed tumour growth, nearly doubling survival time. GREB1 mRNA was expressed in all ovarian tumours examined (N=18) and the TMA also showed frequent GREB1 expression, with no major differences between subtypes (75-85%). GREB1 expression correlated with ESR1 at the mRNA level (QPCR) but not at the protein level (TMA). Interestingly, three subtypes of ovarian cancer were almost always positive for either ESR1 or GREB1 (59/60). This suggests a dependence on estrogen signalling pathways, which may be ligand-independent given the low estrogen levels post-menopause.

Conclusions: GREB1 promotes proliferation and migration in ovarian cancer cell lines, and knockdown slows tumour progression in a mouse model. GREB1 is frequently expressed in epithelial ovarian cancer whereas its expression in normal tissues is mainly confined to the reproductive tract, suggesting that it may be useful as a diagnostic biomarker. Furthermore, targeting GREB1 may inhibit tumour-promoting estrogen signalling pathways downstream of ESR1 and may therefore prove more effective than the current anti-estrogens targeting aromatase or ESR1.

Muscle Regenerative Medicine (1:10 to 2:00)

Moderators: Natasha Chang and Kiran Nakka

3-1 **EARLY-ONSET MOTOR DEFICITS AND REDUCED SKELETAL MUSCLE FORCE IN AN ALPHA-SYNUCLEIN MOUSE MODEL OF PARKINSON'S DISEASE**

Megan E. Fitzpatrick[2], Julianna J. Tomlinson[1], Wei Lin[2], Jean-Marc Renaud[2], Diane C. Lagace[2,3], and Michael G. Schlossmacher[1,2,3]

1. Program in Neuroscience, Ottawa Hospital Research Institute
2. Department of Cellular and Molecular Medicine, University of Ottawa
3. University of Ottawa Brain and Mind Research Institute

Background: In Parkinson's disease, neurodegeneration including in the nigrostriatal dopamine pathway causes motor impairment. Disease pathogenesis is linked to neuronal alpha-synuclein (aSyn) accumulation. Interestingly, in aSyn mouse models, motor impairment exists in the absence of detectable nigrostriatal changes, suggesting that other mechanisms contribute to aSyn-induced motoric dysfunction.

Objectives: To define and characterize the motor impairments observed in a mouse model of Parkinson's disease that overexpresses mutated human aSyn-encoding alleles (SNCA).

Methods: SNCA mice carry four insertions of the human SNCA locus encoding disease-linked Ala53Thr mutation on a murine Snca-/- background, producing 2.5- to 4-fold increased brain aSyn levels. Motoric function was characterized with a battery of behavioural tests between post-natal day 7 (PND 7) and 9 months of age (moa). Following testing, brain and skeletal muscles were collected for histological, biochemical and electrophysiological analysis.

Results: SNCA mice have delayed acquisition of the 'surface righting' reflex from PND 7 and significant deficits on balance beam at PND 14. At 1½ moa, SNCA mice have reduced muscle strength and significantly impaired motor co-ordination (rotarod) that progressively worsens up to 9 moa, in the absence of neuroanatomical abnormalities or detectable aSyn pathology in the brain. Force-frequency measurement from a soleus nerve-muscle preparation provided no evidence of neuromuscular junction abnormalities. There was however a net shift of the force-frequency curve toward lower stimulation frequencies, and lower maximum force, that corresponds to preliminary data showing a shift toward more type I fibers in SNCA mice.

Conclusions: Our data suggest that human aSyn overexpression results in impaired motor function associated with reduced skeletal muscle force at 3 moa. Further electrophysiological, biochemical and histological analyses are ongoing to determine underlying mechanisms. This includes the possibility that SNCA mice have pathological aggregates of aSyn in the brain, spinal cord or skeletal muscles, changes in dopamine release, and/or s that produce early-onset and sustained motoric deficits.

3-2 SMN in Exosomes: A Potential Biomarker for Spinal Muscular Atrophy

Leslie A. Nash[1,2], Emily R. McFall[2], Jodi Warman Chardon[3,4,5] Hugh McMillan[3,5,6], Dylan Burger[7,8], Rashmi Kothary[2,3,5,7], Robin J. Parks[1,2,3,5]

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7. Department of Cellular and Molecular Medicine, University of Ottawa, Ottawa, Ontario, Canada,
8. Kidney Research Centre, Ottawa Hospital Research Institute, Ottawa, Ontario, Canada,

Background: Spinal muscular atrophy (SMA) is an autosomal, recessive disorder that results in the death of motor neurons and subsequent muscle wasting due to deficiency in survival motor neuron (SMN) protein. SMA affects approximately 1 in 6,000 people and is the leading cause of death by a genetic disease in newborns. With no cure and no efficient tool for prognosis, more research is required. Exosomes are nano-sized particles naturally released from all cell types and contain a heterogeneous sampling of proteins and RNA from the cell from which they were derived. Our lab recently discovered that SMN is naturally contained within exosomes, and therefore exosomes may be a useful biomarker for SMA.

Objective: SMN levels in exosomes derived from cell culture and mouse serum will be assessed for their ability to predict the SMA disease state.

Methods: Protein from media was precipitated using 99% trichloroacetic acid and examined using immunoblot. Exosomes were isolated using the commercially available Exoquick kit, or through differential centrifugation and re-suspended in PBS. Characterization of exosomes was accomplished using exosome markers Alix, Flotillin and Tsg101. SMN levels were examined in generic cell lines, primary cells derived from an intermediate mouse model of SMA (SMN2B/-), patient fibroblasts (WT, Carrier, SMA Type 1), and serum from mice.

Results: Media was isolated after incubation with various cell types from mouse and human origin. Examination of the precipitated protein demonstrated naturally high levels of SMN are released from cells into the surrounding milieu. Isolation of microparticles, exosomes and apoptotic bodies revealed SMN is stored within a variety of extracellular vesicles. Exosomes isolated from human and mouse cell lines exhibited SMN levels which corresponded to their parental cell line. Furthermore, exosome SMN levels could be manipulated by creating stable cell lines engineered to overexpress SMN protein or through infection with an adenovirus vector expressing SMN. Exosomes derived from SMN deficient cell lines (Mef2B/- and SMA Type 1 patient fibroblasts) also exhibit reduced quantities of SMN compared to their WT controls. SMN levels in exosomes derived from patient fibroblasts significantly correlated to disease state ($R2=0.922$, $p<0.001$). Finally, serum derived exosomes isolated from WT mice demonstrated greater SMN levels than those found in exosomes derived from the serum of SMN2B/- mice.

Conclusions: Exosomes derived from both cell and animal models reveal SMN levels that are reflective of the disease model. Therefore, serum-derived exosomes from patients may offer a novel prognostic tool for SMA.

3-3 Investigation of microRNA miR-145-5p as a novel MS therapeutic target through its regulation of critical myelination regulator MYRF

Kornfeld, S.F[1,2], S.R. Bonin[1] and R. Kothary[1,2]

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

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

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

Methods: Lentiviral vectors were used to create a stable cell line from immortalized OPCs (Oli-Neus) that inducibly overexpress

miR-145-5p. Cells were characterized while proliferating (P), and on differentiation days 3 and 6 (DD3 and DD6). MYRF expression was analysed by qPCR. MAG expression was visualized by immunofluorescence, and quantified by qPCR and Western blot. Direct targeting of MYRF by miR-145-5p was characterized by dual luciferase assay in HEK 293 T cells.
Cell morphology was visualized by immunofluorescence, and expression of Olig2 and glial fibrillary acidic protein (GFAP) were quantified by Western blot.

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

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

3-4 **Muscle Stem Cell Dysfunction in Duchenne Muscular Dystrophy**

Nicolas A. Dumont[1,2], Yu Xin Wang[1,2], Julia von Maltzahn[1,2,3], Alessandra Pasut[1,2], C. Florian Bentzinger[1,2,4], Caroline E. Brun[1,2], and Michael A. Rudnicki[1,2]

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Background: Duchenne muscular dystrophy (DMD) is a devastating genetic muscular disorder of childhood manifested by progressive debilitating muscle weakness and wasting, and ultimately death in the second or third decade of life. DMD is characterized by the absence of dystrophin, a 3,500aa rod-shaped protein expressed in differentiated myofibers, that connects the myofiber cytoskeleton to the extracellular matrix through the dystrophin-associated glycoprotein complex. In absence of dystrophin, myofibers are extremely susceptible to injury, which leads to cycles of degeneration and regeneration, inflammation, fibrosis and progressive loss of muscle mass and function.

Methods and Results: Here we show that dystrophin is also highly expressed in activated satellite cells (muscle stem cells) where it interacts with the Ser/Thr kinase Par1b, an important regulator of cell polarity. Par1b is known in multiple stem cell types to establish polarity by phosphorylating the Par3 complex leading to its asymmetric distribution. In the absence of dystrophin, expression of Par proteins is perturbed leading to their inability to be polarized on opposite cell cortex prior to cell division. Consequently, dystrophin-deficient satellite cells display a marked reduction in the proportion of asymmetric divisions resulting in a dramatic reduction in the flux between stem cell and progenitor compartments. We also demonstrate that muscle regeneration is impaired in vivo in dystrophin-deficient muscles.

Conclusions: Therefore, we conclude that dystrophin has an essential role in the regulation of satellite cell polarity. Our findings indicate that DMD is not only caused by myofiber fragility, but is also exacerbated by intrinsic dysfunction in dystrophin-deficient satellite cells.

Vascular Biology (4:00 to 4:50)

Moderators: Mohamad Taha and Naomi Read

4-1 **GENETIC BASIS FOR SEX-RELATED HYPER-RESPONSIVENESS TO SEVERE PULMONARY ARTERIAL HYPERTENSION INDUCED BY SU5416 IN A SUBSTRAIN OF SPRAGUE DAWLEY RATS**

Ketul R Chaudhary[1,2], Yupu Deng[1], Kurt Tyson[1], Anli Yang[1], Edwin Cuppen[3], Duncan J. Stewart[1,2]

1. Regenerative Medicine Program, Sinclair Centre for Stem Cell Research, Ottawa Hospital Research Institute
2. Faculty of Medicine, University of Ottawa
3. Hubrecht Institute, Utrecht, The Netherlands

Introduction: Pulmonary arterial hypertension (PAH) is a life-threatening disease that leads to progressive pulmonary hypertension, right heart failure and death. A new experimental model has been introduced that better reproduces the salient pathological

features of human PAH, involving the injection of a single dose of the VEGFR2 antagonist, SU5416 (SU), followed by a 3-week exposure to chronic hypoxia (CH). SU is believed to cause lung endothelial cell apoptosis that, together with CH as a "second hit", results in the emergence of growth dysregulated, "quasi-neoplastic" vascular cells that form characteristic plexiform-like arterial lesions. Our lab has studied strain differences in the SU/CH model of PAH and shown that a specific sub-strain of Sprague Dawley (SD) rats from Charles River labs (Montreal, Canada) developed severe progressive PAH in response to single injection of SU, even in absence of CH.

Objective: In the present study, we investigated whether there were sex related differences in response to SU alone in this hyper-responsive sub-strain of SD rats.

Methods and Results: Male and female rats were injected with SU (20mg/kg, sc) or vehicle. Right ventricular systolic pressure (RVSP) measurement and echocardiography were performed at four and seven weeks after SU injection. We observed that 72% (13 of 18) male SD rats were hyper-responsive (HR) to SU and developed severe PAH with meanRVSP of 96.5±18.4 mmHg in absence of CH whereas only 27% (7 of 26) of the female rats were responsive to SU alone. Furthermore, crossing non-responsive male and female animals drastically decreased the proportion of hyper-responsive animals in the F1-generation (HR 15% and 0% in male vs. female, respectively), highly suggestive of possible genetic basis for the HR phenotype. Exome sequencing was performed to identify genetic mutations involved in the hyper-responsive phenotype. We also studied the SU responsiveness in the oophorectomized female rats to investigate the role of female sex hormones in gender specific HR phenotype. Interestingly, the proportion of HR animals increased drastically in oophorectomized female rats to 71% (10 of 14), compared to normal rats (33%, 4 of 12).

Conclusion: These data are consistent with the existence of as yet unknown genetic modifier(s) that confer responsiveness to SU alone in a specific sub-strain of SD rats in sex specific manner. Moreover, they support a protective role of female sex hormones in this model of SU-induced severe PAH.

4-2 **Vascular-specific deletion of the EP4 receptor promotes hypertension-induced kidney injury**

Jean-Francois Thibodeau[1,2], Chet E. Holterman[1], Ying He[1], Anthony Carter[2], Alex Gutsol[1], Gregory Cron[3] and Christopher R.J. Kennedy[1,2]

1. Kidney Research Centre, Chronic Disease Program, Ottawa Hospital Research Institute, Ottawa, Ontario, Canada
2. Department of Cellular and Molecular Medicine, Faculty of Medicine, University of Ottawa, Ontario, Canada
3. Ottawa Hospital Research Institute, Ottawa, Ontario, Canada.

Background
Cyclooxygenase-inhibition by chronic non-steroidal anti-inflammatory drug use is contraindicated in hypertension as it may impair kidney function through diminished renal blood flow. Accordingly, loss of cyclooxygenase-derived prostaglandin E2 acting via E-Prostanoid 4 receptors which normally dilates the renal vasculature and counteracts pressor hormones such as angiotensin II could account for such non-steroidal anti-inflammatory drug-associated effects on renal function.

Objectives
Our objectives were to determine the vascular EP4 receptor'''''''s contribution to kidney function in a hypertensive setting. We hypothesized that loss of the vasodilatory EP4 receptor in the vasculature would impair its ability to withstand hypertension and would predispose to kidney injury.

Methods
We generated mice with inducible vascular smooth muscle cell-specific EP4 receptor deletion (EP4VSMC-/-) under control of the tamoxifen-sensitive smooth-muscle actin promoter and subjected them to angiotensin II-induced hypertension delivered by osmotic mini-pumps.

Results
EP4 deletion was verified by qPCR of aorta and renal vessels, as well as functionally by the loss of prostaglandin E2-mediated mesenteric artery relaxation by wire myography. After 4 weeks both angiotensin II-treated wild type (EP4VSMC+/+) and EP4VSMC-/-groups were similarly hypertensive, while albuminuria was exacerbated when the EP4 was abolished. AngII-treatment led to severe glomerular scarring and tubulointerstitial fibrosis in EP4VSMC-/- but not in EP4VSMC+/+ mice . AngII significantly lowered glomerular filtration rate in EP4VSMC-/- mice, but not in EP4VSMC+/+ mice. Lastly, AngII-treated EP4VSMC-/- mice showed evidence of capillary damage and reduced renal blood flow as measured by fluorescent bead microangiography and dynamic contrast-enhanced magnetic resonance imaging respectively.

Conclusions
Our data suggest that renovascular EP4 receptors buffer the actions of AngII upon renal hemodynamics and thereby protect against hypertension-associated structural damage.

4-3 **Activation of major signaling pathways in human endothelial progenitors through de-repression of bivalent genes**

Sylvain Fraineau[1,2], Alphonse Chu[1], Elodie Vion[1], Brian McNeill[3], Kristy Rieck[1], Sharmin Nilufar[1], Theodore J. Perkins[1], Erik J. Suuronen[3,4], David S. Allan[1] & Marjorie Brand[1,2]

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Our laboratory recently reported the use of the pan-Histone deacetylases (HDACs) inhibitor Trichostatin A (TSA) to enhance Endothelial Colony forming Cells (ECFCs)-mediated vascular repair through increasing the cells' migratory capacity. Beside acetyltransferases/deacetylases, the histone methyltransferase EZH2 has been proposed to play an important role in the repression of genes that control the migration of endothelial cells. This suggested to us that combining small molecules that inhibit the activities of both HDACs and EZH2 may further improve the regenerative function of endothelial progenitors.

We found that panobinostat (pan-HDACs inhibitor) and GSK-343 (EZH2 inhibitor) treatment improves ECFCs migration, capillary-like tube formation in vitro and enhances blood flow recovery in the murine hindlimb ischemic model in vivo. Interestingly, combined drugs treatment results in a much greater improvement of ECFCs' functions suggesting that these drugs have additive effects. By RNA-Seq analysis, we identified members of major signaling pathways (DLL/NOTCH, WNT/FZD, PGF/VEGFR, CXCL12/CXCR4, SHH) implicated in angiogenesis (DLL1, DLL4, CXCR4, FZD7) as being up-regulated with combined epigenetic drugs treatment. ChIP-qPCR experiments revealed that the promoters of these genes share a common epigenetic signature of bivalent genes consisting of low levels of histone H3 acetylation and high levels of histone H3K27me3 and H3K4me3. Moreover we found that these regions are bound not only by epigenetic repressive enzymes HDAC1 and EZH2 but also by their antagonistic partners P300 and UTX providing a potential mechanism of action of the epigenetic drugs. Further study of the epigenetic status of these bivalent promoters after single or combined epigenetic drugs treatment reveals that panobinostat specifically increases histone H3 acetylation while GSK-343 specifically decreases histone H3K27me3 methylation and combined treatment induced both of these effects without affecting histone H3K4me3 methylation. Interestingly,H3K27me3 demethylation seamed to happened exclusively on promoters where both EZH2 and UTX are bound whereas presence of both P300 and HDAC1 correlates with promoter acetylation after epigenetic drug treatment.

Altogether our data show that panobinostat and GSK-343 combined treatment improves ECFCs function through specific epigenetic modifications of bivalent target-genes promoters.

4-4 **Health care transitions among people with dementia at the end of life**

Amy T. Hsu[1,2,5], Peter Tanuseputro[1,2,3], Mathieu Chalifoux[2], Susan Bronskill[2,4], Douglas G. Manuel[1,2,5]

1. Clinical Epidemiology Program, Ottawa Hospital Research Institute
2. Institute for Clinical and Evaluative Sciences (ICES)
3. Bruyere Research Institute (BRI)
4. Institute of Health Policy, Management and Evaluation, University of Toronto
5. School of Epidemiology, Public Health and Preventive Medicine, University of Ottawa

Background – The median survival following the onset of dementia among seniors is 3.3 years. Currently, we have a limited understanding of the care trajectories for people with dementia, especially towards the end of life. The frequency of care transitions in the months leading to death is often considered a measure of care quality, and has implications for health care spending, as well as the quality of life for patient with dementia and their caregivers.

Methods – Using population-level health administrative data from Ontario, Canada, we examined the health care use among decedents who have dementia (n = 64,628) in the 12 months prior to death. Our decedent cohort was stratified based on whether the individual had received care in a nursing home or in the community (with and without extended home care). Outcomes examined include the use of hospital services and total health care cost in the 3, 30, 90 and 360 days prior to death.

Results – Nearly 70 percent of people who died with dementia received care in a nursing home in the last year of life, while only 7 percent of those without dementia were admitted into nursing homes. 21 percent of decedents with dementia in the community received extended home care in their last year of life. The remaining 11 percent did not receive any extended health care from nursing homes or home care setting. Furthermore, we find that time of nursing home admission may have an impact on the likelihood of hospitalization in the last year of life.

Conclusions – While the majority of people with dementia received care in nursing homes, one-third of individuals who had died with dementia remained in the community until death. Those who were cared for in nursing homes were less likely to use hospital services – resulting in fewer burdensome transitions and similar overall health care costs as people residing in the community in their last year of life. These findings provide important points for consideration in care planning for older individuals with dementia.

POSTER PRESENTATIONS

IMPACT Award

(Identification of Marketable Products, Applications and Commercializable Technologies)
Supported by the BLG (Borden Ladner Gervais) and CCRM (Centre for Commercialization of Regenerative Medicine)

1 **Bio-engineering novel vaccinia virus-based oncolytics for the treatment of cancer**
Brian Keller, Adrian Pelin, Jiahu Wang, Fabrice LeBoeuf

2 **Decreasing deep brain stimulation surgery time and improving patient outcomes through automatic target localization with real-time analysis of intraoperative brain signals**
David Lu

3 **Exosomes: the biological swiss army knife for spinal muscular atrophy**
Leslie Nash

Cancer Therapeutics Program

4 **Withdrawn**

5 **The role of SLK in ErbB2-induced mammary tumorigenesis**
Khalid Al-Zahrani[1,2], Jillian Conway[1,2] and Luc A. Sabourin[1,2].
1. Centre for Cancer Therapeutics, Ottawa Hospital Research Institute.
2. Department of Cellular and Molecular Medicine, University of Ottawa.

Background:
HER2/Neu/ErbB2 is an epidermal growth factor receptor, whose overexpression is implicated in approximately 30% of human breast cancers. The Ste-20 like kinase SLK, plays an important role in cell motility and migration. The role SLK plays in ErbB2-induced mammary tumorigenesis will be the focus of this fellowship application.

Objective:
Although there is an important link between ErbB2 and breast cancer invasion and metastasis, the mechanisms by which it contributes to these processes are not known.
Recently, SLK has been shown to be activated by ErbB2 and required for heregulin chemotaxis, suggesting that migratory signals mediate SLK-dependant pathways. Therefore, we hypothesize that SLK is required for ErbB2-induced mammary tumorigenesis and metastasis in vivo and contributes to the increased invasiveness and metastatic potential of these tumours.

Methods
Mammary tissue specific SLK-deficient mice are being used to study the effect of SLK in ErbB2-driven tumorigenesis, and analyzing the effect of SLK in ErbB2-induced metastasis and invasion.

Results:
SLKfl/fl x NIC mice were generated and tumor onset is significantly delayed accompanied by an overall increase in survival as compared to NIC control mice. These tumors show an increase in epithelial markers accompanied by a decrease in mesenchymal markers suggestion a role for SLK in the epithelial-to-mesenchymal transition.

Conclusions:

As a result of this project, it is our goal to achieve a better understanding of the etiology of this significant cancer phenotype. These results will then hopefully lead to new targets for cancer therapies that will be more successful than those currently employed in treating this cancer, leading to increased quality of life and survival for cancer patients.

6 **Media reporting of practice-changing clinical trials in oncology – a North American perspective.**

Peter Andrew[1], Patricia A. Tang[2], Stephen O'Connor[3], Mario Valdes[4], Michael M. Vickers[1]

1. Division of Medical Oncology, University of Ottawa
2. Department of Medical Oncology, University of Calgary
3. Department of Mathematics, City University
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BACKGROUND: It is primarily through print and electronic media that clinical trial results and their interpretation are first shared with the public at large. Media reporting of clinical trials impacts patient-oncologist interactions.

OBJECTIVE: We sought to characterize the accuracy of media and internet reporting of practice-changing clinical trials in oncology.

METHODS: The first media articles referencing 17 practice-changing clinical trials were collected from four media outlets – newspapers, cable news, cancer websites, and industry websites. Measured outcomes were media reporting score (MRS), social media score (SMS), and academic citation score (ACS). MRS was a surrogate measure for completeness of information detailed in media articles as scored by a 15-point scoring instrument. SMS represented the ubiquity of social media presence referencing 17 practice-changing clinical trials in cancer as determined by the American Society of Clinical Oncology (ASCO) in its annual report, entitled Clinical Cancer Advances (CCA) 2012; SMS was calculated from Twitter, Facebook and Google searches. ACS comprised total citations from Google Scholar plus Scopus databases, which represented the academic impact per CCA.

RESULTS: From 163 media articles, 107 (66%) had sufficient data for analysis. Cohen's K coefficient demonstrated reliability of the MRS instrument with a coefficient of determination of 94%. Per MRS, information was most complete from industry, followed by cancer websites, newspapers, and cable news. The most commonly omitted items in descending order were study limitations, exclusion criteria, conflict of interest, and other. SMS was weakly correlated with ACS. CONCLUSIONS: Media outlets appear to have set a low bar for coverage of many practice-changing advances in oncology, as reports of scientific breakthroughs often omit basic study facts and cautions, which may mislead the public. The media should be encouraged to use a standardized reporting template and provide accessible references to original source information whenever practical. This is feasible across both traditional and new media platforms.

7 **ASSESMENT OF SUBCLINICAL TREATMENT-INDUCED CARDIOTOXICITY USING LEFT VENTRICULAR EJECTION FUNCTION IN BREAST CANCER PATIENTS DURING TRASTUZUMAB TREATMENT**

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BACKGROUND
Cardiotoxicity (Ctx) is a potential devastating sequela of cancer therapy. Early detection of subclinical Ctx and predictive tools to identify breast cancer (BC) patients at risk of Ctx during therapy are needed. In 2008 we established a dedicated cardio-oncology clinic for patients at risk of or experiencing Ctx from their cancer treatment. The purpose of this retrospective study is to identify potential risk factors for LVEF changes and Ctx in women with HER2-positive BC receiving systemic therapy and trastuzumab.

MATERIAL AND METHODS
431 patients with HER2-positive BC (all stages) who received trastuzumab therapy alone or in combination with chemotherapy referred to the cardio-oncology clinic between 2008-2012 were reviewed. Data collected included: age, body mass index, stage of disease, performance status, previous anticancer treatment, and comorbidities. Left ventricular ejection fraction (LVEF) was assessed with ECHO or MUGA at baseline, 3, 6, 9, and 12 months. Subclinical Ctx was defined as decrease of LVEF more than 10% (from baseline) and absence of clinical symptoms related to heart failure. Descriptive statistics, univariate and multivariate analysis, and binary logistic regression were performed.

RESULTS
The majority of patients (278; 72%) experienced no significant drop in LVEF: baseline 62%, 64% after 3, 62% after 6, 61% after 9, and 12 months (Group A). 120 (28%) Patients had a LVEF decrease more than 10% during at least one subsequent assessment. Average LVEF was 67% on baseline, 60% after 3, 59% after 6, 58% after 9, and 56% after 12 months (Group B). Baseline LVEF on

univariate or multivariate analysis did not predict subclinical Ctx in group B.

CONCLUSION
Data from this observational study describes two distinct cardiac risk patterns in patients receiving trastuzumab-based breast cancer therapy. This study was limited by a selected group of patients referred for cardiac oncology assessment and short-term follow-up. New tools to predict patients at risk of Ctx using biomarkers, genetic testing, and novel cardiac imaging are urgently needed. Preliminary data suggests genes that may be useful in prediction of subclinical hypertrophic cardiomyopathy. Future research will determine if such genes may be predictive of subclinical chemotherapy-induced Ctx.

8 **Withdrawn**

9 **PROSPECT eligibility and clinical outcomes: Results from the pan-Canadian rectal cancer consortium**

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Background: The current standard for locally advanced rectal cancer is neoadjuvant chemoradiation therapy (nCRT) followed by surgery. The PROSPECT trial (N1048) is investigating neoadjuvant FOLFOX (5-fluouracil, leucovorin, oxaliplatin) with selective use of nCRT in patients with locally advance rectal cancer undergoing low anterior resection.

Objectif: To evaluate outcomes of PROSPECT eligible and ineligible patients from a retrospective multi-institution database to increase clinicians and patients knowledge on this selected population and encourage accrual on the PROSPECT trial.

Methods: Data from patients with locally advance rectal cancer who received nCRT and had curative intent surgery from 2005 to 2014 were collected from 5 Canadian cancer centres. PROSPECT eligible patients included: cT3N0, cT2N1 and cT3N1 rectal adenocarcinoma, ECOG performance status 2 or less, hemoglobin >80 g/L, age =18 years and receipt of low anterior resection. Overall survival (OS), disease free survival (DFS), recurrence free survival (RFS), and time to local recurrence (TLR) were estimated using Kaplan-Meier method. Cox proportional hazards regression was used to adjust for prognostic factors including circumferential resection margin, performance status, clinical stage, pathological stage and adjuvant chemotherapy, sex, age and radiation dosage.

Results: 1531 patients were included of whom 566 (37%) were considered eligible for PROSPECT. Eligible patients were more likely to have better performance status (p=0.0003), radiation dose =45 Gy (p=0.001), negative circumferential margin (p<0.0001) and distance =5 cm from anal verge (p<0.0001). PROSPECT eligibility was associated with improved DFS (HR 0.75, 95% CI: 0.61-0.91), OS (HR 0.73, 95% CI: 0.57-0.95) and RFS (HR 0.68, 95% CI: 0.54-0.86) in univariate analyses. In multivariate analysis, RFS was improved for PROSPECT eligible patients (HR 0.75, 95% CI: 0.57 – 1.00, p = 0.0499), but not for OS and DFS. TLR was also similar (HR 0.95, 95% CI: 0.31-3.0) adjusting for circumferential resection margin, pathologic stage and adjuvant chemotherapy. The 3-year DFS for PROSPECT eligible patients was 79.1% compared with 71.1% for ineligible patients and the rate of freedom from local recurrence at 3 years was 97.4 v 96.8%, respectively. In comparison, the PROSPECT trial has estimated a 3-year DFS of 69-74% and a 3-year freedom from local recurrence of 96%. Pathologic complete response was 20.3% in PROSPECT eligible patients compare to 22.9% in ineligible patients.

Conclusion: Real world data corroborate the eligibility criteria used in the PROSPECT study, identifying a subgroup of patients in whom recurrence risk is lower and where selective use of nCRT should be actively examined.

10 **Withdrawn**

11 **Combination of Paclitaxel and MG1 oncolytic virus as a successful strategy for breast cancer treatment**

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Background: Breast cancer is the most common malignant disease amongst Western women. The lack of treatment options for patients with chemotherapy-resistant or recurrent cancers is pushing the field toward the rapid development of novel therapies. The use of oncolytic viruses is a promising approach for the treatment of disseminated diseases like breast cancer, with the first candidate soon to be approved for the use in patients. Our hypothesis is that the combination of Paclitaxel, one of the most common drugs used in breast cancer patients, and the oncolytic virus Maraba-Rhabdovirus MG1, a clinical trial candidate in a study currently recruiting late-stage metastatic cancer patients, would improve outcome for breast cancer patients. Indeed, the virus and the drug use different mechanisms to destroy tumors and Paclitaxel was previously identified as a viral sensitizer; a drug that enhances virus replication.

Objective: Our objective was to demonstrate the compatibility of MG1-virotherapy and Paclitaxel for breast cancer treatment.
Methods: The drug and virus combinations were assessed in 3 murine breast cancer models: EMT6, 4T1 and EO771. We used a variant of MG1 expressing GFP to monitor transgene expression as a read-out of viral infection by flow cytometry and microscopy. Plaque assay was used to quantify the infectious particles and efficacy was assessed by tumor measurement and survival of tumor bearing mice.

Results: Our results show that Paclitaxel enhances MG1 in various breast cancer models in vivo and in vitro, thus acting as a viral sensitizer. When combined together, MG1 and Paclitaxel slowed tumor progression and increased survival in the EMT6, 4T1 and EO771 orthotopic models.

Conclusions: Our results demonstrate not only the compatibility of the treatments, but also the synergistic effect of their co-administration.

12 **Palliative chemotherapy (CT) for advanced non-small cell lung cancer (NSCLC): Investigating disparities between patients who are treated versus those who are not**

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Background + Objective
Palliative CT in advanced NSCLC is associated with improved overall survival (OS) and quality of life, yet many patients remain untreated. In this study, we explored the differences between patients who did not receive palliative CT versus those who did, with a goal of better understanding and supporting the untreated.

Methods
We performed a retrospective analysis of all newly diagnosed patients with advanced NSCLC seen at our institution between 2009 and 2012. Demographics, treatment, and survival data were collected. Fisher's exact test assessed the association between CT use and baseline characteristics. Multivariate analysis of overall survival was performed using Cox regression models.

Results
Overall, 528 patients were seen: 291 (55%) received palliative CT, while 237 (45%) received none. Untreated patients were older (median 71 v 64, $p<0.01$) and less fit (ECOG 0-1 in 27% v 69%, $p<0.01$). More had weight loss ($p<0.01$), anemia ($p=0.01$), thrombocytosis ($p<0.01$), leukocytosis ($p<0.01$), and renal impairment ($p<0.01$). Reasons for no treatment included poor performance status (PS) (67%) and patient choice (23%). Median overall survival was shorter among untreated patients (3.9 v 10.7 months, HR 1.80 [95% CI 1.4-2.3], $p<0.01$). In multivariate analysis, in addition to not receiving systemic therapy, factors associated with shorter survival were age, PS, weight loss, leukocytosis and thrombocytosis.

Conclusion
Unsurprisingly, patients who did not receive CT had more poor prognostic features and worse survival. However, it is concerning that, despite being seen in an active academic centre, nearly half of all patients with advanced NSCLC received no anti-cancer treatment, with the most common reason being poor PS. Current research primarily seeks to improve outcomes amongst those receiving systemic therapy, but this suggests that the lung cancer community must urgently advocate for the untreated. This should include more rapid diagnosis prior to functional decline, and development of therapies effective in a sicker population.

13 **SUPPRESSION OF MATRICELLULAR SPARC AS A NOVEL MECHANISM FOR ß1 INTEGRIN MEDIATED PROSTATE CANCER BONE METASTASIS**

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Background
Prostate cancer is the most commonly diagnosed cancer in men with devastating implications particularly deriving from metastasis to the bone. Given the frequency of prostate cancer metastasis we sought to investigate the factors that mediate prostate cancer migration and three dimensional growth. We have previously found that ß1 integrin (ITGß1), one of the primary components of the integrin heterodimer that binds the major bone extracellular matrix collagen I, promotes prostate tumor cell invasion and growth in three dimensions (3D). To better understand ITGß1 regulated prostate cancer phenotypes, we performed preliminary gene expression screens using prostate cancer cell lines in which ITGß1 was depleted. One target of interest which appeared to be ITGß1 regulated was the secreted protein acidic and rich in cysteine (SPARC) gene. As recent studies have established SPARC as an anti-proliferative and anti-invasive protein in prostate cancer1, we investigated whether the impaired prostate tumor cell invasion and growth in 3D observed upon depletion of ITGß1 was due in part to the lack of SPARC suppression.

Objective
We sought to investigate the novel regulation of SPARC by ITGß1 and to determine if SPARC production suppresses prostate cancer 3D growth and migration in the absence of ITGß1.

Methods
SiRNA targeting ITGß1 was used for specific ITGß1 depletion and the invasive and growth properties of ITGß1depleted cells were compared to those transfected with non-targeting siRNA as a control. Similar approaches were used to deplete SPARC using specific siRNAs. Cell migration was assessed using scratch wound-healing assays, and matrigel sphere forming assays were used to compare 3D growth.

Results
We show herein that depletion of ITGß1 results in increased SPARC gene and protein expression. We further show that the ITGß1-mediated suppression of SPARC appeared to be through a JNK dependent mechanism. We further show that SiRNA mediated depletion of ITGß1, results in impaired sphere formation in 3D matrigel assays. This appeared to be dependent on the increased production of SPARC in ITGß1 depleted cells, as additional depletion of SPARC using targeted siRNA in ITGß1 depleted cells led to a restoration in 3D sphere formation.

Conclusions
Our data suggests a novel mechanism whereby ITGß1 promotes prostate tumor cell growth via suppression of SPARC expression, which may contribute to impaired prostate cancer bone metastasis growth and progression.

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14 **TGF Beta-1 Induces an Epithelial-to-Mesenchymal Transition and Increases Ptgs2 Expression to Increase Ovarian Surface Epithelial Stem Cell Characteristics.**

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Background: The ovarian surface epithelium (OSE) is the monolayer of epithelial cells surrounding the ovary. During ovulation the OSE layer is ruptured creating an ovulatory wound site. The processes involved in ovulatory wound repair are poorly understood, as are the consequences of improper healing. Ovulation is the primary non-hereditary risk factor for ovarian cancer and suppressing ovulation (i.e. through oral contraceptive use) reduces ovarian cancer risk.

We have previously identified Stem Cell Antigen 1 (Sca1) expressing OSE cells that exhibit stem cell characteristics such as increased sphere forming capacity. These characteristics were increased upon treatment with Transforming Growth Factor Beta-1 (TGFß1), a factor found in the follicular fluid at the time of ovulation. These data suggest there is a stem cell population in the OSE that may be regulated during ovulation to aid in ovulatory wound repair.

Objective: To elucidate a signaling pathway for the TGFß1 regulation of OSE stem cells.

Methods/Results: To study how TGFß1 is increasing OSE stem cell characteristics, mouse OSE (mOSE) were treated with TGFß1 recombinant protein. TGFß1 treatment increased the stem cell marker CD44 (3.6-fold) and induced an epithelial-to-mesenchymal transition (EMT), as seen morphologically, through an increase in Snai1 (8.3-fold) and a decrease in KRT19 (93%). Snail overexpression also resulted in an EMT and increased sphere formation. A TGFß1 Signaling Targets PCR array was used to identify additional gene targets of TGFß1 treatment. Ptgs2 was increased 9.7-fold in TGFß1-treated mOSE, compared to control mOSE. An ELISA showed that under the influence of TGFß1 treatment, the Ptgs2 increase was accompanied by an increase in Prostaglandin Endoperoxide Synthase 2 (PGE2) protein (1.9-fold), a down-stream product of PTGS2. Treating mOSE cells with PGE2 protein

increases stem cell marker expression Sca1 and CD44 (1.4- and 2- fold respectively), as did the activation of the PGE2 receptor EP4 (6- and 22-fold, respectively). Inhibition of SMAD2/3 phosphorylation completely eliminated the effects of TGFß1 on mOSE stemness, suggesting this proposed pathway is Smad2/3 dependent. Furthermore, the effects of TGFß1 on EMT induction and increasing stemness in mOSE cells is also seen in human OSE cells, suggesting this proposed pathway is translational into humans.

Conclusion: These data suggest that at ovulation, TGFß1 increases the OSE stem cell population through the canonical Smad2/3 pathway to induce an EMT and increase Ptgs2 expression. Expansion of this stem cell population may promote ovulatory wound repair and dysregulation of this pathway may lead to ovarian cancer initiation.

15 **FGL2 as an immunomodulator during early placentation and its role in the pathology of preeclampsia**

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Background
Preeclampsia is a pregnancy-specific disease that can have serious consequences for both the mother and the baby. There is no effective treatment for preeclampsia, and preventative therapies, although helpful, require reliable identification of women at risk. The success of any pregnancy depends greatly on a balance, at the interface of maternal and fetal tissue, between pro- and anti-inflammatory immune mechanisms. The disruption of this balance, early in pregnancy, contributes to impaired placental development, which is at the root of placental dysfunction causing symptoms at later stages. Fibrinogen-like protein 2 (FGL2) is a likely player in the pathology of preeclampsia, as it has known roles in the regulation of immunity and in fibrin deposition, a common feature of preeclamptic placentas. FGL2 is present on both sides of the interface as well as in the maternal blood, making it a potential biomarker and predictor of preeclampsia.

Objective
This study aims to determine if FGL2 regulates the immune environment of early pregnancy and placentation, and if so, by which mechanisms. It also aims to determine if FGL2 could be used, early in pregnancy, as a reliable biomarker predicting the appearance of preeclampsia symptoms at later stages.

Methods
Using novel transgenesis techniques to modulate FGL2 expression in vitro and in vivo in mice, the role of FGL2 in trophoblast function will be defined. Using innovative co-culture models, the influence of FGL2 on maternal uterine immunity (murine and human) will be determined. Bioinformatics analysis of FGL2 expression in human placenta samples will be used to determine its potential as a biomarker for subtypes of preeclampsia.

Results and conclusions
A previous study identified within preeclamptic placenta samples distinct clusters of gene expression, representing molecular subtypes of preeclampsia. Microarray analysis was conducted to compare gene expression in samples classified into these clusters. FGL2 was found to be differentially expressed across clusters and interestingly, to be particularly high in a cluster of samples with "inflammation-related" disease. Bioinformatics correlation analysis was used to identify genes whose expression patterns correlate with that of FGL2. A Gene Ontology (GO) term analysis revealed that most correlated genes are involved in immune response and inflammation. Moreover, genes that better correlate with FGL2 in preeclamptic samples than in control samples are also those involved in immune response. This strongly suggests a role for FGL2 in modulating the immune environment of early pregnancy and placentation.

16 **A Novel Role for SLK in Transforming Growth Factor ß-Mediated Epithelial-to-Mesenchymal Transition**

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

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

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

Results: It was determined that in SLK knockdown conditions, there is a decrease in the cell's ability to progress into EMT, indicating that SLK activity is involved in downstream EMT signaling. In addition, complete loss of SLK results in a significant decrease in the migratory and invasive capacities of cells when EMT is induced using transforming growth factor ß.

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

17 **Snf2h-mediated regulation of cell identity gene expression programs**

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Background
The mammalian ISWI homologue Snf2h is an ATP-dependent chromatin remodelling enzyme, responsible for sliding nucleosomes, affecting the accessibility of underlying DNA to various factors. It has been broadly implicated in transcriptional regulation, DNA repair and replication, and maintaining higher-order chromatin structure. Its expression is relatively ubiquitous, however several studies have highlighted specific roles in distinct cell types, such as the developing cerebellum. Its expression is also required for the development of the early embryo. While its importance is evident, it's currently unclear how Snf2h is utilized to regulate distinct cell states.

Objective
The objective of this study is to explore Snf2h-mediated regulation of cell identity gene expression programs, using the pluripotent state as a model, and to understand the mechanisms governing its recruitment across the genome.

Methods
Using the pluripotent state as a model to study the role of Snf2h in regulating cell identity, we derived a mouse embryonic stem cell line (mESC) from Snf2hfl/fl mice, and induced the deletion of Snf2h by transfecting a Cre recombinase expression plasmid. ChIP-seq data was analyzed with Bowtie and MACS. The following public datasets were used for various analyses GSE60749, GSE36114, GSE71035, and GSE53583.

Results
Deletion in mESCs results in aberrant changes to colony morphology, and coincides with the repression of Oct4, Sox2, and Nanog expression—changes that are typically indicative of the loss of pluripotency. Preliminary ChIP-qPCR experiments suggest these genes are directly regulated by Snf2h. We also used differentiation and reprogramming data and found that Snf2h expression correlates with the pluripotent state. These data suggest that high levels of Snf2h may be required to support the pluripotent state, and its absence may serve as a trigger for differentiation. Interestingly, ChIP-seq data from neural and mammary tissues suggests that Snf2h localization is largely cell type-specific, with unique localization at cell-defining genes. Further work will be done to understand the role of Snf2h in regulating the pluripotent state, and to discover the cell type-specific mechanisms governing the its recruitment across the genome

Conclusion
Cell identity can largely be defined by a cell's unique gene expression program; however, many of the chromatin modifiers that coordinate gene expression are ubiquitously expressed. This work begins to shed light on how Snf2h regulates the pluripotent state, and further work will explore how Snf2h is utilized in cell type-specific contexts to coordinate unique gene expression programs. This knowledge may shed light on how these mechanisms are hijacked to support high disease-driving gene expression.

18 **Distinct gene expression profiles for ovarian carcinoma sensitive to the poly(ADP-ribose) polymerase inhibitor ABT-888 by ex-vivo analysis of fresh tumors from primary surgery**

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Background: Clinical sensitivity to poly(ADP)-ribose polymerase (PARP) inhibitors has been shown in both BRCA-mutated and sporadic ovarian cancer (OC) patients. The objective of this study is to identify a molecular profile that distinguishes sensitivity to the PARP inhibitor ABT-888.

Methods: Following IRB approval, fresh ex vivo tissue from primary surgery and clinicopathologic data were collected on consenting patients at the Ottawa Hospital with ovarian, fallopian tube and peritoneal carcinoma. Tissue viability was assessed and tissue cores were treated ex vivo with ABT-888 +/- cisplatin. Sensitivity to ABT-888 was determined using a two-way ANOVA. Untreated fresh tissue samples were collected for transcriptomic profiling using Agilent 44k gene expression arrays and analysis by GeneSpring GX software.

Results: Of 36 evaluable patients in the study, 7 tumors had limited cell viability at seeding and were excluded from the analysis. The median age of the 29 remaining patients was 63 years (range 37-86). One patient had a known BRCA1 mutation and 57% (17/30) had high-grade serous carcinoma (HGSC). There was a differential response to ABT-888, with 7 (24.1%), 8 (27.6%) and 14 (48.3%) of tumors being highly sensitive, moderately sensitive and non-sensitive to ABT-888, respectively. Microarray gene expression analysis of highly sensitive and non-sensitive OC phenotypes showed distinct gene expression profiles

Conclusions: The ex-vivo OC tissue model represents a novel discovery for putative signatures of PARPi sensitivity. Future validation of the gene profiles in a larger patient cohort is warranted.

19 **Effect of VSe1 Analogues on Innate Antiviral Immunity**

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Background
Oncolytic viruses are selected or engineered to selectively infect and kill cancer cells. Often, oncolytic viruses are attenuated to increase their safety profile, resulting in reduced efficacy in heterogeneous malignancies. This is the case with the recombinant attenuated oncolytic vesicular stomatitis virus VSV?51. A small molecule, Viral Sensitizer 1 (VSe1), has been shown to overcome this problem by selectively enhancing replication of VSV?51 in resistant cancer cell lines. Despite VSe1's potency as a viral sensitizer, the molecule has poor physiochemical properties. Structural analogues of VSe1 were synthesized in the attempt to optimize physiochemical properties while maintaining efficacy. The analogues can be categorized into furans, pyrroles and pyridazines.

Objective
The objective of this study is to identify the mechanism by which these viral sensitizers are able to enhance the replication and spread of VSV?51 in malignant cells. We hypothesize that VSe1 and its analogues interfere with the host cell's innate antiviral immune response.

Methods
The described assays were performed in 7860 renal carcinoma cells. Cells were treated with VSe1 and analogues prior to induction of antiviral pathways (using IFN-ß or infection with VSV?51). Protein or RNA samples were collected and stored. Immunoblotting assays were used to study the effects of VSe1 and its analogues on various key proteins used in antiviral pathways, including STAT1 and IRF-3. Quantitative realtime PCR was used to analyze the transcription of antiviral genes downstream of these pathways.

Results
Furan, pyrrole, and pyridazine analogs were all shown to overcome IFN-induced antiviral activity in cell culture. Western blot and realtime PCR results suggest that the furan viral sensitizers interfere with the JAK/STAT pathway and the transcription of antiviral genes known as 'interferon-stimulated genes' (ISGs). In contrast, pyridazine and pyrrole compounds do not to interfere upstream within the JAK/STAT pathway (i.e. phosphorylation of STAT1). However, preliminary studies suggest that these compounds may inhibit the phosphorylation of IRF-3, which is normally activated following RNA-virus sensing by pattern recognition receptors such as RIG-I. Further studies will address how the different VSe1 analogs lead to disparate effects on antiviral signalling.

Conclusions

Although the pyridazine and pyrrole compounds are structural analogues of VSe1, they seem to differ in mechanism of action. Our data suggests that VSe1 and other furan analogues enhance viral activity by interfering with the activation of STAT1 and the transcription of ISGs. On the other hand, pyrrole and pyridazine analogues interfere with the activation of IRF-3.

20 **Treatment of taxane acute pain syndrome (TAPS) in patients receiving taxane-based chemotherapy for breast and prostate cancers – a systematic review**

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Abstract:

Background: Taxane acute pain syndrome (TAPS) is characterized by myalgias and arthralgias starting 1-3 days, and lasting 5-7 days after taxane-based chemotherapy. Despite negatively impacting patient quality of life, little is known about optimal TAPS management. A systematic review of treatment strategies for TAPS was performed.

Methods: Embase, Ovid Medline(R), and the Cochrane Central Register of Controlled Trials were searched from 1946 to October 2014 for trials reporting the effectiveness of different treatments of TAPS in patients receiving taxane-based chemotherapy for either breast or prostate cancers. Two individuals independently screened citations and full-text articles for eligibility. Outcome measures included: type of treatment, and response of myalgias, arthralgias, pain and quality of life.

Results: Of 1614 unique citations initially identified, 4 studies met the pre-specified eligibility criteria. Two were randomized placebo-controlled trials (225 patients), one was a randomized open-label trial (36 patients) and one was a retrospective study (10 patients). The agents investigated included: gabapentin, amifostine, glutathione and glutamine. Study sizes ranged from 10 to 185 patients. Given the heterogeneity of study designs, a narrative synthesis of results was performed. Neither glutathione (Quality of life, p=0.30, no 95% CI reported) nor glutamine (mean improvement in average pain 0.8 in both treatment arms, p=0.84, no 95% CI reported) were superior to placebo. Response to amifostine (pain response) and gabapentin (reduction in taxane-induced arthralgias and myalgias) were 36% (95% CI, 16-61%) and 90% (no 95% CI data reported) respectively.

Conclusions: Despite its prevalence in patient's receiving taxane-based chemotherapies, TAPS remains poorly researched and few studies evaluate its optimal management. If the management of patients is to be improved, more prospective trials are needed.

21 **Improving patient care with Multiple Myeloma Clinical Management Tool**

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Background: "Apps" for managing medical data are purported to increase healthcare efficiency and improve patient safety. However, according to Commonwealth Fund International Health Policy Survey, Canadian physicians report reduced access to patient test results and hospital records using electronic medical records (EMRs), leaving them poorly equipped to manage patients with chronic diseases. Multiple myeloma (MM) is such chronic illness, followed by monthly laboratory tests and treated by a multidisciplinary healthcare team.

Objective: To develop and implement an easy-to-use, intuitive interface for data management, resulting in improved care for MM patients.

Methods: The Multiple Myeloma Clinical Management Tool (MMCMT) is innovative software suitable for desktops and iPads, derived from paper spreadsheets historically used to follow MM patients. The MMCMT was designed to efficiently display and integrate labs, treatments and events. For patient safety, an alert indicator allows users to enter important patient-specific clinical, treatment, or organizational cautions, or notices for other users. Information including diagnosis, staging, comorbidities, lab results, imaging, chemotherapies, radiotherapies, transplantation history, surgeries, hospitalizations, complications and supportive care measures, have been entered into MMCMT for more than 1,000 patients.

Results: The MMCMT displays patient information on a timeline, presenting the patient's longitudinal course visually, thus facilitating clinical monitoring, decision-making and data documentation. Clinical information is accessible to all members in the

MM clinical group, improving day-to-day management, patient records sharing and communication concerning patients. Furthermore, data has been analyzed to evaluate treatment strategies and overall survival allowing comparison to international benchmarks, thus contributing to continuous quality improvement.

Conclusions: This tool improves patients' quality of care and safety. The MMCMT allows all team members access to data in real time, reduces lost data and filing, ends handwriting errors and provides programmatic data for Continuous Quality Improvement. It is simple to provide a printed timeline to the patient as an educational tool, and to be available to other non-TOH healthcare providers.

Disclosure: Nothing to disclose.

22 **Investigating the role of P-rex1 in glioblastoma**

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Background: The highly invasive nature of the adult brain cancer glioblastoma is a major driver of its malignancy. The loss of tumor suppressor PTEN is the most common genetic alteration in glioblastoma and has previously been implicated with increased motility. Phosphatidylinositol 3,4,5-trisphosphate-dependent Rac exchanger 1 (P-rex1) has been previously shown to promote invasive/metastatic behaviour in multiple cancers through the activation of Rac1. P-rex1 has been shown to be activated synergistically by both phosphatidylinositol 3,4,5-trisphosphates and the g-protein couple receptor (GPCR) beta-gamma subunit. Thus, P-rex1 may be an essential link of PTEN loss to increased migration in glioblastoma.

Objectives: Investigate the role of P-rex1 downstream of PTEN loss in primary glioblastoma cell cultures

Methods: To investigate the molecular basis driving the invasiveness of glioblastoma we utilize primary patient derived cells grown from surgical samples from consented patients undergoing surgery for suspected glioblastoma and cultured in defined neural stem cell media. Cell motility was measured by Transwell invasion chamber assays as well as time-lapse videomicroscopy.

Results: PTEN expression is reduced or lost in all tested glioblastoma tumor initiating cells. The reconstitution of PTEN with a doxycycline inducible lentiviral vector decreased in vitro migration. P-rex1 is expressed in all in all tested glioblastoma tumor initiating cells. Knockdown of P-rex1 with siRNA resulted in decreased in vitro invasiveness as measured by Transwell invasion chamber assays via decreased migration assessed by time-lapse videomicroscopy. To investigate the upstream activators of P-rex1, gallein, a GPCR beta-gamma subunit inhibitor, and BKM120, a phosphoinositide-3-kinase (PI3K) alpha/beta inhibitor were used separately or in combination. Both gallein and BKM120 inhibited migration with no combinatory effects.

Conclusion and future directions:
We have shown that primary glioblastoma cells from multiple patients recapitulate the invasive behavior as intracranial mouse xenografts. My in vitro data suggest that P-rex1 is a potent effector of glioblastoma migration. We are currently exploring shRNAs targeting P-rex1 or dominant negative P-rex1 to explore function in vivo. Gallein and BKM120 phenocopy P-rex1 depletion and thus demonstrate the potential for utilizing inhibitors of P-rex1 activation for inhibiting glioblastoma migration.

23 **ACTIVATING TRANSCRIPTION FACTOR 3 AS A NOVEL MEDIATOR OF DOXORUBICIN RESPONSE IN AGGRESSIVE BREAST CANCER**

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Background: Breast cancer is the second leading cause of cancer related deaths among women. Significant advances have been made in the treatment of advanced breast cancer; however, the 5 year survival of the advanced disease remains very poor. Anthracyclines, such as doxorubicin, are used as first line chemotherapeutics, usually in combination therapies, for the treatment of advanced disease. While these drugs have been successful therapeutic options, their use is limited due to serious drug related toxicities and acquired tumor resistance. Uncovering the molecular mechanisms that mediate doxorubicin's cytotoxic effect may lead to improved therapeutic strategies. Previous findings in our laboratory have identified activating transcription factor 3 (ATF3) as a key mediator of cisplatin cytotoxicity. We have also demonstrated that ATF3 expression, which is disregulated in cisplatin resistance, can be upregulated by doxorubicin treatment.

Hypothesis: The goal of this study is to determine the role of ATF3 in mediating doxorubicin cytotoxicity in breast cancer, as well as

to identify novel ATF3 inducing agents for combination therapies.

Results: ATF3 expression was upregulated in both a time and concentration dependent manner by doxorubicin treatment in the human and murine breast cancer cell lines tested. Doxorubicin induced ATF3 expression occurred through the activation of both JNK and ATM signaling pathways. In ATF3-/- MEFs there was a significant reduction in sensitivity to doxorubicin treatment. Through a 1200 FDA approved compound library screen, several drugs were identified that could enhance doxorubicin cytotoxicity, including the HDAC inhibitor Vorinostat. Additionally, we have demonstrated that doxorubicin can induce ATF3 expression in ex-vivo human breast and ovarian tumor samples which represents a potential novel screening strategy for identifying responsive tumors.

Conclusion: ATF3 is a novel mediator of doxorubicin cytotoxicity and represents a new therapeutic target for the treatment of breast cancer. The use of other ATF3 inducing compounds, such as HDAC inhibitors, can enhance doxorubicin cytotoxicity.

24 **Utility of miRNA signatures to predict rapid versus delayed onset of castrate resistance in prostate cancer**

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Background: The onset of castration-resistant prostate cancer (CRPC) following androgen deprivation therapy (ADT) in metastatic patients can be quite variable with some developing CRPC within 24 months, while others have a delayed development of 36 months or more. Identifying patients who may be more prone to development of CRPC earlier in their disease course is of great clinical importance as this would provide rationale for intensified early treatment with novel therapies that could increase patient survival. To this end, micro (mi)RNAs are an emerging area of interest in biomarker discovery and importantly, show dysregulation in the majority of prostate tumours. The documented stability of miRNAs in paraffin-embedded tissues and plasma make them an ideal robust biomarker candidate for classifying the prostate cancer patients in our study.

Objective: To identify circulating miRNA signatures to predict rapid versus delayed castrate resistance in metastatic prostate cancer patients.

Methods: Preliminary analysis focused on baseline (study entry) plasma samples acquired from metastatic prostate cancer patients prior to initiation of ADT. Patients were subsequently classified as having developed early CRPC (< 2 years since initiating ADT) or delayed CRPC (> 3 years since initiating ADT). MiRNA was isolated from heparin drawn plasma and miRNA profiling was performed for 1066 human miRNAs with the miRNome miScript miRNA PCR array (Qiagen).

Results: A protocol was established for reliable miRNA isolation and quantification from as little as 100 µl of heparin drawn plasma with a 98% success rate (57 of 58 samples produced usable amounts of miRNA suitable for RT-PCR). To date, the plasma miRNome for 29 early and 28 delayed CRPC patients has been obtained. Currently, analysis of data is underway to identify a miRNA signature that may identify patients prone to development of early CRPC, which will be validated in phase II of the study.

25 **Engineering the Vaccinia virus genome using transposon-mediated insertional mutagenesis for the treatment of malignant melanoma.**

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Background: In recent years, checkpoint inhibitors have begun to revolutionize the treatment of malignant melanoma, however a large proportion of patients do not respond adequately. One reason for this is the lack of an initial anti-tumour immune response, which can be rectified through the use of effective oncolytic viruses (OVs) that direct the immune system to initiate an immune response against an OV-infected tumour. Vaccinia virus (VacV) holds promise to deliver a major improvement over the only currently FDA-approved OV, T-Vec. Until now, we have lacked a tool to systematically and comprehensively probe the complex genome of VacV for the most effective OV platform.

Objective: Our objective was to utilize transposon-mediated insertional mutagenesis to create a diverse library of VacV clones, each possessing a single mCherry-transposon insertion that can act to functionally truncate or knock out the respective gene. This

library would represent a novel tool that could be screened for efficacy in cancers, including malignant melanoma.

Methods: We utilized a PiggyBac Transposon System (System Biosciences) to randomly integrate our mCherry-expressing insert into TTAA sites throughout the VacV genome. We cell-sorted the infected cells, performed full viral genome Next-Generation Sequencing to identify our integration sites, and we then plaque purified and expanded our library. Concurrently, we have begun collecting patient melanoma samples, on which we have begun ex vivo infections of tumour cores and the initiation of primary melanoma cell lines. We have also begun complementary library screening techniques that are directed at identifying cancer-targeting clones of interest.

Results: Our transposon-mediated insertional mutagenesis technique has resulted in a library of approximately 100 unique VacV clones that represents over 60 unique VacV genes (almost one third of the VacV genome). We have been collecting patient specimens for approximately 1 year and have shown effective ex vivo infection with our transposon library as well as had success in initiating primary cell lines. We have proven the utility of our screening techniques and are quickly moving forward in selecting clinical OV-candidate clones of interest.

Conclusions: The library that we have created represents a novel approach to the study of viruses and has resulted in a tool that will allow us to more comprehensively and systematically study the VacV genome than previously possible. The experiments we continue to perform will lay the foundation for the in vivo validation of our results and select clones to investigate potential clinical development.

26 **First-in-Class Small Molecule Potentiators of Cancer Virotherapy**

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5. These authors contributed equally to this work

Background: Pre-clinical and clinical studies have shown that oncolytic virus (OV) therapy is safe and well tolerated in humans and can infect a broad range of cancers. However, in clinical studies OV therapy has highly variable response rates. The heterogeneous nature of tumors is widely accepted to be a major obstacle for OV therapeutics and highlights a need for strategies to improve viral replication efficacy. To this end, we have previously identified the synthetic compound Viral Sensitizer 1 (VSe1) that enhanced the spread of oncolytic vesicular stomatitis virus (VSV?51) in resistant cancer cell lines up to a 1000-fold, resulting in synergistic cell killing and improved efficacy in vitro and in vivo. The electrophilic nature of VSe1 prompted us to investigate the scaffold to identify active analogs with more favourable physiochemical properties and explore structure-activity relationships (SAR).

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

Methods: To study the SAR of VSe1, enhancement of VSV?51 expressing firefly luciferase by VSe1 analogs in resistant 786-0 (human renal carcinoma) cells was assessed by a novel high-throughput luciferase expression based viral titration assay. For each analog, plasma stability was assessed by LC-MRM and electrophilicity was assessed by reactivity with glutathione. Selectivity of compounds for cancerous tissue was assessed by ex vivo treatment and infection of murine tissues. In vivo tolerability of VSe1 analogs was assessed in a dose escalation study in Balb/C mice, and in vivo efficacy of VSe1 analogs in combination with VSV?51 was assessed in the CT26 syngeneic murine model of colon carcinoma.

Results: In vitro assays and a rational approach in the design of VSe1 analogs allowed us to identify functional groups that can be modified without hampering activity. Lead compounds increase OV growth up to 2000-fold in vitro and demonstrate remarkable selectivity for cancer cells over normal tissue ex vivo and in vivo. Interestingly, some analogs possess improved potency and stability with reduced electrophilicity. Analogs were significantly better tolerated than VSe1 in vivo and were also able to enhance VSV?51 replication in vivo.

Conclusion: We have developed a novel class of small molecules for enhancing OV replication in cancer tissue. SAR studies led to the identification of compounds with favourable pharmacological properties that significantly enhance OV propagation selectively in resistant cancers in vitro, ex vivo, and in vivo, completing proof-of-concept studies for a pharmacoviral combination approach to enhancing OV therapy.

27 **Premature Senescence in Glioblastoma Tumor Initiating Cells (GTICs)**

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Background: Glioblastoma is the most common primary malignant brain tumor. Unfortunately, despite current advances in treatment the median survival after diagnosis is 14 months. Recently, a population of tumor cells called Glioblastoma Tumor Initiating Cells (GTICs) were discovered to be the key drivers of glioblastoma. Senescence is the irreversible growth arrest of cells with continued metabolic activity and is best detected by the Senescence Associated Beta Galactosidase (SA-B-Gal) assay. Premature senescence, senescence that is independent of telomere attrition, has been shown to decrease the malignant potential of various cancers. Although premature senescence has been shown to occur in glioblastoma it has not been demonstrated in GTICs. Treatment of GTICs with Fetal Calf Serum (FCS) is known to cause aberrant differentiation and I recently discovered that some subtypes of GTICs can undergo premature senescence in addition to differentiation in response to FCS.

Objective: Determine the molecular mechanisms of serum induced premature senescence in GTICs.
Methods: RNA was harvested from untreated GTICs and GTICs treated with serum for 24 hrs and sent for microarray analysis. Bioinformatics analysis was provided by Ted Perkins at the OHRI. GTICs were treated with 20 ng/ml Transforming Growth Factor-Beta (TGF-B) or 10% FCS for seven days. GTICs were treated with the TGF-B inhibitor SB431542 at 10 uM for seven days. Senescence was determined by the SA-B-Gal assay and protein expression was detected by immunoblotting.

Results: Genetic expression profiling of GTICs treated with FCS demonstrated the increased expression of genes in the TGF-B pathway, such as Thrombospondin1 (THBS1), compared to untreated GTICs. TGF-B treatment caused 50% SA-B-Gal positivity compared to 5% in untreated GTICs. Serum treatment of GTICs led to increased expression of phosphoSMAD2 (downstream signalling of TGF-B) 2 hours after treatment without any change in total SMAD2. Furthermore, co-treatment of GTICs with serum and the TGF-B inhibitor SB431542 led to a 30% decrease in SA-B-Gal positivity compared to serum only treated cells. In addition, SB431542 treatment of serum treated GTICs led to a decrease in phosphoSMAD2 without any change in total SMAD2 compared to serum only treated GTICs.

Conclusions: Together, these data suggest TGF-B is a key mediator of serum induced senescence in GTICs. Future studies will aim to elucidate TGF-B in mediating senescence in GTICs such as its activation from the latent to active form by THBS1.

28 **The role of Focal Adhesion Kinase in breast cancer mediated osteolysis**

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Background:
Breast cancer most commonly metastasizes to the bone, where it disrupts bone homeostasis leading to osteolytic lesions. This occurs through the secretion of soluble factors from breast cancer cells that both inhibit osteoblast maturation, and induce severe bone degradation by osteoclasts. Although there are current treatments for bone metastasis including RANK-L inhibitors and bisphosphonates, an increase in patient survival has not been seen. There is therefore a need for novel therapeutics in the treatment of bone metastasis. Focal adhesion kinase (FAK), has emerged as a novel therapeutic in the treatment of cancers, including breast. FAK is a non receptor tyrosine kinase shown to be involved in tumor progression and metastasis. FAK is also expressed in all of the cells involved in breast cancer mediated osteolysis. We therefore hypothesized that the inhibition of FAK would not only cause direct anti-tumor effects, but would also lead to a restoration in bone homeostasis through osteoblast maturation and inhibition of osteoclast bone degradation.

Objective:
Given the need for novel anti-tumor agents effective in blocking tumor progression in bone metastatic breast cancer patients, we sought to evaluate the role of FAK blockade in breast cancer mediated osteolysis.

Methods:
Using in vitro siRNA-targeted depletion of FAK in breast cancer cell lines, we evaluated the expression of multiple factors involved in the induction of osteoclastogenesis and osteolysis by PCR, Western blot and ELISA. Further, in in vitro osteoblast and osteoclast co-cultures, the ability of FAK-depleted versus FAK-expressing breast cancer conditioned media to induce osteoclastogenesis was evaluated. We also assessed the effects of treatment with FAK tyrosine kinase inhibitor, PF-562,271 on the expression of osteoclastic factors in RANK-L differentiated RAW 264.7 cells.

Results:
We found that an essential osteoclastogenic factor, macrophage colony stimulating factor was decreased in FAK depleted breast cancer cells. Further, through an osteoblast/osteoclast co-culture, it was determined that FAK depleted conditioned media resulted in impaired osteoclastogenesis compared to the control media from FAK expressing tumor cells. Lastly, we found that direct pharmacological inhibition of FAK in mature osteoclasts resulted in decreased production of osteoclastic enzymes such as Cathepsin K.

Conclusion:
This data suggests that FAK inhibition could prove to be a beneficial therapeutic in the treatment of bone metastasis, by preventing bone degradation while also providing its known direct anti-tumor effects.

29 **MEVALONATE PATHWAY INHIBITORS ENHANCE EGFR-TKIS EFFICACY IN HEAD AND NECK SQUAMOUS CELL CARCINOMAS**

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Epidermal growth factor receptor (EGFR) is highly expressed in head and neck squamous cell carcinomas (HNSCC) it is associated with advanced tumor stage. Tarceva, the EGFR tyrosine kinase inhibitors (TKIs), showed its effect in HNSCC in targeting EGFR by preventing ligand induced receptor activation. However, some cancers were resistant to those molecules. Novel therapeutic approaches are urgently required. Thus, expanding the therapeutic repertoire of EGFR-TKIs will likely require the addition of an agent(s) that in combination will more efficiently target the EGFR. Our laboratory was the first to demonstrate that statins, inhibitors of the mevalonate pathway, inhibit the activation and downstream signaling of the EGFR in a mechanism distinct from EGFR-TKIs. Hence, combining statins with EGFR-TKIs enhanced EGFR inhibition and induced synergistic cytotoxicity in HNSCC cells. This work led to Phase I clinical trial combining Rosuvastatin with Tarceva that has just been completed. This trial showed the activityty of this approach, however, with GGTI-298 can induce synergistic cytotoxicity with Tarceva in HNSCC cells. We believe that targeting this GGTase I enzyme may alleviate the toxicities of statins but retain the efficacy in combination with EGFR inhibitors.

30 **A genome wide CRISPR/Cas9 screen to identify novel genes that regulate onvolytic virus infection and growth specifically in cancer cells**

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Background: Oncolytic virotherapy is an emerging therapeutic strategy that uses replication competent viruses to selectively kill tumor cells without damaging normal tissues. Despite the clinical successes of oncolytic viruses (OVs), like most therapeutics they show efficacy only in a subset of patients, limiting their wide-spread application. The recent identification of the easily programmable RNA-guided CRISPR/Cas9 endonuclease to modify specific genomic loci by introducing loss of function mutations represents a new way to interrogate gene function on a genome-wide scale.

Objective: The objective of this work is to identify novel genes and signaling pathways that enhance OV growth specifically in cancer and associated stromal cells but not normal tissues.

Methods: In an attempt to identify novel genes involved in OV infection and growth, a genome-wide CRISPR/Cas9 knockout screen with over 100,000 synthetic guide-RNAs that direct gene specific inactivation by Cas9 will be performed in vitro in cancer cell lines resistant to OV infection and normal cell lines. Cells will be subsequently treated with Maraba-MG1 for 48 hours and surviving cells sequenced to reveal depleted genes that specifically sensitize cancer cells to viral growth. Guide-RNAs found to enhance OV growth will be validated by targeted knockout and promising candidates engineered into the MG1 backbone. The ability of these viruses to infect and kill cancer cells both in vitro and in vivo will then be assessed.

Results: Treatment of a panel of pan-cancer cell lines with MG1 revealed a spectrum of sensitivity to OV infection. The most resistant cell lines were transfected with lentivirus encoding the Cas endonuclease and clonal populations generated. We confirmed that transfection of cell lines with Cas expressing plasmids does not significantly alter cell growth or morphology and has no effect on OV infection or growth. Optimization of viral titers and duration of infection identified a low multiplicity of infection (0.1) and medium time period (48 hours) to be ideal for maximum cell death. Surviving cells following OV infection are currently being sequenced.

Conclusions: In light of recent clinical successes, OVs are making a resurgence in cancer therapeutics. This study will generate fundamental knowledge regarding the susceptibility of cancer and normal cells to OV infection, which can be immediately applied to engineer more effective virus strains and make a substantial leap forward in the design of potent oncolytics for cancers typically resistant to OV therapy

31 **Deletion of the Ste20-Like Kinase Results in Impaired Muscle Function, but Rescues Terminal Differentiation in Response to TGFb Signaling**

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The Ste20-Like Kinase (SLK) is ubiquitously expressed throughout all tissues of the body, but shows predominant expression throughout embryonic and adult skeletal muscle. Expression of a dominant negative SLK construct in C2C12 myoblasts decreased fusion and myotube formation. In order to assess the functional role of SLK in muscle development, we generated a muscle specific knockout using the Myf5-Cre Recombinase and SLK conditional knockout mouse models. Knockout animals were born at expected ratios with no overt defects upon weaning. At 6 months of age we observed the presence of centrally located nuclei and decreased force generation in knockout animals compared to control littermates. Additionally, we noticed defects in the localization and activation of Paxillin, which had been previously shown to be a target of SLK. Smaller myofibers were also observed in older animals, but was not dependant on increased expression of atrophy associated proteins. Further investigation in cultured C2C12 myoblasts suggested that the deletion of SLK conferred resistance to inhibition of terminal differentiation in response to TGFb. Therefore, SLK is necessary for normal development and function of skeletal muscle tissue, but may be detrimental in times of increased TGFb signalling, such as atrophy or muscular dystrophy.

32 **Ontario Tumour Bank Initiative at The Ottawa Hospital**

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

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

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

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

33 **Gene expression analysis in pancreatic cancer xenografts**

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Background: Pancreatic ductal adenocarcinoma (PDAC) is one of the leading lethal cancers in the world. Most patients (80-85%) are diagnosed in late stages, presenting with locally advanced or metastatic disease. Treatments such as radiation and chemotherapy have limited effectiveness in treating pancreatic cancer due to poor response; therefore, advanced PDAC has a very poor prognosis with a five year survival rate of only 3-5%. Thus, research into more effective targeted therapies must be

investigated. Patient derived xenografts maintain the genetic and histological characteristics of the patient's original tumor and can serve as predictive models of drug response.

Objective: Investigate and identify gene and miRNA targets in PDAC versus neuroendocrine (NE) xenografts using tumor profiling sequencing.

Methods: Formalin-fixed paraffin embedded (FFPE) xenografts (n=7 PDAC, 1 NE) were sectioned at 5µm onto microscope slides. An area of 12 to 25mm2 of tissue was scraped off the slide using a razor blade into a tube. Sample libraries were prepared on the HTG platform (HTG Molecular Diagnostics) for tumor profiling using the HTG EdgeSeq Oncology Biomarker Assay (2560 genes, 24 pathways) and the HTG EdgeSeq miRNA Whole Transcriptome Assay (2084 miRNAs). Samples were then sequenced on a MiSeq (Illumina). Data was normalized to total number of reads and to endogenous references.
Results: The HTG EdgeSeq assays successfully profiled all eight xenografts. For miRNA sequencing, there were 189 miRNAs with robust signals (>300 reads). Within these data, significant differences were observed between the PDAC and NE tumor profiles. There were several miRNAs that were significantly upregulated (>6-fold) in the PDAC tumors compared to the NE: miR-199a, miR-221, miR-222, and miR-29a. Significantly downregulated miRNAs in the PDAC tumors included: miR-148a, miR-193b, miR-30b, miR-30d, miR-335, and miR-375.

Conclusions: Gene expression analysis of patient derived xenografts identified previously known and novel genes and miRNAs in pancreatic cancer xenografts. We found different tumor profiles between the PDAC and NE xenografts. We plan to assess miRNA and genetic expression in these PDAC xenografts pre- and post-treatment with oncolytic virus.

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34 **Developing Biomarkers to Select Potential Patients for Clinical Trials of Oncolytic Virotherapy**

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Background
Oncolytic virotherapy is a promising new take on cancer therapy exploiting viruses that selectively target and kill tumor cells without causing harm to normal cells. Pre-clinical data indicate that oncolytic viruses such as the attenuated rhadovirus Maraba MG-1 can be curative; however, heterogeneous infection of tumors and subsequent therapeutic response is evident. Therefore, it would be advantageous to gain insight that would allow for the selection of cancer patients most likely to respond to the therapy prior to trial enrolment.

Objective
We hypothesized that assessing ex vivo infectivity of tumors and the tumor microenvironment could help identify biomarkers to help select clinical trial candidates. Our objectives were to screen ten different types of syngeneic mouse tumor models to: (1) examine the correlation between ex vivo and in vivo susceptibility to MG-1 by plaque assay, and (2) determine the histopathology of sensitive and resistant tumors using immunohistochemistry.

Methods
Ten different types of mouse syngeneic tumors were implanted in immunocompetent mice. For the in vivo experimental arm, MG-1 was injected either intratumorally or intravenously into tumor bearing mice. At 24 hours post infection, tumors were excised, sliced, and cored. For the ex vivo experimental arm, tumors were excised and cored before the administration of MG-1. At 24 hours post infection (ex vivo) or following tumor tissue processing (in vivo), cores were homogenized; then, virus yield was titered by plaque assay. Also, uninfected ex vivo cores were fixed and stained for CD31 and Blimp1.

Results
Ex vivo infectivity of isolated tumors did not correlate with in vivo infection irrespectively of the route of virus delivery. However, our preliminary data indicate that MG1 was delivered more efficiently in vivo when tumors were better vascularized; the number of CD31+ blood vessels positively correlated with in vivo infectivity. Therefore, high tumour vascularization was determined to be a potential biomarker for in vivo infection. Also, most of the CD31+ tumor endothelial cells expressed Blimp1, a key transcriptional regulator that induces antagonism of interferon antiviral signaling pathway, suggesting that upregulation of Blimp1 allowed more oncolytic virus infection.

Conclusions
High tumor vascularization is a potential biomarker for in vivo infection using MG-1. If we are able to predict the in vivo infectivity from ex vivo tumor assessments, this research has the potential to help clinical oncologists to select patients and predict outcomes of MG-1 clinical trials, which will facilitate the effective use of oncolytic virotherapy in the clinic.

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35 **Identifying Chemical Enhancers of Adenoviral Transgene Expression for Gene Therapy Applications**

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Background: Gene therapy is a therapeutic approach that aims to treat genetic illnesses at the source, by replacing or correcting deleterious genes that are causal for disease. Adenoviruses are a desirable vector for gene therapy due to their ability to transduce both replication-competent and –deficient cells, and the fact that the viral genetic material does not integrate into the host genome. However, these therapeutics can be rendered ineffective by inadequate infection of target cells and restricted transgene expression.

Objective: To identify adeno-sensitizing drug compounds that improve target cell infection and or the efficiency of adenovirus transgene expression.

Methods: A high-throughput screening assay was optimized to screen 1280 FDA approved compounds in a human A549 lung carcinoma cell line. Candidate compounds were identified by measuring the expression of a luciferase transgene from a non-replicating adenoviral vector. Drugs that dramatically enhanced the observed luminescent signal were selected to proceed to the validation stage.

Results: Several of the identified drugs were validated in multiple in vitro cell lines, as well as in ex vivo murine tissue samples. The drugs appeared to significantly increase the level of adenovirus transgene expression, in addition to maintaining relatively low levels of cytotoxicity at their active doses.

Conclusion: The compounds identified in this screening process demonstrate the possibility of combining small molecule treatment to potentiate the efficacy of adenoviral gene therapy. Ideally, these approaches will contribute to more potent treatment options for several human diseases, including cancer. Future aims involve transitioning these pharmacoviral systems to a relevant in vivo model, as well as exploring different transgenes.

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36 **A correlative study of NCIC CTG OV.16 to evaluate AQUA-derived PTEN score as a biomarker in epithelial ovarian cancer**

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Background: Platinum resistance continues to be the dominant cause of poor long-term outcomes following primary surgery and chemotherapy in advanced ovarian cancer (OC). One of the mechanisms of platinum resistance in OC is the inhibition of apoptosis through phosphatidyl inositol 3 kinase (PI3K) pathway activation. Phosphatase and tensin homolog (PTEN) is an important negative regulator of the PI3K pathway and thus its expression in newly diagnosed patients may be of prognostic and predictive significance.

Patients and Methods: In 238 patient tumors from the first-line NCIC CTG trial OV.16, PTEN protein expression was quantified by Automated Quantitative Analysis (AQUA). Cox model was used to study the association between PTEN expression and clinical outcomes using a minimum p-value approach in univariate analysis. Multivariate analysis was used to adjust for clinical and pathological parameters. The ability of PTEN expression to predict progression-free survival (PFS) and overall survival (OS) was summarized using Harrell's concordance (C)-index

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Results: PTEN scores (range 13.9-192.3) of the 202 samples that passed quality control were included in the analysis. In univariate analysis, there was a trend suggesting an association between PTEN expression as a binary variable and PFS (low vs high PTEN HR=0.77, p=0.083), and in multivariate analysis, PTEN expression had a marginally significant association with PFS (low vs high PTEN HR=0.74, p=0.059). Neither continuous nor binary PTEN expression measures were associated with OS. Subgroup analysis by

debulking status (residual disease (RD) <1cm or = 1cm) demonstrated a significant inverse relationship between PTEN expression and PFS (p=0.01 for the interaction between binary PTEN level and debulking status), with a numerically superior but non-significant PFS in patients with higher PTEN (median PFS: 14.9 vs 23.5) when RD<1cm (p=0.19) and a trend to better PFS with lower PTEN values (median PFS: 13.0 vs 11.8) when RD=1cm (p=0.08).

Conclusions: The AQUA technique is a novel method for the quantitative measure of PTEN expression in OC with appealing biomarker characteristics. In our study, PTEN expression showed a modest prognostic relationship with PFS but these data require confirmation. The value of PTEN expression by AQUA technique as a predictive biomarker may also be of interest in future studies.

Chronic Disease Program

37 **Withdrawn**

38 **Profiling antimicrobial peptides in the human and murine genitourinary tracts: a search for novel anti-infective agents**

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Background:
Antimicrobial peptides (AMPs) are small, cationic and amphipathic molecules, important for innate immunity and host defense. Due to their broad spectrum microbicidal activity and natural presence within environmentally exposed epithelial cells and innate immune cells, these molecules have been presented as intriguing, low-risk candidates for antimicrobial development. The WHO has now deemed antibiotics resistance as a true global threat. Therefore, obtaining a better understanding of the presence and roles of AMPs in the genitourinary (GU) tract is of paramount importance as they potentially offer a local, broad spectrum, and low resistance alternative for the treatment and prophylaxis of GU tract infections. Profiling AMPs in the GU tract and its secreted materials (urine and seminal plasma) is therefore the first step towards the use of AMPs in a clinical setting.

Objective:
To profile antimicrobial peptides within the human and murine genitourinary systems

Methods:
Human semen samples were collected from healthy male volunteers, aged 21-35, with no history of sexually transmitted infection or infertility. Healthy sperm parameters were assessed based on WHO guidelines for motility, abundance and morphology. AMPs were isolated into three distinct fractions using differential centrifugation: microparticles, exosomes and exosomes supernatant. Protein profiles were assessed using Immunoblotting, MS/MS and LC-MS. Mass spectrometry data was analyzed, scored and sorted, using MASCOT software. Tissue-specific protein profiles of the murine GU Tract were obtained from healthy, 10 week old male mice, and assessed by immunoblotting.

Results:
Preliminary analyses of our results confirm the presence of LL-37, RNase 7, hBD2, clusterin, lactotransferrin, and semenogelin in human seminal plasma, in addition to CRAMP and mBD1 in the murine GU tract. It appears that AMPs in the seminal plasma may be secreted, stored and transported through body fluid in protected, membrane-enclosed trafficking vesicles; microparticles and exosomes. Nonetheless, the mechanisms through which AMPs are released, processed, and activated, require further elucidation. Induction of AMP expression by LPS and uropathogenic bacteria will be studied next to obtain a better understanding of AMPs' potential uses as anti-infective agents.

Conclusion:
In conclusion, our studies so far on human seminal plasma and the murine GU tract have unveiled a variety of AMPs with known microbicidal activity. The presence of AMPs within the GU tract suggests an important role in innate immunity and may represent an effective, lower-risk alternative to conventional antibiotics.

39 **A primary culture of Sertoli cells from adult mice: unique differences in their properties compared to Sertoli cells from young mice.**

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BACKGROUND:
Sertoli cells in the seminiferous tubules (SFTs) play important roles in spermatogenesis. They provide physical and nutritional support to developing germ cells. Sertoli cells' blood-testis barriers (constituted mainly of tight junctions) also safeguard germ cells from harmful humoral attacks. To further understand the roles of Sertoli cells, their primary cultures are needed. However, it is a challenge to isolate Sertoli cells from adult animals, as they constitute only 5% of the total SFT cells. Consequently, primary cultures of Sertoli cells from 20-day old mice/rats have been used as surrogates of adult Sertoli cells for research studies. Since the first round of spermatogenesis is not complete and levels of LH/FSH/testosterone, essential for spermatogenesis, have not maximally attained in 20-day old mice/rats, Sertoli cells from young animals likely have different properties from "adult" Sertoli cells.

OBJECTIVE:
Establish an efficient method for the primary culture of Sertoli cells from adult mice.

METHODS AND RESULTS:
Sertoli cells were separated from germ cells, co-isolated from engzyme-digested SFTs, through their adherence to the extracellular matrix (ECM) coated substratum. However, instead of Matrigel used for culturing Sertoli cells of young mice, the natural ECM, laminin, was used in the adult Sertoli cell culture. On laminin, adult Sertoli cells adhered tightly, allowing germ cell removal simply by medium replacement instead of their disruption by hypotonic solution treatment (used in 20-day old cultures). This hypotonic shocking actually created irreversible injury to adult Sertoli cells. Day 8 yielded 90-95% pure sertoli cells, as revealed by their "tripartite nuclei" morphology and presence of a specific Sertoli cell marker, Wilm's tumor protein. The isolation yield was ~ 2.0e+6 Sertoli cells/three adult mice. Like young Sertoli cells, the adult cultures expressed tight junction proteins, claudin-11 and ZO-1, and secreted prosaposin, ABP and clusterin. However, lipidomic and proteomic analyses of Sertoli cells from the two ages exhibited significant differences. Adult Sertoli cells possessed higher levels of triacylglycerols, cholesteryl esters and seminolipid, corroborating higher numbers of intracellular lipid droplets. Several proteins were preferentially expressed in either adult or young Sertoli cells. Most significantly, cultured adult Sertoli cells were revivable post-cryopreservation.

CONCLUSIONS:
We showed herein for the first time an efficient method to culture Sertoli cells from adult mice. This culture system will provide not only a better understanding of the etiology of male infertility/subfertility emerging in adulthood but also a platform for studying spermatogenesis in vitro.

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40 **Cholesterol is primarily in the inner leaflet of live cell plasma membrane, which suppresses the formation of liquid-ordered domains.**

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Background
The complex composition of the mammalian cell plasma membrane is tightly regulated to maintain cellular homeostasis. Both lipids and proteins that comprise the plasma membrane are known to have a different composition between the inner and outer bilayer leaflets. Disruption in this membrane asymmetry results in cellular dysfunction, leading to cell death. Although the bilayer asymmetry is well characterized for proteins and lipids, the cholesterol distribution, however, is not known. Cholesterol represents 30% of the plasma membrane lipids and in addition to maintaining cell structure, it is involved in regulating membrane trafficking, viral infection and signal transduction. The close interaction of cholesterol and certain membrane lipids, such as sphingomyelin, is thought to create small, transient ordered regions (domains) within the otherwise fluid plasma membrane, which facilitates protein-protein interactions.

Objective
The purpose of this project is to characterize the cholesterol trans-bilayer distribution in mammalian cells in order to better understand how plasma membrane asymmetry regulates cellular homeostasis. We are examining how plasma membrane organization and composition influences cell function.

Methods
In order to accomplish our objectives, we have used a combination of biochemical and biophysical approaches to characterize cholesterol in both model membrane systems and live mammalian cells. We have generated a variety of asymmetric membrane vesicles, mimicking the plasma membrane, to examine how lipid composition affects cholesterol distribution and membrane domain formation. Furthermore, we have performed knockdown experiments in live cells to characterize how manipulating specific

lipid species will alter the cholesterol distribution.

Results
We have found, for the first time, that cholesterol is primarily (80%) found in the inner leaflet of the mammalian cell plasma membrane. In addition, through bly of membrane domains.

Conclusions
Cholesterol is maintained with an asymmetric trans-bilayer distribution to suppress the formation of membrane domains and maintain homeostasis. By increasing outer leaflet cholesterol content, more cholesterol is available to facilitate protein-protein interactions. This basic cholesterol asymmetry should now be a starting point to better understand plasma membrane organization and membrane-related cellular processes and disease

41 **A RETROSPECTIVE LONGITUDINAL WITHIN-SUBJECT RISK INTERVAL ANALYSIS OF IMMUNOGLOBULIN TREATMENT FOR RECURRENT ACUTE EXACERBATION OF CHRONIC OBSTRUCTIVE PULMONARY DISEASE**

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Background: Recurrent acute exacerbations of chronic obstructive pulmonary disease (AECOPD) are common, debilitating, costly and often difficult to prevent.

Methods: We reviewed records of patients who had COPD and immunoglobulin (Ig) treatment as adjunctive preventative treatment for AECOPD, and documented all AECOPD episodes for one year before and after initiation of Ig treatment. We graded AECOPD episodes as moderate for prescription of antibiotics and/or corticosteroids or for visit to the Emergency Department, and as severe for hospital admission. We conducted a retrospective within-subject self-controlled risk interval analysis to compare the outcome of annual AECOPD rate before and after treatment.

Results: We identified 22 cases of certain COPD, of which three had early discontinuation of Ig treatment due to rash and local swelling to subcutaneous Ig, and five had incomplete records leaving 14 cases for analyses. The median baseline IgG level was 5.9 g/L (interquartile range 4.1 – 7.4). Eight had CT radiographic bronchiectasis. Overall, the incidence of AECOPD was consistently and significantly reduced in frequency from 4.7 (± 3.1) per patient-year before, to 0.6 (± 1.0) after the Ig treatment ($p = 0.0001$). There were twelve episodes of severe AECOPD (in seven cases) in the year prior, and one in the year after Ig treatment initiation ($p = 0.016$).

Conclusions: Ig treatment appears to decrease the frequency of moderate and severe recurrent AECOPD. A prospective, controlled evaluation of adjunctive Ig treatment to standard therapy of recurrent AECOPD is warranted.

42 **Hexokinse II-mediated Warburg effect is required for chemoresistance in ovarian cancer cells.**

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Background: Cancer recurrence and resistance to cisplatin(CDDP) is a major hurdle to the successful treatment. Cancer cells utilize aerobic glycolysis for obtaining additional energy for cellular growth and function, commonly called Warburg effect. Hexokinase II (HKII) catalyzes the first committed step in glycolysis, converting glucose to glucose-6-phosphate. In ovarian cancer (OVCA), high HKII expression is associated with low progression free survival rate of patients. However, HKII association with CDDP chemoresistance, overall survival rate, and the regulatory mechanism of HKII were unknown.

Objectives & Hypothesis: The proposed research will address 1) the role of HKII in accelerated glucose metabolism in chemoresistant OVCA 2) how HKII-mediated Warburg effect is required for anti-apoptosis and CDDP resistance 3) regulatory role of p53 upon HKII and its mechanism in signaling cascade. We hypothesized that HKII is a key molecule contributing to chemoresistance and anti-apoptosis beyond its metabolic function in OVCA.

Methods: Using paired CDDP sensitive (A2780s) and counterpart resistant (A2780cp) ovarian cancer cell line model, we examined the protein content and mRNA level of HKII in chemosensitive and chemoresistant OVCA cells in response to CDDP. For cellular metabolism, glucose consumption and lactate level were examined. Using gain- and loss-of-function approaches, the role of HKII in the regulation of apoptosis and of its dependence on p53 was examined in context of cellular metabolism.
Results: CDDP decreased mRNA level and protein content of HKII in chemosensitive cells but not in chemoresistant cells in vitro in a culture duration- and concentration-dependent manner. Also, level of glucose consumption is significantly higher in chemoresistant cells than chemosensitive cells regardless of CDDP treatment. In chemoresistant cells with different p53 status, both 2-DG inhibitor and HKII siRNA treatments specifically sensitized p53-wt chemoresistant cells (Hey) to CDDP whereas no significant change was observed in 53 null (SKOV3) and p53 mutant (A2780cp) cells. Also, p53 knocked down in chemosensitive cells attenuated the CDDP-induced decreases in HKII protein content level. Both HKII knockdown (siRNA) and p53-wt reconstitution significantly sensitize p53 deficient chemoresistant cells to CDDP, confirming that functional p53 is required for chemosensitization in HKII down-regulated chemoresistant cells.

Conclusion: Collectively our data suggests that CDDP activated p53 is required for HKII regulation and cellular energy metabolism in chemoresistant OVCA cells. In conclusion, HKII is a key molecule engaged chemoresistance via accelerated aerobic glycolysis, highlighting that HKII could be a promising therapeutic target to overcome CDDP resistance in ovarian cancer.

43 **Withdrawn**

44 **The ubiquitin E3 ligase ring finger protein 6 (RNF6) regulates follicular cell proliferation via a modulation of androgen receptor (AR) activity.**

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

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

Methods: To examine the expression level of AR and RNF6 in the follicular development, ovarian sections were assessed by immunofluorescence (IF). To examine if AR and RNF6 expression in the GCs is regulated by androgen (DHT; 5a-dihydrotestosterone) and if this regulation is dependent on the stage of follicular development, GCs from pre/early antral follicles and from late antral were cultured in the presence of DHT (0-10 μM) for 24 hours and AR and RNF6 content was examined by WB and IF. GCs proliferation and apoptosis were examined using cell cycle analysis. In addition, to determine if AR and RNF6 are co-localized in the GCs, IF data (AR and RNF6) was analyzed by ImageJ(JACoP).
Results: Immunohistochemistry studies indicate that AR in ovary tissue was strongly observed in the GCs but not in the oocyte and theca cells of all follicular stages. Similarly, RNF6 was strongly expressed in granulosa cells, and its expression was low in the theca cells. In vitro studies showed that, AR and RNF6 expression is depending on the concentration of DHT (0-1μM), increased but decreased with high concentration (10μM). Cell cycle analysis indicates that DHT increased cell proliferation and decreased apoptosis in GCs from pre/early antral follicles, but opposite responses were observed in the GCs from the more mature follicles. IF for AR and RNF6 co-localization indicates that GCs treated with DHT (1uM) increased percentage of AR/RNF6 localization at the nucleus from pre/early antral GCs.

Conclusion: This study demonstrates, for the first time, that the expression of RNF6 during ovarian follicular cell growth and that this response is regulated by the actions of androgen in a follicular stage-dependent manner.

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45 **Role of FSH on the Regulation of Mitochondrial Dynamics in Polycystic Ovarian Syndrome**

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BACKGROUND
Polycystic ovarian syndrome (PCOS) is characterized by early antral follicle growth arrest, chronic anovulation, and suppressed granulosa cell proliferation. FSH is an anti-apoptotic gonadotropin that promotes follicular growth and steroidogenesis, and the dysregulation of FSH via the adipokine Chemerin may be involved in the pathogenesis of PCOS. Whether follicular growth arrest is a consequence of chemerin-induced granulosa cell apoptosis is unknown.
Mitochondria are highly dynamic organelles, constantly dividing (fission) and elongating (fusion) to form a network, which maintains mitochondrial function and regulates apoptotic cell death. Dysregulation in the proteins involved in mitochondrial fission (Fis1, Drp1) and fusion (Opa1, Mfn1/2) has been linked to the pathogenesis of human diseases. However, it remains unclear as to how mitochondrial dynamics regulate granulosa cell survival and whether dysregulation of mitochondrial fission play a role in PCOS. The role of FSH and chemerin in this process also remains uncertain.

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

METHODS
Granulosa cells from early antral rat follicles will be cultured with chemerin and/or FSH, and will be investigated to ascertain the importance of mitochondrial fission/fusion proteins in granulosa cell apoptosis leading to follicular growth arrest using apoptosis assays, Western blot, ELISA, confocal microscopy and electron microscopy. These techniques will also be used for our DHT-induced rat model to determine the link between dysregulated mitochondrial dynamics and PCOS. This model will be treated with gonadotropin to determine its effect on the PCOS phenotype.

RESULTS
Chemerin failed to induce apoptosis in granulosa cells from early antral follicles nor attenuate FSH-induced cAMP production. Of the mitochondrial dynamic proteins, FSH significantly decreased phospho-Ser616 Drp1 (stimulates fission) and increased phospho-Ser637 Drp1 (inhibits fission) content, while chemerin had no effect. Using the DHT-induced rat model, DHT treatment significantly increased phospho-Ser616 Drp1.

CONCLUSIONS/FUTURE DIRECTIONS
Chemerin does not directly induce granulosa cell apoptosis and FSH was found to have a suppressive effect on Drp1 activity in granulosa cell culture. Further investigation of the potential role of mitochondrial dynamics in PCOS (using the DHT-induced rat model with/without gonadotropin) will be accomplished.

46 **Putrescine supplementation during in vitro maturation of aged mouse oocytes improves the quality of blastocysts**

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Mouse ovaries exhibit a transient peri-ovulatory rise of ornithine decarboxylase and its product putrescine, concurrent with oocyte maturation. Older mice exhibit diminished levels of both the enzyme and putrescine in the ovaries. Peri-ovulatory putrescine supplementation in drinking water increases ovarian putrescine levels in older mice; similar putrescine supplementation reduces embryo resorption and increases live pups in older mice. However, given the systemic nature of putrescine supplementation, it is unknown if peri-ovulatory putrescine supplementation directly acts in the ovaries to improve oocyte maturation. This study examined the impact of putrescine supplementation during oocyte in vitro maturation (IVM) on the developmental potential of aged oocytes. Cumulus-oocyte complexes were isolated from 9-12 month old C57BL/6 mice and subjected to IVM with or without 0.5 mM putrescine. This was followed by in vitro fertilization and embryo culture to blastocyst stage (96 hours post fertilization). Putrescine supplementation during IVM did not influence oocyte maturation rate, fertilization rate, or blastocyst formation rate. However blastocysts derived from putrescine-treated IVM oocytes had more total cells (44.5±1.9, compared to 36.5±1.9 for control; P=0.003) and a greater proportion with OCT4-positive inner cell mass (38.3%, compared to 19.8% for control; P=0.005). Our results demonstrate that putrescine supplementation during oocyte maturation significantly improves the developmental potential of aged mouse oocytes, and provides proof of principle for possible application of putrescine supplementation in human

IVM procedure to improve oocyte quality in older women.

47 **Podocyte Oxidative Stress Coupled With Ubiquitin C-Terminal Hydrolase Deletion Exacerbates Renal Damage**

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Background
Ubiquitin C-terminal hydrolase L1 (UCHL1) may promote antioxidation by hydrolyzing ubiquitin-thioester bonds on glutathione (GSH) thereby protecting GSH from degradation. Podocyte UCHL1 is upregulated in diseased glomeruli where it may maintain redox balance.

Objective
UCHL1-deleted mice overexpressing podocyte-specific NOX5 should exhibit exacerbated glomerular damage due to unregulated oxidative stress.

Methods
COS7 cells were infected with AdGFP or AdNOX5 and UCHL1 levels determined by immunoblot. UCHL1 mRNA was quantified by qPCR. UCHL1+/- and NOX5pod+ mice were crossed to generate NOX5pod+/UCHL1-/- mice. Albuminuria (ACR) was measured by ELISA. Renal mass was normalized to tibia length. Glomerular filtration rate (GFR) was assessed by FITC-Inulin. Immunofluorescence with anti-PCNA and anti-renin was performed on renal sections. Blood pressure was determined by tail cuff plethysmography.

Results
UCHL1 protein increased 1.9-fold in COS7 cells infected with AdNOX5 vs. AdGFP-infected controls. Similarly, glomeruli from 12-week-old NOX5pod+ mice showed limited UCHL1 mRNA induction (1.5-fold). ACR increased in NOX5pod+/UCHL1-/- mice at 12 weeks (40ug/mg) but not in nonTG, UCHL1-/- and NOX5pod+/UCHL1-/- mice (24, 28 and 25ug/mg). Renal mass was reduced by 10% in both UCHL1-/- and NOX5pod+/UCHL1-/- mice as compared to nonTG mice. Interestingly, GFR was elevated in NOX5pod+, UCHL1-/- and NOX5pod+/UCHL1-/- mice (283, 362 and 352ul/min) vs. nonTG (198ul/min). In UCHL1-/- mice regardless of NOX5 expression, hypercellularity was evident in the juxtaglomerular region. However, no differences were found in renin or PCNA expression, suggesting that the increased GFR is independent of the renin-angiotensin system. Blood pressure increased in NOX5pod+/UCHL1-/- mice (121mmHg vs. 113 and 109mmHg for nonTG and UCHL1-/- mice), but was not different from NOX5pod+ mice (127mmHg) at 12 weeks.

Conclusion
UCHL1-null mice have and mass. When intercrossed with NOX5pod+ mice, UCHL1 deletion combined with unchecked oxidative stress promotes filtration barrier damage.

48 **Latent HIV Infection is Associated with Defects in the Type I IFN Response**

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Introduction: During productive HIV infection, several viral-mediated immune evasive strategies, such as the impairment of the antiviral type I IFN (IFN-I) response, facilitate viral replication and spread. Whether similar impairment occurs in latently HIV infected cells is as of yet unknown. Presently, HIV latency represents a major barrier to complete viral clearance, highlighting the need for innovative treatment strategies. Defects in the IFN-I response, if present during HIV latency, have the potential to be exploited in a novel therapeutic manner for the targeted eradication of latently infected cells.

Objectives: To address our hypothesis that IFN-I mediated signaling pathway defects are present during HIV latency, we will measure the induction of IFN stimulated genes (ISGs) in latently HIV infected CD4+ T cells following treatment with exogenous IFN-I.

Methods: An established in vitro model of HIV latency using primary CD4+ T cells isolated from healthy blood was employed to investigate IFN-I response defects during latent HIV infection. Mock-infected CD4+ T cells were included as controls. First, surface

expression of the IFNa receptor subunit 1 (IFNAR1) was measured on mock and latently HIV-infected CD4+ T cells. Next, cells were treated with various doses of IFNa for 24 hours, after which the expression of various ISGs, including PKR, ISG15, and MHCI was measured by flow cytometry.

Results: No difference in IFNAR1 expression was observed between latently infected and mock-infected cells. However, the induction of PKR, ISG15, and MHCI expression in response to IFNa treatment was found to be lower in latently HIV-infected CD4+ T cells in comparison to mock-infected control cells.

Conclusions: Our results suggest that in an in vitro model of latently HIV infected CD4+ T cells, the induction of key antiviral ISGs in response to IFN-I may be defective. Therefore, the specific impairment of key IFN-I-induced antiviral proteins may serve as a target for the design of novel treatment strategies aimed at eradicating the latent viral reservoir.

49 **Peri-ovulatory putrescine supplementation reduces embryo resorption in older mice**

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Background: Rodents exhibit a transient rise of ornithine decarboxylase (ODC) and putrescine in the ovaries during ovulation. Older mice exhibit reduced ovarian ODC activity during ovulation. Supplementation of in vitro maturation medium with putrescine reduces oocyte aneuploidy rates of older mice.

Objective: The study was to investigate if peri-ovulatory putrescine supplementation of older mice improves oocyte quality and reduces the incidence of embryo resorption.

Methods: The rationale was to correct ovarian putrescine deficiency in older mice by peri-ovulatory putrescine supplementation in drinking water and to observe the reproductive consequences of this intervention. Older mice (9–11 months of age) were given regular drinking water (control) or drinking water with 1% putrescine dihydrochloride (62 mM) for 2-4 days before mating. Plugged mice were then withdrawn from putrescine supplementation. Blastocysts were retrieved on 3.5 days post coitum (dpc) for the determination of cell numbers. For resorption analyses, mice were killed on 9.5 dp or 12.5 dpc, and implantation sites were dissected to determine the embryo status. For birth studies, mice were examinedevery morning between 16.5 and 23.5 dpc. Births were recorded as live or stillbirth.

Results: We demonstrated that deficiency of ovarian putrescine in older mice can be restored byperi-ovulatory putrescine supplementation in drinking water. Putrescine supplementation in older mice increased blastocyst cell numbers (from 40 to 54 P= 0.0001, t-test), reduced embryo resorption rates (from 41.1 to 15.4% in old C57BL/6 mice, P , 0.0001, Fisher's exact test from 14.2 to 6.4% in old CF1 mice, P=0.004, Fisher's exact test), and doubled the number of live born pups. Furthermore, exogenous putrescine exhibited rapid absorption and excretion, and showed no toxicity to mothers or fetuses.
Conclusions: Peri-ovulatory putrescine supplementation in older mice improved oocyte quality, as indicated by increased blastocyst cell numbers and reduced the incidence of embryo resorption. This study demonstrates a natural and simple remedy to improve oocyte quality in older women.

50 **The SERCA inhibitor Saikosaponin-d Sensitizes Chemoresistant Ovarian Cancer Cells to Cisplatin in vitro**

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Background
Cisplatin (CDDP) and its derivatives are first line drugs for ovarian cancer (OVCA), but chemoresistance remains a major hurdle to successful therapy and is associated with poor prognosis. Mitochondrial fission is associated with the induction of apoptosis and autophagy (mitophagy) in cancer cells, and can be augmented via post-translational modifications of dynamin related protein 1 (Drp1). It has been previously reported that Ca2+/calmodulin–dependent protein kinase I (CaMKI) phosphorylates and activates Drp1 which in turn induces mitochondrial fission. Saikosaponin-d (Ssd), derived from medicinal plant, exhibits anti-inflammatory, anti-bacterial, anti-viral, and anti-cancer activities. Recent studies further revealed that its anti-cancer effects could be mediated by calcium signaling, apoptotic, and autophagic pathways. However, whether Ssd regulates chemosensitivity of OVCA cells is not

known and needs to be elucidated.

Objective
The overall objective of the present studies is to examine the action and interactions of Ssd and CDDP on Ca2+ signaling, mitochondrial fission and autophagy in chemoresistance.in OVCA

Methods
Chemosensitive (A2780s) and chemoresistant cells with different p53 status A2780cp (p53-mutant), Hey (p53-wild type) and SKOV3 (p53-null) were incubated with CDDP in the absence and presence of Ssd. Apoptosis, CaMKI and Drp1 contents and mitochondrial morphology were assessed by Hoechst nuclear staining, Western blotting, and confocal microscopy, respectively.

Results
As expected, CDDP alone significantly increased apoptosis in chemosensitive but not chemoresistant OVCA cells in concentration- and time-dependent manner. Ssd alone (2 μM) also induced apoptosis in the chemoresistant OVCA cells, a response independent of p53 status. Ssd (1 μM) significantly enhanced the CDDP response in the sensitive cells and sensitized the chemoresistant cells to CDDP. Ssd in combination with CDDP increased phospho-CaMKI protein content in both chemosensitive and chemoresistant OVCA cells. These treatments also down-regulated phospho-Drp1 (ser616) and phospho-Drp1 (ser637) protein contents, and increased the proportions of both OVCA cells with fragmented mitochondria.

Conclusions
These results support the concept that Ssd could be therapeutic agent, alone or together with CDDP, for chemoresistant OVCA via the interaction between Ca2+ signaling and mitochondrial fission. The relationship between autophagy and sensitivity of OVCA to Ssd remains to be determined (supported by CIHR).

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51 **Effect of high glucose exposure on endothelial microparticle formation and composition**

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Background: Diabetes is a group of metabolic disorders arising from the body's inability to produce or properly use insulin leading to chronic hyperglycemia. Diabetes is associated with significant target organ damage and a high premature mortality predominantly due to the impact of chronic complications of the macro and microvasculature. Endothelial dysfunction is a hallmark of the early macrovascular and microvascular complications of diabetes. Microparticles (MPs) are small membrane-derived vesicles that are secreted ubiquitously following cell stress and/or death. We have previously reported that endothelial MPs are active signaling agents that induce endothelial cell injury. However, the impact of high glucose on the formation and composition of endothelial MPs is unknown.

Methods: MPs were isolated via differential centrifugation from the media of cultured human umbilical vein endothelial cells (HUVECs) treated with normal D-glucose (5.6 mM), 25 D-glucose, or L-glucose as osmotic control (5.6 mM D-Glucose + 19.44 mM L-Glucose). MP levels were then quantified using nanoparticle tracking analysis (NTA). To assess the protein composition of endothelial MPs formed under each condition, MP protein lysates were separated by SDS-Page and the tryptic digests were analyzed by liquid chromatography-mass spectrometry (LC-MS). Relative differences in protein levels between groups were assessed by label-free spectral counting and candidate proteins were verified by Western blot analysis.

Results: MP formation increased following D-glucose (25mM) treatment relative to the normal D-glucose (3-fold, $P<0.05$). LC-MS analysis identified 14,631 unique peptides of which resulted in 220 independent proteins with at least 2 peptides per protein with an average sequence coverage of 16%. Interestingly, there was a 4-fold enrichment in thrombospondin-1 in the 25mM D-glucose treated cells which was verified via Western blot.

Conclusions: High glucose increases the formation of endothelial MPs and leads to alterations in their molecular composition including enrichment in thrombospondin-1. Such alterations may contribute to the development of vascular injury in diabetes.

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52 **Exosome-Mediated Delivery of Pro-Survival MicroRNA-486-5p in Acute Kidney Injury**

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BACKGROUND

We recently showed that administration of human endothelial colony forming cells (ECFCs) and their exosomes to mice with ischemic acute kidney injury (AKI) attenuated renal damage. Our data also indicate that ECFCs release exosomes that are highly enriched in micro-RNA (miR)-486-5p and protect against hypoxia-induced endothelial cell apoptosis. MiR-486-5p targets phosphatase and tensin homologue (PTEN), which may enhance the pro-survival Akt pathway.

OBJECTIVE
To examine the role of exosomes and miR-486-5p on the Akt pathway in vivo, and to test hypothesis that exosomes transfer miR-486-5p to endothelial cells in vitro.

METHODS
Mice with ischemic AKI were injected (i.v.) with exosomes at reperfusion. Kidneys were subjected to immunoblots and RT-PCR for miR-486-5p. Transfer of ECFC exosomes and miR-486-5p was studied in human umbilical vein endothelial cells (HUVECs).

RESULTS
Bioinformatic analysis of the 10 most abundant miRs in ECFC exosomes revealed that they were all involved in the Akt pro-survival pathway. In mice with AKI, exosome treatment significantly increased renal miR-486-5p levels ($P<0.01$ vs ischemia alone, n=6-7), associated with decreased PTEN expression, and increased Akt phosphorylation. In cultured HUVECs, ECFC exosomes labeled with the fluorescent dye PKH-26 localized to the perinuclear compartment. Treatment of HUVECs with ECFC-derived exosomes or co-culture of ECFCs with HUVECs caused a 40-fold increase in levels of miR-486-5p. Transfection of ECFCs with pre-miR-486-5p, followed by co-culture with HUVECs was associated with a further marked increase (~20-fold) in miR-486-5p levels in HUVECs ($P<0.001$ vs control, n=3). This effect was blocked by pre-incubation of HUVECs with ethylisopropyl amiloride (an inhibitor of exosome uptake).

CONCLUSIONS
These data suggest ECFC-derived exosomes exert renoprotective effects in AKI, possibly via the transfer of miR-486-5p to endothelial cells. Exosome-mediated transfer of miRs could represent a strategy to target pro-survival pathways in the injured kidney.

53 **Preclinical assessment of SC66 in ovarian cancer therapy**

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Epithelial ovarian carcinoma (EOC) is the most lethal gynecologic malignancy. Despite the advent of surgical cytoreduction and combination chemotherapy, the majority of patients will ultimately recur and will succumb to disease. This emphasizes the need for novel therapies aimed at targeting cancer cells most resistant to initial therapy.

We previously investigated the importance of Collagen type XI alpha 1 (COL11A1) in EOC. Our findings indicate that COL11A1 may promote cell aggressiveness via the TGF-β1/Ets-1/MMP3 axis and that the nuclear transcription factor Y alpha (NF-YA) binding site in the COL11A1 promoter is the major determinant of TGF-β1-induced COL11A1 expression. Recently, we elucidated the mechanisms by which COL11A1 promotes cancer cell sensitivity to anticancer drugs, and we observed that, in ovarian cancer cells, chemoresistance developed via activation of the Akt/c/EBP-β pathway in concert with increased PDK1 degradation. The findings from our cell-based models were further supported by clinical evidence in EOC patients. Collectively, our findings highlight the importance of COL11A1 in EOC tumour progression and chemoresistance, and suggest that future therapies targeting COL11A1 or the Akt signaling pathway might provide new opportunities for therapeutic intervention in chemoresistant EOC.

The novel AKT inhibitor SC66 has been demonstrated to promote cell death in cervical cancer through disruption of mTOR signaling and has antitumor effects on HCC cells. Recently, our preliminary findings showed that SC66 exhibits similar killing effect on chemoresistant cancer cells and their chemosensitive parental counterparts, implicating that SC66 have the potential to overcome and kill cancer cells with chemoresistance. In this study, we will explore the therapeutic effects of combining SC66 with chemotherapy drugs and to demonstrate the detailed mechanism of SC66 in overcoming chemoresistance.

The X-linked inhibitor of apoptosis protein (XIAP) is a potent inhibitor of apoptosis; however, it is also involved in other biological behavior of cancer cells. The XIAP RING domain has E3 ubiquitin ligase activity and is able to promote the degradation of proteins by marking them with ubiquitin molecules. Through its E3 ligase, the XIAP RING domain activates signaling cascades influencing cell death, inflammation, and cell migration.

The objective of this study is to examine if and how XIAP and its E3 ligase played a crucial role in regulation of PDK1 content in ovarian cancer cells. Our hypothesis is SC66 inhibits COL11A1-mediated chemoresistance via increased XIAP expression and increased PDK1 degradation and subsequently down-regulation of the Akt signaling pathway.

Specific Objective:

A. To examine if SC66 increase cell sensitivity of chemosensitive (A2780s) and chemoresistant (A2780cp) ovarian cancer cells to

cisplatin *in vitro*.

B. To investigate if SC66 induces apoptosis in in chemosensitive and chemoresistant ovarian cancer cells.
C. To compare the influence of SC66 on XIAP content in chemosensitive and chemoresistant ovarian cancer cells.
D. To compare the ubiquitination of PDK1 and phosphorylation of Akt after XIAP knockdown or overexpressed in chemosensitive and chemoresistant ovarian cancer cells after SC66 treatment.
E. To investigate if SC66 inhibit COL11A1 expression in chemosensitive and chemoresistant ovarian cancer cells.

Clinical Epidemiology Program

54 **Ambulatory Electrocardiograms Quality Analysis**

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BACKGROUND: Every year 500,000 to 900,000 patients die due to cardiovascular complications following a noncardiac operation. In attempt to reduce cardiac complications after a surgery, ß blockers are administered to the patient; however, a large multi-center trial demonstrated that while ß blockers can reduce cardiac complications, it increases the incidents of stroke, hypotension, bradycardia, and death. Rather than a general prophylactic to all postsurgical patients, the Perioperative Ischemia Reduction Study (PROSE) aims to provide ß blockers to patients that are experiencing myocardial ischemia, a reduction of blood to the heart that precedes major cardiovascular complications. Myocardial ischemia can be detected as deviations in the ST segment of the electrocardiogram (ECG). Post-surgical patients are encouraged to ambulate to promote recovery, ambulatory ECG is employed; however, a commercial implementation of the ST monitoring solution was found to have a high false alarm rate, rendering the system impractical for use.

OBJECTIVES: The objective of the research thesis is to develop a parallel ECG biomedical quality analysis system that can be used to gate false alarms due to unreliable ECG data and ST estimates.

METHODS: A system was designed to gate false alarms by disabling the alarm generating algorithm in the case of low ECG signal quality, which can be quantified using a signal quality index (SQI). Four SQIs based on estimates of signal to noise ratio (SNR) were considered: the mean, median, 25th percentile and minimum SNR. To validate the false alarm gating system, every 15 min of the two records, s20051 and s20111, from the European ST-T database were contaminated by 5 min segments of motion artifact from the MIT-BIH Noise Stress Test database; both databases are found on Physionet. SQIs based on 30 sec segments were generated every 5 sec to match the behaviour of the alarm generating algorithm of commercial bedside monitor. Alarms generated from ECG signal with 25th percentile SQI less than 5 dB were gated.

Results: Without alarm gating, the records had 9 true alarms and 14 false alarms and the system had precision of 0.41 and sensitivity of 0.90. With the alarm gating system, the false alarms dropped to 1 alarm, while only missing 1 true alarm, and the system had precision of of 0.82, while maintaining sensitivity of 0.90.

CONCLUSIONS: The system successfully gated false alarms while maintaining sensitivity. Future work includes exploring SQIs based on Wavelet transform to improve precision.

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55 **Comparative Effectiveness and Harms of Screening for Vitamin D Deficiency, Vitamin D Supplementation, Hypothyroidism Screening, and Hypothyroidism Treatment in Low Risk Pregnant Women: Systematic Reviews of Literature**

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Background: To ensure a successful pregnancy, quality antenatal care with timely identification of risk factors is essential.

Objective: To assess the comparative effectiveness and harms of the following interventions in low risk pregnant women:
1- Vitamin D deficiency screening versus no or targeted testing
2- Vitamin D supplementation versus placebo/no treatment, or lower doses of vitamin D supplement
3- Hypothyroidism screening versus no or targeted testing
4- Hypothyroidism treatment with Levothyroxine, or Selenomethionine versus placebo, no treatment, lower doses of the drugs, or alternative treatment in subclinical hypothyroidism

Methods: We conducted systematic reviews of the literature commissioned by Belgium Health Care Knowledge Center to update the Belgian national clinical practice guideline 2004 on antenatal care in low risk pregnant women. MEDLINE, Embase, and the

Cochran library were searched for evidence. We screened literature through two steps: 1) title and abstract, 2) full text. To assess the quality of evidence, we used a 9-point modified AMSTAR for the selected systematic reviews; Cochran Risk of Bias for randomized controlled trials, and a generic assessment of selection, information, and confounding biases for observational studies. We used the GRADE methodology to rate the quality of the body of evidence using the GRADEpro software.

Results:
No comparative experimental or observational evidence addressed objective 1.

Available evidence of very low quality favored vitamin D supplementation with respect to the outcomes birth weight <2500 grams and NICU admission but not other childhood and maternal outcomes.

For hypothyroidism screening, there was no statistically significant association:
• between universal screening versus no screening for the childhood outcomes assessed (control standardised IQ at age 3; IQ< 85, child behavior checklist T-score; behavior rating inventory of executive function, preschool T-score; and preterm birth), and maternal Becks Depression Inventory.
• between universal screening versus targeted screening for the neonatal outcomes assessed (miscarriage; perinatal/neonatal death; NICU admission; birth weight> 2500g; preterm birth), maternal outcomes (gestational hypertension; preeclampsia; and congestive heart failure), and adverse outcomes.

For subclinical hypothyroidism treatment with levothyroxine, very low quality (due to low applicability and power) evidence favored treatment for the outcome of miscarriage and preterm birth, but not for other childhood and maternal outcomes. Inconclusive evidence exists on comparative effectiveness of selenomethionine treatment in sub-clinical hypothyroidism.

Conclusions:
Overall effectiveness and harms of vitamin D supplementation, hypothyroidism treatment, universal screening of hypothyroidism remain uncertain for most of the maternal and neonatal outcomes due to under powered evidence of limited validity and mostly low-very low quality.

56 **Improving Travelers' knowledge, decision-making, and adherence to malaria chemoprophylaxis: Examining the impact of incorporating the "Ottawa Malaria Decision Aid" into the pre-travel consultation process."**

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Background: >400 cases of malaria, and often a couple of deaths occur in Canada each year. This is mainly a result of travelers not using chemoprophylaxis as directed, and it may also be attributed to the conflict of choosing the appropriate chemoprophylaxis. Many Canadians visit chloroquine-resistant areas. In order appropriately protect against malaria in these areas travelers have to decide between three alternative antimalarial drugs.

Methods: We have developed the Ottawa Malaria Decision Aid (OMDA), a bilingual online resource to support malaria chemoprophylaxis decision-making using plain language fact-based information. The OMDA project is a randomized control study that was launched at the Ottawa Hospital (TOH) Travel Clinic, the National Capital Region (NCR) Travel Clinic and through the International Association for Medical Assistance to Traveller's (IATMAT) in 2014. Eligible travelers who enrol in the study are assigned to one of two arms: standard care, or standard care plus OMDA. Both groups complete three questionnaires: 1) before clinic visit/ travel: 2) after clinic visit and before travel; and 3) after travel.

Objectives: This project aims to evaluate whether the OMDA can be integrated into the pre-travel consultation process to: 1) improve a traveller's knowledge of malaria and prevention strategies; 2) improve traveller's decision-making; 3) decrease decisional conflict; 4) affect levels of adherence to malaria chemoprophylaxis. Hypothesis: 1) decision aids will improve the quality of decision-making about malaria chemoprophylaxis by decreasing decisional conflict and increasing knowledge about malaria and malaria pills; 2) better decision quality will result in greater levels of adherence to malaria chemoprophylaxis. It is hoped that the malaria cases arriving in Canada can decrease by using different methods of presenting information, resulting in an overall decrease in unnecessary deaths and healthcare dollars.

Conclusion: We aimed to recruit >100 individuals to complete all three surveys from since the launch of the study in 2014. To date we have 674 online web visits, 59 excluded, 20 refusals, 40 registered and randomized, and 6 completed all three surveys. There have been ongoing issues with recruitment of participants. This is due in part to changes in policy for approaching patients for research purposes at TOH as well as the passive nature of questionnaire completion. The questionnaires are completed

independently at leisure of the participants and often forgotten. Limited reminder emails are permitted by TOH. Study recruitment and enrolment is ongoing until the end of 2015.

57 **Peer researchers' knowledge, confidence and self-reported readiness to administer spirometry after training.**

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Background: Community Based Participatory Research (CBPR) though gaining popularity, is still rare. In CBPR, community members participate in all or some research phases. This framework presents multiple advantages and challenges. Methods and amount of community involvement to optimize the research and health outcomes are still unclear.

Objective: To assess the peer researchers' preparedness to administer spirometry testing, before and after training, and obtain feedback on the training process.

Method: For the Participatory Research in Ottawa: Management and Point-of-care of Tobacco dependence (PROMPT) study, we recruited 4 peer researchers. The peers represented the PROMPT study target population (current or ex-multi-drug users, tobacco smokers, homeless or marginally housed and living in Ottawa). We trained the peers to administer PROMPT study questionnaires and spirometry testing. They underwent spirometry training as per the Canadian Cohort of Obstructive Lung Disease (CanCOLD) study guidelines. To assess peer researchers' knowledge and comfort level with spirometry testing, we administered pre- and post-training questionnaires. The questions attempted to measure self-reported knowledge of lung function, confidence in administering spirometry and knowledge with skill testing questions. Secondary outcome was to obtain participant's opinions of the training process.

Results: All 4 peer researchers underwent 1 one-on-one and 6 group training sessions. The group sessions included didactic presentations, discussions and practice. There was improvement in both self-perceived knowledge and confidence in administering spirometry between the pre- and post-training questionnaires. The final post-training questionnaires showed peer researchers felt very confident in being able to administer a good quality lung function test and disagreed that they needed further training. Skill testing score averages improved from the pre- to the post-training questionnaires.

Discussion /Conclusion: Standardized spirometry training of non-healthcare, community peer researchers, was effective in increasing knowledge, confidence and self reported readiness to administer spirometry. Implementation of this training protocol to community peer researchers in future studies will resources and encourage enrollment of vulnerable, hidden populations in research studies. Further research is required to assess peer researchers' effectiveness in implementing acceptable quality spirometry testing to study participants.

58 **Robust and Efficient Medical Image De-identification; sniffing for needles in the haystack using Optical Character Recognition**

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Background:
Image sharing between institutes is an essential part of modern medical research. Increasingly, ethics committees and legislation require that no Protected Health Information (PHI) such as patient names and MRNs be transferred outside of a medical institution. To facilitate multi-centre clinical trials and mitigate breach of confidentiality risks, robust and efficient medical image de-identification tools must be developed. The RSNA has met this challenge by developing the Clinical Trial Processor (CTP): a free, open-source platform that uses rule sets to de-identify image data. Unfortunately, CTP can be expected to näively miss PHI in datasets it hasn't been explicitly programmed to support. Optical Character Recognition (OCR) software may therefore extend the robustness of CTP, by providing an independent PHI "check" in binary image data. In this study, we aim to extend our de-identification capability via OCR software (GOCR), to capture identifiers in binary image data.

Methods:
A set of 57 echocardiography studies (image and video) from 7 vendors and 11 models were evaluated. Each study contained image data with a pseudo-name, and 48 studies had a pseudo-MRN. GOCR with post-processing returned a set of "high-confidence" text string characters found in the image, including typical permutations (e.g. "0" vs. "O"). These results were scored as

pass or fail depending on whether string permutations exactly resolved the identifier information.

Results:
Overall, GOCR name recognition scored 49/57 (86%), while MRN recognition was 38/49 (78%). In a subset of modern, high resolution, non-lossy images, GOCR performed better scoring 38/39 (97%) for name recognition and 32/37 (86%) for MRN.

Conclusions:
GOCR name and MRN recognition performance on medical images is sufficient for: 1) screening of large data volumes in real-time as a second order test and, 2) as an automated tool for developing new de-identification rules to improve future CTP performance.

59 Mechanical Exoskeleton Ankle Design

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Background: Lower body exoskeletons allow people with lower extremity paralysis to walk again. However, existing technology is limited in terms of gait speed, time of use, and stability. This existing technology could allow closer to normal walking speed but the lack of stability and extensive use of crutches may prevent users from achieving such speeds. The biped locomotion literature has shown that ankle joints are invaluable for system stability and energy efficient walking. Considering existing exoskeletons have modest ankle designs, improvements in ankle design could enhance stability and efficiency (i.e., biological, prosthetic, and biped robot ankles).

Objective: In the aim of improving stability and efficiency of lower body exoskeletons, a completely mechanical low weight energy dissipating and returning foot and ankle design is proposed that is inspired by the mechanical behaviour of the biological ankle and foot. This new robotic exoskeleton ankle will provide three basic functions: fast plantar flexion at foot strike to dissipate kinetic energy, stance phase dorsiflexion resistance and energy return at toe off, as well as toe and ankle dorsiflexion during swing phase for toe clearance.

Methods: The Anybody musculoskeletal modelling software will be used to model and evaluate parameters of the proposed design in order to optimize the design, to evaluate the potential advantages of electrical control vs. mechanical control, and to compare the performance of the proposed design to that of existing exoskeleton ankles.

60 A Novel Multi-Modality Perfusion Phantom for Nuclear Imaging Studies

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Objectives: Physical phantoms are routinely used to evaluate nuclear imaging technologies, but few options are available for dynamic imaging for pharmacokinetic modeling. We modified a Dynamic Contrast-Enhanced (DCE) CT/MRI perfused cylinder phantom for use in nuclear imaging. We evaluated the reproducibility and accuracy of PET and SPECT image derived kinetic model parameters in a series of experiments, and compared to computer simulations.

Methods: The DCE (Shelley Medical) phantom was modified by addition of a large inlet (Ø = 1.9 cm, length = 5.5 cm) cylinder to accommodate the spatial resolution of PET/SPECT. The phantom was imaged using 82Rb on a high count-rate PET/CT (GE 690) and low count-rate PET/CT (GE 600). Likewise, 99mTcO4 projections were acquired on a cardiac SPECT (GE 530c) and reconstructed with and without CT (acquired separately) attenuation corrections (AC) and Scatter Correction (SC). Twenty scans were acquired on each scanner under varying inlet flow-rates, Q, (100-300 mL/min) and flow ratios to the cylinder, R, (20-100%) versus perfusing tubing. Images were analyzed to define regions of interest on the inlet and cylinder which were used to sample input and output time-activity-curves (TACs) respectively, and on which a 1-compartment model with wash in and wash out parameters (Qin and Qout) was fitted. Computer simulated TACs were used to validate the kinetic model. Qin and Qout were compared against the control variable Qcyl = Q*R.

Results: PET imaged derived parameters agreed with the simulations results with Pearson correlation coefficient r2 = 1.00 and unity slopes. GE690 and GE 600 parameters had excellent agreement with true values as shown in the Table. For SPECT agreement was reduced, revealing saturation of the camera during tracer administration, however analysis of late time frames with a mono-exponential model resulted in good Qout agreement. Introduction of attenuation and scatter corrections reduced image noise, without significantly impacting quantification accuracy.

Conclusion: The DCE phantom can be used to validate quantitative nuclear imaging applications in PET and SPECT.

Table 1: Agreement between Image Derived Parameters and control variable for PET and SPECT modalities
Method Qin Qout
r2 Agreement slope r2 Agreement Slope
GE690 0.97 1.02 0.94 1.00
GE600 0.95 1.02 0.94 1.01
GE530C 0.60 0.82 0.53 1.33
GE530C AC 0.80 0.82 0.94 1.37
GE530C ACSC 0.85 0.83 0.94 1.31
GE530C Mono-exp - - 0.98 0.97
GE530C AC Mono-exp - - 0.98 1.04
GE530C ACSC mono-exp - - 0.98 1.10

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61 **Developing WHO Rapid Advice Guidelines (RAG) in the Setting of a Public Health Emergency**

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Background: In response to a public health emergency, WHO must provide an evidence-informed guideline produced at times within 1-3 months. Such guidance must follow the basic steps for full guideline development but with modifications to meet the accelerated timeline. Until now, WHO has lacked specific guidance on how best to accelerate guideline development across the planning phase conduct of the evidence reviews used to inform recommendations and the mobilization of a Guideline Development Group (GDG) to formulate recommendations.

Objectives: To describe newly established guidance for guideline developers at WHO on the process and procedures for developing evidence reviews within a shortened time frame of 1- 3 months; guidance that complements, and is consistent with, the recently revised WHO Handbook on Guideline Development (2nd Edition).

Methods: Guidance was informed by in-depth discussions of issues related to rapid guideline development with WHO staff (n=6) who deal most often with public health emergencies. Further, we relied on established rapid review methods, which were incorporated into the current WHO guideline development structure.

Results: We will highlight potential criteria used to consider whether or not a RAG is apt or feasible; and roles of the Guideline Review Committee (GRC) Secretariat, and the GDG across the RAG phases. We will focus on the methods and steps involved in doing rapid reviews, which are prone to post hoc changes given the fluid and iterative nature of the RAG process.

Conclusions: Important differences exist between developing a standard guideline and a RAG, which involves a shorter timeline, narrower scope, and use of abbreviated methods of the associated review. In turn, this may affect the risk of bias of the review's conclusions. However, the core principles and standards for WHO guidelines apply to RAGs including aims to minimize bias; apply transparent processes and use of explicit methods.

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62 **Bruyère Best Evidence Review Group (BBERG)**

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Background:
Bruyère Continuing Care (BCC) needs rapid tailored best evidence to provide the best quality of care to its patients. The Bruyère Research Institute (BRI) is committed to aligning its research assets to assist Bruyère Continuing Care achieve this goal.

Objectives:
To present a progress report of the activities of the BBERG team.

Methods:
Clinical or program questions are submitted by a local champion who can work with the BBERG team to ensure relevance and for capacity building around the clinical program or team. Questions will be prioritized by the Senior Leadership Team in consultation

with the BBERG team who will inform regarding feasibility. The BBERG team will carry out a rapid review of best evidence and provide a summary and analysis of the field to the clinical champion or team. The clinical program will then review the findings contained in the review and identify practice gaps. In the event of gaps the Methods group can be requested to participate in further evaluation and/or the development of care maps, etc.

Results:
Ten rapid reviews have been completed or are in progress on various topics including: falls prevention in long-term care and continuing care; respiratory therapy and care; concept mapping to build coordinated, person-centred, and high-quality care; process for complaint management; living environments for people with dementia; patient satisfaction; and COPD management.

Two have led to funding from Canadian Foundation for Healthcare Improvement (CFHI), and three are being used by senior management to design quality improvement in the hospital setting. We have had external requests for information from Ontario Long Term Care Association, Alzheimer Society and the Champlain Dementia Network. We also have two academic publications.

Conclusions:
BBERG represents an exceptional vehicle to contribute to evidence-based clinical care design and performance as well as help in clinical leadership capacity building.

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63 **Suspension system for limb prostheses based on the static magnetic field**

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Background:
Individuals with limb loss are fully dependent on prosthetic limbs for the daily living activities. No matter how advanced, the prosthesis must be attached securely and efficiently to the residual limb or prosthetic care has failed.

Objective:
To evaluate and compare a newly designed prosthetic suspension system with common suspension system in the market.

Methods:
A new prosthetic suspension system has been developed based on a static magnetic field. The mechanical design was successfully tested through tensile loading as well as clinical use by amputees. Pressure changes over the residual limb pistoning within the prosthetic socket and kinetic, kinematic, and spatiotemporal gait parameters during various activities were studied using the new system and compared to another commonly-used system. Participant subjective feedback for satisfaction, problems with the new system, and comparison to other commercially available suspension methods were obtained. Gait deviation index was calculated to compare between able-bodied individuals and amputee prosthetic and sound limbs.

Results:
The new system successfully tolerated the average centrifuge forces applied to the lower limb during normal walking. The traction that causes pain and skin problems with competing designs was reduced with the new magnetic system. Walking pattern and pistoning were in the acceptable range for lower limb prostheses. The participants were generally highly satisfied with the system in terms of donning and doffing, pistoning, walking comfort, pain, and pressure within the socket. The gait deviation index showed significant differences between amputee and able-bodied groups with all studied systems, but there was no significant difference among them.

Conclusions:
The new magnetic suspension system is being commercialized after clinical testing on transtibial amputees. Being purely mechanical, this design is low maintenance, durable, low-cost, and the fabrication procedure is up to the standards of prosthesis fabrication in terms of time and ease of technique.

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64 **Defining an equity-informing randomized trial: a deliberative process**

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Background: Disparities in opportunities for health within society are described as a "health inequities". Health equity is achieved with the absence of avoidable differences in health within and across populations. Randomized controlled trial (RCT) studies can provide evidence about the impact of an intervention, and the effects of the intervention on health equity. Therefore, RCTs are

useful for equity-informed decision making which can be described as decisions which influence opportunities for access and uptake of health services. There remains, however, a gap in the literature about how to identify when an RCT may be equity-informing.

Methods: A process for identifying equity-informing RCTs in a way defined as both useful and relevant by those interested in and seeking evidence for equity-informed decision making. Potential knowledge users include researchers conducting intervention studies, journal editors publishing research results, decision makers seeking to apply research evidence for equity-informed decision making, and populations for whom the evidence is meant to benefit.

Results: An international advisory board and study executive, consisting of a broad range of interdisciplinary and user group representatives, engaged in an iterative and deliberative reflective process for identifying when a research study is equity-informing. An integrated knowledge translation process was structured using participatory research principles and conducted using moderated discussion and examples to co-create knowledge. Outcomes were articulated in a mutually agreed upon conceptual model identified by the advisory board and study executive as useful for identifying equity-informing RCTs.

Conclusions: Ongoing activities including publications and presentations with user groups are anticipated to prompt further deliberation about understandings of RCTs in relation to health equity, and to result in the development of further iterations of the conceptual model.

Acknowledgements: CONSORT Equity Advisory Board members and Study Executive for their participation and contributions.

65 **Perioperative glycemic control: continuous insulin infusion protocol for diabetic or pre-diabetic patients undergoing infra-inguinal bypass surgery**

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Background:
The importance of hyperglycaemia in the surgical patients has been debated extensively in the past two decades. A growing number of evidence indicates that hyperglycaemia is an independent risk factor for morbidity and mortality in the perioperative period. Hyperglycaemia is associated with increased risk of infection, renal failure, prolonged hospital stay and greater consumption of hospital resources. To date, the optimal perioperative glucose target for enhanced clinical outcomes is unclear. Therefore, we designed a protocol with the aim of improving outcomes for hyperglycaemic patients undergoing elective peripheral vascular surgery.

Objective:
To evaluate the safety of the continuous insulin infusion protocol; it's efficacy for glycemic control and the impact on perioperative complications.

Methods:
Between 2010 and 2012, 70 patients at TOH underwent elective infra-inguinal bypass surgery while on the continuous insulin infusion protocol. The protocol aims to maintain blood glucose levels between 6.0 and 10.0mmolL-1. Data on perioperative glucose levels, insulin requirements and 30 days perioperative complications was reviewed retrospectively. From the 70 patients in the study, 19 were excluded from the analysis due to protocol violations, leaving 51 for analysis.

Results:
The average blood glucose level on day of surgery was 8.6mmol/L and the average on postoperative day one was 8.4.mmol/L. No patient had a daily average glucose level under 6.0mmolL-1 but 14(27%) at some point, had a daily average glucose level over 10mmolL-1. There were 3 wound infections (6%), 8 patients with one or more episodes of hypoglycaemia (16%), 4 amputations (8%), 3 myocardial infarction (6%) and 2 deaths (4%) while in hospital.

Conclusions:
The protocol seems safe in preventing hypoglycaemia. It will be interesting to compare the outcomes of the study group to a control group with conventional perioperative glucose management.

66 **Quantitative Susceptibility Mapping of the Motor Cortex in ALS and PLS Patients: A Biomarker for Upper Motor Neuron Dysfunction**

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Background:
Motor Neuron Disease is a progressive neurodegenerative disease characterized lower- (LMN) and upper motor neuron (UMN) dysfunction in ALS (Amyotropic Lateral Sclerosis) and mostly UMN dysfunction in PLS (Primary Lateral Sclerosis). The diagnosis is currently based on clinical assessment, electrodiagnostic studies and exclusion of other diseases. Electromyography effectively detects LMN degeneration but there is no definite technique for demonstrating UMN involvement and UMN findings on clinical examination may not occur until late in the disease course. A method that detects early UMN involvement and accurately monitors disease progression is highly desirable especially for future clinical trials and strategies for early intervention.

Objective:
In this study we investigated the importance of quantitative susceptibility mapping (QSM) analysis in ALS and PLS patients and compared the QSM values with presence of UMN signs in these patients.

Methods:
MRI was performed on ten ALS and three PLS using a 3D gradient-echo sequence at 3 Tesla. QSM images were calculated from the filtered phase maps and magnitude data using the iterative Susceptibility Weighted Imaging and Mapping (iSWIM) algorithm with a software package developed in-house. To evaluate the susceptibility changes, ROIs were drawn into the right (RMC) and the left motor cortex (LMC). Control ROIs were in the anterior border of precentral gyrus on the right (RCT) and left (LCT) side. We correlated the susceptibility values between the primary motor cortex (in the hand knob area) and the anterior border of precentral gyrus with presence of UMN signs (spasticity and hyperreflexia).

Results:
Increased susceptibility values were found in the grey matter of primary motor cortex and not in the adjacent cortex including the anterior border of precentral gyrus. This is seen in a subgroup of ALS patients and all three PLS patients. Patients with spasticity symptoms have significant higher susceptibility values in the motor cortex area than those who do not ($p<0.043$). In the patient group showing symptoms of spasticity the susceptibility in the control cortex area is significant lower than in the motor cortex area ($p<0.001$).

Conclusions:
Our study showed a significant correlation between QSM values and presence of UMN signs (spasticity). The results suggest QSM could be a quantitative tool to detect changes in the UMN as they are present in ALS and PLS. Larger prospective studies will be needed to find the incidence, sensitivity and specificity of this sign in ALS patients and to establish its prognostic value.

67 **Reproducible tracer injection profile improves the test-retest repeatability of myocardial blood flow quantification with 82Rb PET**

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Background
Myocardial blood flow (MBF) imaging with 82Rb PET is gaining interest for improved diagnosis of coronary artery disease (CAD).

This study evaluated whether the administration of 82Rb tracer using a constant-activity-rate (CA) vs constant-flow-rate (CF), bolus shaped, injection profile, may improve the test-retest repeatability of MBF measurements on a high count-rate 3D-PET scanner.

Methods
22 volunteers underwent sequential test-retest PET imaging during rest and dipyridamole stress, with 82Rb injected using a custom infusion system.
In 13 patients, test-retest tracer injections were both administered using the CA mode (CA-CA).
In the remaining 9 patients, CA-CF modes were used for test-retest imaging. MBF was quantified using a 1 tissue-compartment model with a dual spillover correction.

The injection profile effects on absolute and relative repeatability coefficients (RPC and RPC%) were compared between injection profile cohorts. Tissue-to-blood contrast and signal-to-noise ratios of uptake images (2-6 minutes) were also compared between infusion modes.

Results
The CA-CA injections produced MBF values that were more repeatable than the CA-CF group (RPC = 0.38 vs 0.54 mL/min/g, $p=0.004$; RPC% = 25% vs 38%, $p<0.001$).

In terms of uptake image quality, tissue-to-blood contrast was slightly higher for CA vs. CF images (1.88 vs 1.76, $p<0.001$).

The signal-to-noise ratio was not significantly different (9.97 vs 9.81, $p=0.45$).

Conclusion
Myocardial blood flow quantification with 82Rb PET can be influenced by the time-activity injection profile of the tracer. Constant-activity infusion of 82Rb produces more repeatable profiles and decreases the test-retest variability of MBF measurements, when compared to constant-flow-rate administration of 82Rb.

68 **User testing and evaluation of Evidence for Equity: Systematic review summaries for policymakers**

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Background: Most systematic reviews are written using technical language, are too long, and do not describe contextual information important for policy makers and other users making decisions about how to use the evidence. Evidence for Equity (E4E) is a special collection of evidence summaries of interventions that are effective at reducing inequities.
Objective: To develop and evaluate a special collection of evidence summaries of systematic reviews of interventions that can reduce inequities across the factors described by the PROGRESS acronym.
Methods: Two rounds of user testing of one summary in each of five topic areas (HIV/AIDS, malaria, mental health, nutrition, and public health) will be conducted. Each round of user testing will test one summary with two policymakers per topic for a total of 20 user tests. 20 policymakers and/or researchers will participate in user testing.to assess usefulness, credibility, value, and relevance of the summaries
Results: We have recruited international stakeholders to participate in user testing in early 2016.
Conclusions: Improving policymakers' understanding of an intervention using evidence from high quality systematic reviews can ensure that their decisions are evidence-informed. User testing of E4E will ensure that the summaries and website are useful and usable. In addition, the brief evidence summaries included in E4E are focused on interventions that can help reduce inequities which will help improve health outcomes for disadvantaged populations. The improved E4E Summaries will be freely available online and can provide evidence to policymakers interested in interventions to reduce health inequities.

69 **Nurses' perception of the impact of a model of care on organizational climate and patient safety.**

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Background
The Ottawa Hospital Model of Nursing Clinical Practice (TOH MoNCP) was developed in-house by a multidisciplinary team. It emphasizes the five tenets of working to full scope of practice, autonomy, accountability, continuity of care, and engagement of patients in decision making. As it was developed to organize the way nursing care was delivered, it became important to assess how individual nurses perceived the hospital safety climate safety policies, procedures, and practices and quality of patient care as a way to understand how effective the model has been.

Objective
To examine the impact of re-implementing TOH MoNCP on nursing staff perception of patient safety climate and quality of care in two surgical units.

Methods
The study used a quasi-experimental longitudinal design. Data was collected through a number of standardized self-reported questionnaires in two surgical units at the Ottawa Hospital. The questionnaire captured nurses' perceptions of patient safety climate and global patient care and safety at three time points: prior to implementation of the model, and one and two years post implementation. Data was analyzed using SPSS. Data analysis consisted of frequency distribution, chi-square tests, and T-tests.

Results
92 nurses (Baseline N=52, Y1: N=26, Y2: N=14) completed the survey. Respondents were predominantly registered nurses (98%), female (84%), married (61%), working full-time (82%) with an average age of 38 years. Nurses' perception of patient safety improved significantly one-year after re-implementing the model ($t(74)=2.281$, $p=0.025$). Sixty-one percent of nurses at one-year post re-implementation strongly agreed that their suggestions to management about safety would be acted upon. Nurses' perceptions of global patient care and safety improved significantly two years after the model re-implementation ($t(62)=2.312$, $p=0.025$). Sixty-three percent and 57 percent of nurses rated overall quality of patient care and patient safety, respectively, as excellent two years post re-implementation.

Conclusions
The findings indicate that adopting the model of nursing clinical practice has a positive impact on nurses' perception of patient safety and quality of patient care.

70 **Withdrawn**

71 **Automatic Detection of Patient Motion Using Fiducial Markers in Dynamic Cardiac PET Images**

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Purpose: Tracking of patient motion in a dynamic cardiac positron emission tomography (PET) image sequence can be difficult due to the changing tracer distribution from blood to perfused tissues. Fiducial markers may help identify cases with patient motion during dynamic image acquisition. This work evaluates two automated algorithms for detection of fiducial marker motion.

Method: 65 patients undergoing dynamic cardiac Rubidium-82 PET imaging at rest and stress had a 1uCi Sodium-22 fiducial marker affixed to their chest. 130 images were visually inspected for the presence motion. Inter-frame motion was determined automatically as greater than 2.7mm distance change in marker center of mass (COM) between successive frames. Intra-frame motion was determined as pixel intensity gradient broadening radially outwards from the marker COM by fitting a modified Gaussian function.

Results: Intra-frame motion was present in 604 of 1950 frames. The intra-frame algorithm sensitivity and specificity was 0.80 and 0.90 respectively. Inter-frame motion was present in 367 of 1820 frames. The inter-frame algorithm sensitivity and specificity was 0.91 and 0.91 respectively.

Conclusion: The intra and inter-frame algorithms may be suitable for automated fiducial marker tracking to identify scans with patient motion, in which myocardial blood flow quantification may not be reliable.

72 **Inductive Risks and Values in Clinical Trials Composite Outcome Measures**

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Background:
The use of composite outcomes is becoming widespread in clinical trials. By combining individual outcome measures into a composite, researchers claim a composite can increase statistical precision and trial efficiency, expediting the trial by reducing sample size and cost, and consequently enabling researchers to answer questions that could not otherwise be answered. Another rationale given for using a composite is that it provides a measure of the net effect of the intervention that is more patient relevant than any single outcome measure. Critics, on the other hand, argue that the use of composite threatens the scientific objectivity of the trial by introducing new risks, and that in practice components are inconsistently defined, unreasonably combined, and inadequately reported. Conceptual scrutiny can help shed light in these disagreements and disentangle ethical from epistemological issues of composites in clinical trials.

Objectives:
I examine common use of composites in cardiovascular trials, and show how composite results can be problematic, particularly misleading, if the proper range of scientific decisions and their consequences are ignored when judging the composite. I propose ways of making explicit the range of scientific decisions and their risks, remedying composite issues in clinical trials.

Methods:
Ethical inquiry based on practical reasoning, and selected case studies. Applied logics, and philosophies of science (e.g., inductive logic, error statistics, casuistry) that are principled on normative versions of the argument from inductive risk (predictable consequences of error) can help explicate and mitigate issues with composites.
Results:
(a) Recommendations for making explicit the rationale for using composite outcome measure in the particular clinical trial.
(b) Recommendations for making explicit ascertainments for selecting and combing multiple individual outcome measures into the composite measure.
(c) Recommendations for making explicit interim analysis and decisions based on composite outcome results.
(d) Recommendations for making explicit the reporting of evidence based on composite outcome results.
Conclusions:
The conceptual work makes clear important competing risks for using composites in clinical trials, and that, in turn, proper evaluations of composite results demand making explicit the range of scientific decisions, their inductive risks, and both epistemic and non-epistemic values that are embedded in composite selection and outcome analysis. This work has the potential of better informing physicians, patients, systematic review authors, and health policy decision makers when having to make difficult choices based on composite results, and taking risks responsibly.

73 **Mesenchymal Stromal Cell Therapy for Sepsis in Preclinical Animal Models: A Systematic Review**

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Background
Mesenchymal stromal cells (MSCs, 'adult stem cells') represent a potential novel treatment for septic shock. Preclinical animal studies of sepsis suggest that MSCs modulate inflammation, enhance bacteria clearance and tissue repair, and reduce organ failure and death1-5. We performed a systematic review and meta-analysis to answer the question, "In preclinical animal models of sepsis what is the effect of MSCs (versus control treatment) on mortality?".

Objectives
To determine the effect of MSC treatment on mortality in preclinical models of sepsis, as compared to diseased controls.

Methods
A systematic search of MEDLINE, Embase, BIOSIS and Web of Science was conducted in May 2015 to identify studies that compared the treatment of sepsis with MSCs versus diseased controls in pre–clinical sepsis models. Data were collected for the mortality and for general study characteristics to enable subgroup meta-analyses. Results were pooled and expressed as odds ratios (OR) and 95% confidence intervals (CI) using a random effects model; heterogeneity was measured with the I2 statistic. Methodological quality of included studies was assessed using the Cochrane Risk of Bias tool. A funnel plot was generated for mortality to examine publication bias.

Results
3114 citations were screened; 41 studies met our inclusion criteria with 18 studies (describing a total of 20 experiments) reporting the primary outcome of mortality. Meta-analysis indicated that treatment of pre-clinical sepsis with MSCs as compared to control significantly reduced the overall odds of mortality (OR 0.27 [95% CI 0.18-0.40], I2=32%) as well as mortality at pre-defined time intervals: =2 days (OR 0.31 [95% CI 0.21-0.47], I2=35%), >2 to =4 days (OR 0.23 [95% CI 0.13-0.43], I2=41%) and >4 days (OR 0.18 [95% CI 0.11-0.32], I2=0%). Subgroup analyses suggested efficacy is maintained across a range of experimental conditions (e.g. animal characteristics, sepsis model, MSC characteristics, MSC administration, and control group). The Cochrane Risk of Bias items of sequence generation, allocation concealment, and blinding of outcomes assessors were considered unclear for all 18 studies. Funnel plot analysis showed evidence of asymmetry, suggesting potential publication bias.

Conclusion
Our results indicate that treatment of preclinical systemic sepsis with MSCs reduces the odds of mortality across a range of conditions. However, unclear reporting of Risk of Bias elements and the suggestion of publication bias limit the strength of our conclusions.

74 **Withdrawn**

75 **BETWEEN-CENTRE VARIATION IN THE MANAGEMENT OF TRANSPLANTATION: A SYSTEMATIC REVIEW**

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Background: Chronic kidney disease (CKD) affects an estimated 3 million Canadians When CKD progresses to kidney failure either transplantation or chronic dialysis is necessary to sustain life. Kidney transplantation is the most effective therapy for these patients with an improved quality of life and lower risk of death compared to dialysis. Although one-year graft and patient survival rates are now above 95% there has been little improvement in long-term outcomes. While certain patient-level factors that influence graft and patient survival (such as recipient and donor demographics) have been established, the presence of variation in the management of kidney transplant patients and its influence on patient outcomes remains largely unknown.

Objective: This systematic review will focus on centre and provider factors that impact patient and graft survival post renal transplantation.

Methods: A comprehensive and systematic search of published articles was performed by a trained and experienced librarian using both MEDLINE and EMBASE. Key words such as “kidney transplantation”, “centre effect” and “centre variation” “mortality” and “graft failure” were used. We included all study design types. The primary outcome was patient survival post-transplant (this includes all cause mortality) and graft failure (defined as required return to dialysis or retransplantation). Information on study characteristics, comparisons and results were abstracted by two individuals independently.

Results: Our search yielded 6196 abstracts 236 full texts were assessed for eligibility and 30 studies were included in this abstract. These studies were published between 1985 and 2013. Of the 30 included studies 26 studies were retrospective and 4 were prospective. The number of centres in each study ranged from 1 to 258 with patient sample sizes ranging from 152 to 141,406. Different centre factors were assessed in the studies which included transplant volume, cohort era, kidney sharing policy, prior mortality rate and follow up management. Studies varied in their approach to data analysis and length of follow up.

Conclusion: Variation in patient and graft survival post kidney transplant is not a new concept and has been studied since the 1990s.There continues to be variation in graft and patient survival rates at different centres that is not always explained by patient factors.

76 **Underreporting of sex and/or gender in randomized control trials: an environmental scan of reporting considerations**

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Background: Sex and gender analysis is critical for the safe and effective use of drugs and technology. Governments internationally have called for both inclusion of women in trials and sex-disaggregated analyses of data. Research in the USA has shown that funding requirements have not led to improvements in sex-disaggregated analysis. There is a lack of research on the practices regarding sex and/or gender analysis in trials conducted in Canada, where there is an impetus for change.
Objective: The purpose of this study was to assess the extent to which sex and/or gender is reported in Canadian randomized controlled trials (RCT).

Methods: We searched MEDLINE in June 2014 using the McMaster University filter for RCTs combined with terms for "Canada" and "Canadian". We a Canadian organization or had a first or last author in Canada. We collected data on reporting of sex and/or gender analysis using a pre-tested data extraction form.
Results: The trials had a mean sample size of 120 (range 12-4752). Forty percent were multi-site trials, 18% were industry funded and 92% were individually randomized RCTs. Among the 100 trials, 93% reported the sex of the included populations and 24% of the mixed-sex studies conducted subgroup analyses (21/87). Only 8% (7/87) discussed implications of sex and/or gender analysis results.

Conclusions: This environmental scan identified a gap in reporting between conducting sex and/or gender analysis and discussing the implications of these analyses for policy, practice and research in the Canadian context. This suggests that the current policies to foster sex/gender analysis are not necessarily sufficient to ensure the translation of findings into safe and effective treatments across sex and/or gender.

Neuroscience Program

77 **Predicting decision outcomes from single realizations of lateral prefrontal cortex neuronal activity**

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Background: Neurons in the lateral prefrontal cortex (LPFC) encode sensory and cognitive signals, as well as commands for goal directed actions. Thus, this brain region might be a good signal source for a goal-selection brain-computer interface (BCI) that decodes the intended goal of a motor action previous to its execution.
Objective: Toward the development of a goal-selection BCI, we set out to determine if we could decode saccade goals from single-trial LPFC neuronal activity.

Methods: We recorded neuronal spiking activity from microelectrode arrays implanted in area 8A of the LPFC of two adult macaques while they made visually guided saccades. The rewarded target was indicated by a colour cue and we changed periodically the association between colour and rewarded direction. We extracted neuronal firing from single-trial LPFC activity in the pre-saccade period and predicted saccade targets using support vector machines with 10-fold cross-validation. Binary decision outcomes were decoded from pre-saccadic single-trial LPFC activity with better-than-chance accuracy.
Results: Decoder performance remained high when examining groups of trials with identical visual stimuli but different behaviour (>80%). Further, some LPFC activity was dependent on contextual information independent of visual information and saccade performance.

Conclusions: These results provide further evidence that LPFC neurons encode decision processes and suggest that LPFC activity can be used as a signal source for a goal-selection cognitive BCI.

78 **Translational lipidomics: Harmonizing LC-ESI-MS targeted lipidomic methodologies in clinical study of Dementia with Lewy Bodies**

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The emerging field of neurolipidomics seeks to understand how dynamic changes in membrane composition regulate brain cell function and how these changes can be used as biomarkers to predict disease outcome and track disease fate. Commonly conceptualized as undulating fields of identical molecules, neuronal membranes are, in fact, made up of hundreds of chemically and molecularly diverse lipid species. For the first time, significant technological advances in liquid chromatography (LC), electrospray ionization (ESI), and mass spectrometry (MS) are enabling membrane composition to be profiled comprehensively at the molecular level. Coupled with subcellular fractionation and careful consideration of extraction protocols that enrich for different phospholipid families, species that vary by only one double bond, a single methylene group, or carbon chain linkage can now be quantified directly in synaptic preparations. These advances are allowing for discovery of novel biomarkers of disease transition, progression, and fate and new mechanistic insight into the determinative roles of lipid metabolism in neurodegenerative disease. Yet, as with imaging biomarkers, accuracy and reproducibility are fundamentally dependent on how lipid biomarkers are measured. We seek to profile the changes in the circulating plasma and cerebrospinal fluid (CSF) sphingolipidome that, either alone or in combination with changes in a-synuclein, identify antecedent cognitive impairment in DLB patients. To do so, we present here data highlighting the challenges in harmonization of protocols, analyses, and lipid identification required for multi-centre replication and validation of biomarker results obtained through neurolipidomic investigations.

79 **IRF2BP2 necessity in innate immune response affecting stroke recovery in mice**

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Background. Immune response and inflammatory signaling are intertwined events after stroke, the second most frequent cause of death and debilitating illness. Microglia, the resident immune cell in brain can be polarized into M1 (pro-inflammatory) and M2 (anti-inflammatory) phenotypes after brain injury. Initial M1 inflammatory signaling subsides by turning on M2 reparative actions but nonresolving inflammation causes further tissue damage. Members of the IRF family of transcription factors play a major role for innate and adaptive immune responses regulating genes encoding IFNa, IFNß and IFN?, including IFNs-inducible genes. Supporting evidence showed IRF2 activates M1 polarization, while IRF2 gene expression and transactivational activities was known to be inhibited by IRF2 binding protein 2 (IRF2BP2).

Objective. In this study, we hypothesize that IRF2BP2 serves as modulator/suppressor of IRF2 action on microglia/macrophages polarization. Using mouse model lacking IRF2BP2 in myeloid cell-lineage that includes peripheral macrophages and CNS microglia, we are testing whether IRF2BP2 action is neuroprotective or –destructive after CNS focal ischemia.

Methods. We used Cre-mediated deletion of IRF2BP2 in myeloid precursor cells. Two months old mice male or female are prepared for CNS focal ischemia by photothrombosis with coordinates set up at 0.7 mm anterior-posterior (AP) and 2.0 mm midline (ML) from bregma for 10 min irradiation. Animals were sacrificed at 24 h, 4 d and 8 d post-stroke. Fluorescence-activated cell sorting (FACS) was used to identify microglia phenotypes. Behavioral, histological, molecular and immunocytochemistry assays were used to evaluate post-stroke damage. Values are presented as the mean standard error (S.E.). Differences among groups were identified by one-way analysis of variance (ANOVA) (Tukey's method). A significant difference was accepted at (*) $p<0.05$.

Results. The infarct volume of IRF2BP2-/- mice 4 days after photothrombosis was significantly larger compared to IRF2BP2+/+ mice. This was supported by behavioral analysis using adhesive removal test, showing IRF2BP2-/- mice have significant deficit of the contralateral forelimb usage compared to IRF2BP2+/+ mice. In FACS after 4d stroke, M1 phenotype was found to be significantly higher in IRF2BP2-/- compared to IRF2BP2+/+, indicating inflammatory microglia remains high which is correlated to a bigger infarct volume.

Conclusions. Our study shows that the loss of IRF2BP2 can result into increased brain cell death and affects microglia polarization that dictates the extent of damage after stroke. Hence, we provide novel evidences of IRF2BP2 critical role after stroke. Further study of IRF2BP2 mechanisms of action in stroke may provide beneficial therapeutic ways of dealing with recovery.

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80 **Investigating Parkin's role in oxidative stress and calcium regulation in recessive Parkinson disease.**

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Curing Parkinson disease (PD) is hindered by our lack of understanding of the molecular mechanisms that lead to the death of neurons. For the majority of cases of PD, we don't know the exact cause. It is thought to be a combination of genetic susceptibility coupled to a yet to be identified environmental factor. However, in a small number of people, PD can be caused by a mutation in one specific gene. The advantage is that studying these genetic cases can provide important insights into what drives neuronal death. My research focuses on autosomal-recessive, early-onset Parkinsonism caused by mutations in the Parkin gene (Kitada et al., 1998). These mutations cause a 'loss of function' of the parkin protein; therefore suggesting that parkin normally protects neurons. We know that parkin is protective against oxidative stress, something that is toxic to vulnerable neurons. However, how parkin does this is still unknown, although certain models have been put forward including its involvement in keeping mitochondria healthy. My overarching hypothesis is that Parkin's neuroprotective role is to protect vulnerable neurons against increased oxidative stress. I hypothesize that it does this in two specific ways: 1) by acting to neutralize reactive oxygen species (ROS). ROS are produced normally by the cell, but cause oxidative stress and cell death if uncontrolled. Mitochondria are critical to providing energy to neurons, and they are particularly vulnerable to ROS damage. 2) By regulating calcium signaling which is a critical component of healthy mitochondria function (and sensitive to ROS production). My project focuses on an exciting new model of how parkin protects neurons, which will combine genetic mouse models that lack parkin expression, as well as increased oxidative stress paradigms. My project is focused on characterizing Parkin-dependent changes and dissecting the molecular pathways that underlie them with the goal to bring closer cause-directed therapeutics for PD.

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81 **IRF2BP2, an innate immune modulator, affects hepatic lipid homeostasis**

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Background:Metabolic disorders (obesity/nonalcoholic fatty liver disease/diabetes) are a modern pandemic with hallmarks of chronic systemic inflammation and disrupted cholesterol homeostasis by the liver. Emerging evidence reveals that transcription factors of the innate immune response modulate hepatic cholesterol homeostasis. A recent GWAS (genome-wide association study) has tied IRF2BP2 to elevated plasma total and LDL-cholesterol. IRF2BP2 interacts with interferon regulatory factor 2 (IRF2) to limit interferon signaling, known to promote steatosis and suppress cholesterol synthesis. IRF2BP2 function in the liver is not known and will be elucidated here.

Hypothesis: We hypothesize that IRF2BP2 regulates hepatic gene expression and the response to metabolic and inflammatory stress.

Method: We generated LKO mice (AlbCre/IRF2BP2flox) that ablate IRF2BP2 in hepatocytes and then evaluate how LKO mouse response to metabolism and inflammatory stress differently.

Results: we discovered that IRF2BP2 is important in hepatocytes to limit the liver inflammatory response to HFD/HFSCD-induced changes and maintain hepatic insulin sensitivity. We use a yeast two-hydrid screen of a human liver cDNA library discovered JDP2 is a novel interacting partner of IRF2BP2. This is important because JDP2 may no longer suppress the expression of the gluconeogenic gene PEPCK and account for excess hepatic glucose production. In addition, by hindering AP-1-dependent gene expression, JDP2 plays an anti-inflammatory function.Our data further suggest that IRF2BP2 may control hepatocyte interaction with liver innate immune cells, the NKT cells. We found that IRF2BP2 is required for the expression of the RAET1 ligands of NKT cells that are known to sustain insulin signaling and to suppress inflammation in the liver. Importantly, IRF2BP2 expression in hepatocytes is suppressed by high fat diet or a saturated fatty acid (palmitate), suggesting that loss of IRF2BP2 may be a general mechanism underlying the susceptibility to hepatic inflammation leading to insulin resistance in response to high fat diet.

Conclusion:Our IRF2BP2 LKO mouse is an informative to identify therapeutic targets for NAFLD and T2DM. Promoting IRF2BP2 function to limit inflammation in combination with conventional therapies (metformin) may be the next phase to prevent NAFLD development and T2DM.

82 **CCAR-1 ortholog LST-3 plays a role in regulating motor neuron positioning in C. elegans**

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Background
CCAR1/2 are important regulators of the cell cycle and apoptosis. Its ortholog in C. elegans, lst-3, also plays a role in controlling neuron spacing. Mutations in the lst-3 gene produces motor neuron spacing phenotypes similar to mutations in other genes of the canonical Wnt pathway and the planar cell polarity pathway.

Objective
The objective is to understand how LST3/CCAR regulates motor neuron position.

Methods
lst-3 will be crossed into various canonical and non-canonical Wnt gene mutants to provide insight into how lst-3 interacts with these genes. Transcriptional and translational fusions to GFP injected into wild-type and mutant worms will show the expression and localization of lst-3. In addition, the transgenic worms will undergo RNAi treatment specifically targeting GFP, allowing visual confirmation as to which neurons are expressing this protein and whether the mutants affect their positioning.

Results
From preliminary results, it is seen that the lst-3;vang-1 double mutant has a phenotype similar to lst-3. The transcriptional GFP fusion transgene expresses high levels of GFP throughout the body of a larval nematode with certain areas particularly showing brighter levels of fluorescence. These areas are the pharynx, the distal tip cells of the anterior and posterior gonads, the vulva and the tail. GFP was also seen in neurons along the ventral cord. Finally, the translational GFP fusion protein is shown to localize in the nucleus of various epidermal cells.

Conclusions
Preliminary results suggest that the similarity of the lst-3;vang-1 double mutant phenotype to lst-3 may indicate that these two genes are working together in the same pathway. Also, the nuclear localization of the translational GFP fusion protein is consistent

to CCAR's role as a transcriptional regulator.

83 **Dendritic amplification of activity-dependent calcium signals drives local associative spine plasticity during clustered synapse development**

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Pyramidal neurons establish thousands of synaptic connections during early postnatal brain development, marking a key period in neural circuit assembly. Despite the eminent role of calcium in synapse regulation, remarkably little is known about intracellular calcium dynamics during synaptogenesis. Using whole-cell electrophysiology, two-photon calcium imaging and glutamate uncaging in acute hippocampal slices, we found that synaptic NMDA receptor activation during a narrow developmental epoch triggered ryanodine receptor-dependent calcium-induced calcium release (CICR) at CA1 pyramidal cell dendrites. This NMDAR-mediated CICR was a major determinant of spine calcium kinetics, and drove local calcium signal propagation to nearby spines. Moreover, NMDAR-CICR coupling enabled dendrites to biochemically encode a range of distinct spatiotemporal features of synaptic input. We hypothesized that this mechanism may spatially regulate the development of synaptic ensembles in vivo. To test this prediction, we mapped synapse maturation by probing the functional AMPAR and NMDAR content of neighbouring spines and found unequivocal evidence for clustered synapse development along individual dendritic segments. Our results reveal novel developmental features of NMDAR-dependent calcium dynamics suited to instruct the assembly of synaptic microcircuit motifs apt to exploit the nonlinear regimes of dendritic information processing. Dysregulation of these mechanisms may be involved the genesis of subtle microcircuit disturbances underlying neurodevelopmental disorders.

84 **Withdrawn**

85 **Sigma-1 receptor involvement in an animal model of Alzheimer's Disease**

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Background: Alzheimer's disease (AD) is characterized by the progressive loss of neurons in the cortex and hippocampus, leading to cognitive decline. While a small percentage of AD is hereditary, the vast majority of cases are sporadic, composed of both genetic and non-genetic risk factors. Despite decades of research, mostly focused on the amyloid beta (Abeta) synthesis pathway, there is still no cure and current therapies fail to significantly alter the disease course. Thus, new targets for therapeutics are desperately needed. One intriguing area of investigation has focused on the sigma 1 receptor (Sig1R). Sig1Rs are proposed to play a role in the pathogenesis of AD for a number of reasons: 1) genetic polymorphisms of the Sig1R confer risk for AD; 2) Sig1R expression is reduced in AD; 3) Sig1R agonists are neuroprotective and anti-amnesic in AD mouse models; and finally 4) Aricept, the most prescribed drug for AD patients, has nanomolar affinity for Sig1R. Together these results led us to question whether loss of Sig1R function is involved in the underlying etiology of AD.
Rationale and Hypothesis: The role of Sig1Rs in regulating calcium homeostasis under cellular stress is particularly interesting given the importance of calcium homeostasis for proper cell functioning. Moreover, alterations in calcium homeostasis and synaptic function are observed during AD and normal aging. We hypothesize that loss of Sig1R will exacerbate the cognitive and synaptic deficits in AD by potentiating perturbations in calcium homeostasis. To verify our hypothesis we used an in vivo knockout (KO) mouse model, the Sig1R KO mice.
Preliminary data: We explored how loss of Sig1R affects calcium regulation, physiology, and behavior in the Sig1R KO mice. We used the Abeta25-35 infusion model as it recapitulates the process of sporadic AD, including impairments in learning, synaptic plasticity, Abeta plaque deposits, and cell loss in the hippocampus. Wild-type (WT) and Sig1R KO mice were infused intracerebroventricularly with Abeta25-35. We found that Abeta25-35 impairs performance on the Morris water maze, reduces the magnitude of long-term potentiation, alters neuronal excitability, and increases the AHP. Future experiments will determine how calcium homeostasis is altered and whether reversing changes to calcium will prevent changes to physiology and behaviour.

86 **Genes involved in embryonic motor neuron positioning along the AP axis**

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Background

The six dorsal D (DD) motor neurons are part of a class of embryonically-derived motor neurons that normally appear evenly spaced in the ventral cord which extends along the antero-posterior (AP) axis in C. elegans. It is known that axis formation occurs very early on during embryonic development. A pathway that is integral to axis formation is the planar cell polarity (PCP) pathway. Here we show that gene mutations in components of the PCP pathway, like prkl and vang, can disturb the spacing of the DD motor neurons in the ventral cord. Previous work shows that the DD neurons undergo a process of intercalation during embryogenesis, leading to the proper migration and polarization the DD neuronal cell bodies in the ventral cord. We observed that in C. elegans with mutations in the core PCP component prkl, the intercalation process delayed, leaving us with the question as to what is responsible for controlling the proper spacing of the DD motor neurons. For the purposes of our research, we believe that by using a forward genetic screen to identify DD neuron positional defect mutants, we gain insight into the specific genes as well as genetic pathways that regulate the intercalation process resulting in the regular distribution of the DD motor neurons in the ventral cord.

Objective
The discovery-driven investigation into the genetic mutations responsible for DD motor neuron positional defects that result in their abnormal spacing along the AP axis.

Methods
A forward genetic screen was conducted using the mutagen ethylmethane sulphonate. Visualization of the DD motor neurons for screening and subsequent characterization of the mutant phenotypes was enabled by the fluorescent DD reporter, ynIs37 flp-13p::GFP.

Results
Sex-1 and arl-6 are involved in regulating the migration and polarization of the DD motor neurons, as well as in the process of intercalation.

Conclusions
There are many genes involved in the planar cell polarity pathway that are capable of influencing the migration and polarization of the DD motor neurons. Beginning with a forward genetic screen for DD motor neuron positional defects, this research aims to gain more insight into the corresponding genes that are involved in controlling the spacing of the DD neurons in the ventral cord. By focussing on characterizing our strongest mutants, namely sex-1 and arl-6, we hope to contribute novel gene functioning and subsequent signalling pathways to the regulation of the processes of intercalation and DD neuron spacing.

87 **Lrrk2 - immunology side of a Parkinson related gene**

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Parkinson disease (PD) is a 'complex disease' with unknown etiology. However a combination of genetic risk AND (an) environmental trigger(s) are believed to be major contributing factors for its onset and development. For example, amino-acid exchanges in LRRK2 protein (such as G2019S) are associated with increased risk of developing PD.
LRRK2 is a protein kinase, expressed at low levels in neurons and whose function remains elusive. Our discovery that LRRK2 is highly expressed in the immune system led us to reassess our understanding of the function of LRRK2 and propose that the primary role of LRRK2 is not in neurons, but in the immune system and is this is critical to disease onset. This suggests an environmental trigger may be a xenobiotic which in combination with a dysregulated immune response, can lead to disease. A reasoning consistent with the concept that PD actually begins in the gut and olfactory system (which are in direct contact with the environment) years prior to motor symptoms are present.

Hypothesis: Because LRRK2 is an interferon-inducible gene and many xenobiotics upregulate interferons, we predict that reduced LRRK2 activity will be neuroprotective in our reovirus model. This is a model that we use to modulate nerve cell loss in a proof-of-principle study.
In support of our understanding, our lab has recently demonstrated that mice lacking the LRRK2 gene are more susceptible to 'reovirus' infection [Hakimi et al, in prep]. REO virus is transmitted through the blood stream, ultimately leading to neuronal infection and encephalitis causing death in mouse pups. We next found that nasally infected G2019S Lrrk2 knock-in mice: i) control reovirus better in their lungs; ii) yet have higher mortality from encephalitis than wild-type littermates; and iii) reveal altered regulation of brain inflammation [Shutinoski et al., in prep.]. An outcome associated with altered cytokine levels, which is indication of a dysregulated immune response.

Due to lack of effective treatments the neuronal loss in Parkinson disease remains progressive and incurable. Delay in the onset and progression of neurodegeneration would be beneficial considering the current treatment limitations. Mouse models that traditionally have been used to assess function of the immune system are found to be protective in key paradigms of human neurodegenerative disease. Incorporating the knowledge that a xenobiotic AND dysregulated immune system in G2019S LRRK2

carriers is a predisposition for PD represents novel understanding that can be potentially translated into innovative therapeutics for neurodegenerative diseases.

88 **Investigating the Role of Parkin on Oxidative Stress Mitigation**

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BACKGROUND: Parkinson's Disease (PD) is a devastating neurodegenerative disease that affects >110,000 Canadians and is the second most prevalent neurodegenerative disease. Early-onset PD is associated with specific autosomal recessive mutations in the DJ-1, PINK1 and PARKIN genes, which are linked to mitochondrial dysfunction and oxidative stress (OS). The PARKIN gene encodes a 465aa protein having 35 cysteines, accounting for 7 % of its total amino acid content (general protein cysteine content is 2 %). Current theories proposing Parkin's role in PD include: 1) protein clearance via polyubiquitination, and 2) mitochondrial recycling, or mitophagy. Unfortunately no specific role for Parkin has been validated that can explain the specific loss of dopaminergic cells in PD. It has been proposed that oxidative degradation of dopamine itself is the main reactive oxygen species (ROS)- producing culprit in dopaminergic cells.
OBJECTIVE: The present research focuses on understanding the role of Parkin in protecting cells from OS induced by H2O2 and oxidized dopamine.
HYPOTHESIS: It has been demonstrated that PD-affected human brains have increased signs of OS. Based on the redox hypothesis (defined by Jones), OS occurs when normal thiol-redox regulation in a cell is disrupted, and results from an increase in oxidants. Having a large number and ratio of cysteines which are susceptible to oxidation, we propose that Parkin is able to protect cells from OS by regulating redox changes in these cells and accomplishes this by altering the redox state of its own thiol groups.
METHODS: To investigate this hypothesis, the present study focuses on Parkin's ability to directly and indirectly mitigate H2O2 and oxidized dopamine insults. 1) Direct migitation of ROS (H2O2 and oxidized dopamine) will be tested using recombinant Parkin protein and two ROS measuring assays: a modified chemiluminescence assay and Amplex Red assay. 2) Parkin's ability to reduce OS in human embryonic kidney cells (HEK293) and human neuroblastoma cells (SH-SY5Y) is determined using the 2'',7''-dichlorodihydrofluorescein diacetate (H2DCFDA) assay and by measuring total thiol changes.
RESULTS AND CONCLUSIONS: Recombinant Parkin has been successfully generated and preliminary data suggests that Parkin is more effective than equimolar amounts of glutathione in migitating H2O2. Also, over-expression of FLAG-tagged Parkin in HEK293 cells suggests that FLAG-Parkin may have a protective role against H2O2-induced redox changes. Further optimization of Parkin expression in SH-SY5Y cells and the effect of Parkin on dopamine oxidation will be conducted to validate these preliminary findings.

89 **Aberrant Receptor Function, Mobility, Subcellular Distribution and Unfolded Protein Response in Cells Expressing the Mutated Sigma-1 Receptor Underlying ALS16**

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Background:
Amyotrophic lateral sclerosis (ALS) is an adult-onset neurodegenerative disease characterized by the selective degeneration of upper and lower motor neurons (MNs). While an array of triggers exists for ALS, they all converge on one of two main pathological mechanisms, RNA metabolism or proteostasis. One form of familial ALS (ALS 16) arises from a point mutation (E102Q) in the ligand-binding domain of an ER chaperone protein, the sigma-1 receptor (Sig-1R). In addition, a splice site mutation leading to a partial deletion of the cytoplasmic loop of the Sig-1R leads to distal hereditary motor neuropathy (dHMN), suggesting that the Sig-1R may play a role in the etiology of motor neuron disease.
As the Sig-1R is a protein chaperone, it is thought to modulate proteostasis during endoplasmic reticulum (ER) stress by modulating the Unfolded Protein Response (UPR), which is upregulated in ALS and could contribute to MN degeneration. The Sig-1R is also known to modulate voltage-gated K+ channels, which could contribute to the increased excitability of MNs observed in ALS.

Objective:
To investigate the subcellular dynamics and function of wild-type (WT) and mutant Sig-1Rs underlying motor neuron disease.

Methods:
We examined the subcellular localization, mobility and function of WT and Sig-1R mutants using confocal imaging, semi-quantitative RT-PCR and electrophysiological techniques.

Results:
Induction of ER stress, by either DTT or Tunicamycin, resulted in an increased mobility of WT Sig-1R, and sequestration into

immobile puncta. Co-localization analysis suggests that these puncta are ER quality control compartments. In contrast, the E102Q mutant has a significantly higher mobility and tendency to cluster in the absence of ER stress. Activation of wild-type Sig-1R by the agonist SKF-10,047 attenuated the expression of apoptotic markers and promoted recovery from DTT-induced ER stress in NSC-34 cells and MEFs derived from WT mice. This was not observed in NSC-34 overexpressing E102Q or MEFs from Sig-1R-/- mice. In addition, the Sig-1R mutants drastically differ in the modulation of inward rectifier K+ channels (Kir 2.1), which are known to regulate neuronal excitability.

Conclusion:
Aberrant subcellular dynamics of the Sig-1R mutant proteins leads to dysregulation of adaptive mechanisms of the UPR during ER stress and differential modulation of K+ channels. These mechanisms may contribute to MN death in ALS.

90 **Complementing functional and structural evidence to understand the roles of serine and threonine residues in first intracellular loop and transmembrane 2 for human dopamine D1 receptor**

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For many G protein-coupled receptors (GPCRs), the role of their first intracellular loop (IL1) and its transition into transmembrane (TM) domains are very much unknown. Notably, this region harbours conserved Ser and Thr residues that make recurrent molecular contacts in crystallized GPCR structures. To investigate their functions, we have designed human dopamine D1 receptor (hD1R) mutants whereby all or individual Ser and Thr spanning IL1 and IL1/TM2 juncture were replaced by Ala and Val, respectively. Compared to hD1R, the triple mutant hD1-ST1 demonstrated diminished dopamine affinity, abolished constitutive activity, and reduced dopamine potency by 260-fold—pharmacological changes that were recapitulated in the hD1-T59V mutant receptor. Such warranted loss in G protein coupling by hD1-ST1 and hD1-T59V can be explained by the crystallized ß2-adrenoceptor-Gs complex, in which Thr2.39 (Ballesteros-Weinstein nomenclature) interacts with Tyr3.60 to allow the proper insertion of Phe3.58 into a hydrophobic pocket of Gs. Interestingly, despite drastically losing dopamine potency, the hD1-ST1 demonstrated over 2-fold elevation of dopamine-mediated maximal stimulation of adenylyl cyclase (Emax) that was recapitulated in the hD1-S65A mutant receptor. Predicting from solved GPCR structures, further mutagenesis and functional studies verified the elevated Emax of hD1-S65A was mimicked by breaking the molecular interactions of Ser65 with either Asn113 or Trp148. Additionally, we demonstrate the elevated Emax of hD1-S65A was not due to higher cell surface expression or an impaired ability to desensitize in the presence of agonist. Rather, ELISA assays report that hD1-ST1 and hD1-S65A exhibited an impaired ability to undergo agonist-mediated internalization. Taken together, our work highlights the functional and structural importance of Thr59 and Ser65, which may constitute as unrecognized molecular motifs critical for GPCR signalling in general.

Regenerative Medicine Program

91 **Demethylation of Myf5 DNA marks the transition from satellite stem to progenitor cell.**

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Background: Myogenic satellite stem cells, which have never expressed the myogenic transcription factor Myf5, functionally engraft after transplantation, are able to repopulate the muscle stem cell niche and extensively replicate and contribute to myogenesis. Committed myogenic satellite cells, which have expressed Myf5, cannot contribute to long-term myogenesis after transplant. Myf5 expression is under the control of Pax7, which is expressed in all satellite cells however, it is unclear why Pax7 does not activate Myf5 expression in satellite stem cells. DNA methylation blocks gene expression and can be actively removed via Tet hydroxylases. Myf5 DNA is methylated early in development but this methylation is lost during myogenesis suggesting that DNA methylation at Myf5 may be a demarcation between satellite stem and progenitor cells. Wnt signaling via Wnt7a/Fzd7 has an inhibitory effect on activation of Myf5 expression although the specific mechanism of action is unclear.

Objective: This work aims to understand the transition form satellite stem cells to myogenic satellite cells and how Wnt signaling inhibits this transition.

Methods: Methyl-DNA immunoprecipitation was used to determine changes in DNA methylation at the Mfy5 gene. Cultured mouse derived MEF and myoblasts were used for initial analysis of Myf5 DNA methylation. To determine the precise stage during differentiation when methylation at Myf5 is lost, satellite stem and myogenic cell populations were isolated from mice via FACS using a Myf5 lineage tracing knock-in (Myf5-Cre / Rosa YFP). Cultured cells, myoblasts and myofibers were used to examine specific determinants influencing activation of Myf5 expression.

Results: Myf5 DNA is methylated in MEF and all other non-myogenic lineages examined, but is not methylated in myoblasts. Myf5 DNA methylation was also found in satellite stem cells but not in myogenic satellite cells. Methylation at Myf5 was not found after Myf5 expression stops: when myoblasts were differentiated or when Pax7 expression was lost via conditional knockout. Knockdown of Tet3 was found to prevent activation of Myf5 expression. Tet3 was also found to bind Dvl3, part of the Wnt signaling pathway which acts as a cytoplasmic-nuclear shuttle. Wnt7a treatment was found to result in loss of Dvl3 protein and differences in Dvl3 expression were seen between satellite stem and myogenic cells.

Conclusion: Methylation at Myf5 DNA was found to be a unique stem cell characteristic which is lost during the transition from stem to progenitor cell. Wnt7a/Dvl3 may regulate Tet3 based demethylation of Myf5 DNA.

92 **Shh signaling controls the lineage choice of the satellite stem cells.**

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Background: The regenerative capacity of skeletal muscle relies on a subpopulation of muscle stem cells, termed satellite stem cells. Tracking the activation of Myf5 with Myf5-Cre:ROSA26-YFP mice revealed that YFP-negative satellite stem cells can perform symmetric cell divisions, which give rise to two identical daughter cells allowing self-renewal and satellite cell pool expansion, or asymmetric divisions, which generate one self-renewing stem cell and one YFP-positive committed cell that express Myf5 and will progress through the myogenic lineage. However, it remained unknown whether a satellite stem cell could give rise directly to a committed cell without expressing Myf5.
Objectives: We propose to investigate the differentiation potential of satellite stem cells.
Methods: Myf5-Cre:ROSA-YFP mice are used to sort both YFP-negative and YFP-positive satellite cells and compare their molecular profiles and phenotypes during proliferation and differentiation in vitro. EDL myofibers are also isolated to analyze satellite cell divisions and differentiation.
Results: By immunostaining on cultured myofibers, we discovered that YFP-negative satellite stem cells can generate committed cells that remain YFP-negative and express MyoD and myogenin. Moreover, freshly sorted YFP-negative cells are able to differentiate in vitro. Cellular and molecular characterization of the differentiation potential will be presented. Our preliminary data support the hypothesis that multiple myogenic lineages coexist. Expression analysis by RNA-sequencing revealed that Sonic hedgehog (Shh) signaling genes are differentially expressed in YFP-negative stem cells and YFP-positive committed cells. Shh is a key regulator of myogenesis since the signaling pathway triggered by Shh leads to the activation of Gli transcription factors and expression of Myf5. We showed that activation of Shh signaling enhances asymmetric satellite cell divisions while its inhibition leads to satellite stem cell expansion.

Conclusion: We proposed that Shh signaling pathway could regulate the satellite stem cell fate decision to give rise to two distinct types of committed cells.

93 **Regulation of satellite stem cell self-renewal by p38-gamma MAP kinase**

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Background
During skeletal muscle regeneration, resident satellite cells activate and give rise to myogenic progenitors that rebuild muscle tissue. Concurrently, satellite stem cells maintain the satellite cell pool through their ability to undergo self-renewal via both symmetric and asymmetric cell divisions. The intrinsic molecular mechanisms that underlie these cellular fate decisions, however, have remained elusive. The transcription factor Pax7 is required for specification of satellite cells and controls their entry into the myogenic program through its ability to target the myogenic regulatory factor Myf5. Transcription of Myf5 serves as an indicator of myogenic commitment and is regulated during asymmetric satellite stem cell division by the methyltransferase Carm1.

Objectives
Activation of Carm1 represents a critical step during asymmetric cell division of satellite stem cells. To identify regulators of satellite stem cell fate determination, we set out to identify regulatory kinases of Carm1.

Methods
Employing a candidate kinase approach, we identified p38-gamma as a Carm1 regulatory kinase. To study the role of p38-gamma in satellite cells, we used siRNA to effectively knock-down p38-gamma protein in satellite cells cultured on single myofibers and performed muscle regeneration studies in mice with satellite cell-specific deletion of p38-gamma.

Results
Depletion of p38-gamma in satellite cells revealed a preference for asymmetric cell division and inhibited symmetric satellite stem cell divisions. Moreover, knock-down of p38-gamma abrogated satellite cell engraftment potential. Along these lines, inactivation of p38-gamma specifically in satellite cells in vivo resulted in a significant reduction in satellite cell number and impaired muscle regeneration following muscle injury.

Conclusion
In contrast to Carm1, which is necessary for asymmetric cell division, p38-gamma is required for satellite stem cell self-renewal via symmetric expansion. Ultimately, insight into the molecular pathways that regulate satellite stem cell self-renewal is essential for advancement of therapeutic strategies to treat muscle degeneration.

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

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Skeletal muscle regeneration is mediated by a small population of self-renewing satellite stem cells. This self-renewal is facilitated by both symmetric and asymmetric expansion of Pax7+Myf5- satellite stem cells. During asymmetric expansion, the stem cell pool is maintained while Pax7+Myf5+ committed progenitors are simultaneously produced to continue through the myogenic lineage, facilitating skeletal muscle regeneration. The stem cell pool is also maintained during symmetric expansion of satellite stem cells when two Pax7+Myf5- daughter cells are produced. We have developed an image based high content screen of satellite cells using flexor digitorum brevis (FDB) fibers to facilitate the identification of chemical targets that s (Symmetric vs. Asymmetric). This is a versatile platform, which may be used to screen compounds, validate findings, and establish dose-response curves.

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

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Background: Hutchinson-Gilford Progeria Syndrome (HGPS) is a rare accelerated aging disorder in which affected children develop deficits associated with aging and succumb to vascular complications in their teen years due to deterioration of vascular smooth muscle cells (VSMCs). HGPS is caused by a mutation in LMNA gene that activates a cryptic splice site, leading to the accumulation

of mutant protein Progerin in the nuclear lamina. Although mechanistic studies have not uncovered the HGPS disease process in HGPS VSMCs, evidences have shown that HGPS fibroblasts exhibit increased DNA damage and global disruptions to epigenetic marks as well as changes in gene expression. Furthermore, we and others have found that Progeria cells express high levels of reactive oxygen species (ROS), which is similar to increased ROS in aging vasculature and could be an underlying cause for replicative stress in HGPS VSMCs.

Hypothesis: We hypothesize that high ROS in aged HGPS VSMCs could be driving replicative stress and causing defects in epigenetic inheritance.

Methods: To test this hypothesis, we have generated HGPS patient-specific iPSCs and differentiated them into VSMCs to model mechanisms driving epigenetic inheritance in these cells in vitro.

Results: We found that HGPS VSMCs exhibit elevated levels of ROS and DNA damage during replication. We utilized techniques such as iPOND and Proximity Ligation Assay and found that DNA damage accumulated at sites of nascent DNA at late passages, suggesting that HGPS VSMCs are undergoing replicative stress.

Conclusions: In general, we hope to learn more about aging using HGPS model by assessing molecular role of ROS and its link to epigenetics, and potentially uncover novel drug targets to treat the aging population.

96 **Elucidation of Wnt7a mechanism of action during muscle regeneration**

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Background
Duchenne Muscular Dystrophy (DMD) is a devastating genetic muscular disorder of childhood manifested by progressive muscle weakness and wasting, and ultimately death. In mice, a small subset of satellite cells have never expressed Myf5 and are considered as satellite stem cells. Satellite stem cells can undergo either symmetric divisions to give rise to two stem cells or alternatively undergo an asymmetric division to give rise to a stem cell and a committed cell. Previous work in the Rudnicki group has demonstrated that Wnt7a treatment stimulates the regeneration of muscle by acting at multiple levels. Indeed, Wnt7a drives the symmetric expansion of the satellite stem cells through the planar cell polarity (PCP) pathway. Wnt7a also stimulate myofibers hypertrophy and markedly stimulates regeneration in mdx mice, a mouse model for DMD.

Objective
The overarching goal of the project is to elucidate the mechanism of action of Wnt7a at the molecular level and identify downstream target gene in stimulating the function of wild-type (wt) and dystrophin-deficient (mdx) satellite cells.

Methods
Satellite cells on myofibers will be isolated from the EDL muscles of wt or mdx mice and exposed to 100ng/mL Wnt7a for 36, 42h or 72h in order to assess respectively the regulation of the centrosome, the proportion of asymmetric vs symmetric divisions and the progression of the myogenic program. We will identify the protein complexes induced by Wnt7a by proximity ligation assay, Co-IP and Western-Blot.

Results
We have found that Wnt7a strongly induces the interaction between NuMA1 and Dvl-2 in satellite cells after 42h of culture. Interestingly, NuMA1 is a key effector of PCP regulation of centrosome positioning in both Drosophila and Zebrafish and is required for PCP-specification of spindle orientation. In addition, Dvl-2 is interacting with p38 gamma MAPK and Wnt7a treatment induces the phosphorylation of Dvl-2. Further experiments are needed to decipher the chronology of all of these molecular events and to understand how the signaling cascade induced by Wnt7a will modify the satellite stem cell fate.

Conclusion
These experiments will advance our knowledge of Wnt7a signaling in muscle and illuminate the therapeutic potential of Wnt7a as a protein biologic to stimulate intrinsic repair in a muscle-wasting disease like DMD.

97 **Altered Repair Potential And Gene Expression Profile Of CD146+ Endogenous Lung Mesenchymal Stromal Cells in Experimental Bronchopulmonary Dysplasia**

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Background: Bronchopulmonary dysplasia (BPD), a common adverse outcome of extreme preterm birth, can be caused by oxygen-related lung injury and is characterized by arrested alveolar development. Given the regenerative potential of exogenous (bone marrow-/cord-derived) MSCs, it is unclear why resident lung MSCs (L-MSCs) do not support lung repair and growth in BPD.

Objective: To determine whether hyperoxia perturbs gene expression in CD146+ endogenous lung MSCs in oxygen-induced BPD in neonatal rats.

Methods: Rat pups were exposed to 21% or 95% oxygen from postnatal day 0 to 10 and sacrificed on day 12. CD146+ L-MSCs were isolated by enzymatic digestion, Ficoll-purification and magnetic bead mixed lymphocyte reaction assay.

Results: Hyperoxia exposure decreased CD73 expression in CD146+ L-MSCs. Gene expression of the axonal guidance cue pathways increased after in vivo hyperoxia, whereas genes of the JAK/STAT pathway were decreased. CD146+ L-MSCs promoted epithelial wound healing and inhibited T-cell proliferation, regardless of exposure. Angiogenesis potential of endothelial cells was impaired in the presence of hyperoxia CD146+ L-MSC conditioned media.

Conclusions: In vivo hyperoxia exposure in L-MSCs lowered CD73 and JAK/STAT expression, indicating decreased immune function, promoted inhibition of angiogenesis, and changed the gene expression of critical alveolar development pathways. These changes in L-MSCs likely reflect their role in BPD pathogenesis, and must be further explored to optimize exogenous MSC-derived therapies.

98 **Investigation of Oligodendrocyte Differentiation in the Inhibitory Multiple Sclerosis Lesion Microenvironment In Vitro**

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Background
In multiple sclerosis (MS), oligodendrocyte precursor cells (OPCs) migrate to lesion sites to repair damaged myelin. Throughout MS disease progression, the ability to repair such damage diminishes considerably. This reduced capacity to regenerate is thought to be a consequence of lesion-associated inhibitory factors (LAIFs) that perturb OPC maturation into myelinating oligodendrocytes (OLs). These LAIFs include, but are not limited to, chondroitin sulfate proteoglycans (CSPGs) and myelin debris.

Objective
The current study aims to further characterize the OL response to CSPG exposure, as well as explore the molecular pathways involved in CSPG-mediated inhibition of OL differentiation and myelination.

Results
We have validated the impact of CSPGs on OL maturation using a primary mouse OL culture system. Exposure to CSPGs impaired the ability of the OL to extend processes, and also negatively impacted the size of the myelin sheet produced in culture. Additionally, this work explores the potential involvement of glycogen synthase kinase 3ß (GSK-3ß) signaling in mediating the inhibitory effects of CSPG exposure on OL morphological differentiation. Pharmacological manipulation of GSK-3ß signaling does not appear to rectify the morphological impairments seen in primary OLs on CSPGs.

Conclusions
Results of this study indicate that CSPG-mediated inhibition of OL growth is not primarily dependent on GSK-3ß signaling. Further investigation of the mechanism underpinning the CSPG-OL interaction will provide a better understanding of how the lesion microenvironment contributes to MS pathophysiology, and elucidate avenues to promote remyelination within the inhibitory milieu.

99 **Expression of p14 Fusion Associated Small Transmembrane Protein in an Oncolytic Adenovirus for Improved Vector Efficiency**

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Background
Adenovirus (Ad) vectors are the most commonly used delivery system in gene therapy applications. The ability of Ad to efficiently infect many different cell types and relatively large cloning capacity, without integration into the host genome makes them an excellent candidate for anti-cancer gene therapies. One of the main drawbacks of Ad viral vectors is their relatively poor ability to spread through a tumor mass following intratumoral injection. A particular class of fusion-associated small transmembrane (FAST) proteins have been investigated for their potential anti-cancer properties. Over-expression of these proteins in the Ad viral vector system could increase virus spread in the tumor through cell-cell fusion, as well as amplify virus oncolytic activity.

Objective
1-Produce therapeutic amounts of Ad virus expressing the FAST protein in the late transcription region of the Ad genome regulated by the major late promoter and a splice acceptor.
2-Further examine the potential for FAST-expressing Ad as an oncolytic agent in tissue culture and murine models of cancer.

Methods
The growth of AdFAST in three human cell lines (A549, 293, HeLa) was compared to achieve highest possible viral titer. To validate the AdFAST gene construct, an Ad with and without the MLP splice acceptor and a downstream red fluorescent protein (RFP) will be grown in tissue culture. RFP intensity will be quantified to determine the extent of protein product increase in the splice acceptor construct.

Results
AdFAST failed to grow well in 293 cells likely due to rampant cell fusion interfering with cell viability. The titer of virus recovered from A549 and HeLa was improved relative to 293 cells. The use of the splice acceptor construct is currently being validated with the RFP Ad.

Conclusions
Low yield of AdFAST is likely the result of FAST expression decreasing the cell viability through fusion-associated cell death and membrane instability before the virus is able to complete its lifecycle. Fusion resistant cell types are being investigated for the growth of the AdFAST virus. A cell line with shRNA for the FAST protein mRNA could also potentially mitigate the detrimental effects of the FAST expression on virus production.

100 **Evidence of a neural crest origin for Lymphangioleiomyomatosis: loss of TSC2 function enhances neural crest differentiation.**

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Background: Lymphangioleiomyomatosis (LAM) is a low grade, but progressive neoplasm of the lung that affects only women. LAM is characterized by the formation of multiple thin walled cysts throughout the lung interstitium, caused by the abnormal proliferation of smooth muscle-like cells (LAM cells). These cysts eventually rupture, leading to parenchymal destruction and eventual respiratory failure. LAM results from inherited or spontaneous, heterozygous germ line mutations in the TSC1 or TSC2 genes, with mutations in TSC2 resulting in more severe symptoms. Loss of functional TSC2 results in the inability to regulate mTOR signalling through the TSC1/2 complex, leading to uncontrolled growth and proliferation of tumour cells. The cell of origin for LAM is unknown; however, the expression of melanocyte-specific markers in LAM tumours suggests that the cell of origin for LAM is the neural crest.

Objective: Use the pluripotent nature of human embryonic stem cells (hESCs) and CRISPR/Cas9 genome editing to specifically investigate the role of TSC2 signaling in a candidate cell of origin for LAM, the neural crest.

Methods: H1 (male) and H9 (female) TSC2 mutant hESC lines were generated using CRISPR/Cas9. These cells were differentiated in vitro through the neural crest fate to smooth muscle cells. Differentiation was performed in vivo via intramuscular injection into NOD/scidIL2R?null (NSG) mice. The resulting tumour cells were cultured and serially injected into the lungs of NSG mice.

Results: The TSC2 mutant hESCs no longer express functional TSC2. These TSC2 mutant hESCs can be efficiently differentiated through the neural crest fate into smooth muscle cells. TSC2 mutant hESCs exhibit an increased capacity for neural crest cell generation and display a disorganized migratory phenotype compared to wild-type and heterozygous mutant cells. TSC2 mutant

H9 hESCs differentiated in vivo result in homogeneous and extensively pigmented tumours, which is not observed using TSC2 mutant H1 hESCs. Serial injection of the tumour cells into the lungs of NSG mice results in the engraftment and growth of tumour cells reminiscent of LAM cells.

Conclusions: Enhanced neural crest differentiation observed both in vitro and in vivo demonstrates that TSC2 signalling plays an important role in neural crest cell fate determination. Furthermore, the generation of pigmented tumours from only H9 hESCs suggests a possible role for gender in cell fate decisions upon loss of TSC2 function. Importantly, successful engraftment of LAM-like cells into the lungs of NSG mice is a major step in establishing the first humanized preclinical model for LAM.

101 The Human Pluripotent Stem Cell Facility

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The goal of the the Human Pluripotent Stem Cell Facility is to provide resources that otherwise might not be available to every researcher as well as expertise on how to work with pluripotent stem cells

Services available: iPSC and ESC training
Reprogramming of fibroblasts
Differentiation into multiple cell types
Characterization and analysis
Teratoma assays
Genome Editing

102 Characterizing synaptic alterations within the central nervous system of a mouse model of Spinal Muscular Atrophy

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Background
Neuromuscular junctions are the primary sites of pathology in Spinal Muscular Atrophy (SMA). However, central synapses might also be affected in this disease. For example, it is known that SMN is highly expressed in the mouse CNS during development and SMN depletion leads to cell death and pathological foci in the mouse telencephalon during this period. There are also reports of impairment of spinal circuits in SMA mouse models.

Objective
Using the Smn2B/- mouse model of SMA, here we are investigating the synaptic alterations in the mouse CNS. Specifically, we are preparing synaptosomes from cortices and spinal cords to assess proteomic changes upon SMN depletion.

Methods
Smn2B/- mice were sacrificed at P11 (pre-symptomatic) and P16 (early symptomatic), and their cortices and spinal cords were dissected immediately. Enriched synaptosomal fractions were prepared by ultra-centrifugation of crude synaptosome preparations on the top of non-continuous Percoll gradients. In the first phase of this study, a biased approach was used to investigate the alterations of specific synaptic proteins in Smn2B/- mice.

Results
Interestingly at P11, the level of some synaptic markers (including Rab3, amphiphysin and PSD95) was ed in spinal cord synaptosomes. Cortical synaptosome preparations from Smn2B/- mice also showed minimal to moderate changes in some of the synaptic markers at both P11 and P16. We also assessed some cellular signaling proteins within synaptosome preparations at P11 and P16 Smn2B/- mice and found some alterations in the level of synapsin, ERK1/2 and eIF2a within spinal cord synaptosome preparations. Currently, we are looking at morphology and organization of neuronal processes, density of dendritic spines and also distribution of synaptic vesicles within mouse cortex and spinal cords using Golgi-Cox and immunofluorescent staining.

Conclusions
We conclude that the central synapses of Smn2B/- mice might undergo pathologic changes before the onset of disease. In the next phase of the study, we will use an unbiased proteomic approach to determine the phospho/proteomic profile of synaptosomal fractions prepared from Smn2B/- mouse spinal cord and cortex. The results will be analyzed to identify signaling pathways affected in Smn2B/- mice. By studying early affected signaling pathways in Smn2B/- mice, we will be able to identify

novel targets for treatment of SMA.

103 Molecular regulation of satellite cell fate switching

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Background:
The prevalence of obesity and its related disorders presents a growing obstacle for the medical community worldwide. Where white adipose is the main reservoir for excess energy, brown adipose has the ability to convert excess energy to heat, and in turn poses a promising therapy for obesity. Brown adipose and muscle are derived from a common progenitor and adult muscle stem cells (satellite cells) remain permissive to brown adipogenic signaling. A key switch involved in the myogenic commitment of satellite cells involves the muscle enriched microRNA-133 (miR-133), that blocks brown adipogenic signaling. Antagonizing miR-133 during muscle regeneration leads to de novo brown adipocyte generation, promotes energy expenditure and impedes diet-induced obesity. Screening regulators of miR-133 uncovered the tumour suppressor p53 as a potential regulator of miR-133.

Objective:
To study how p53 affects fate choices in satellite cells and identify pathways involved in promoting brown adipogenesis.

Results:
Using a luciferase based assay to screen small molecule inhibitors of miR-133, we uncovered the involvement of p53 in lineage reinforcement within satellite cells. We found Pifithrin-a (a p53 transactivation inhibitor) to be a potent inhibitor of miR-133 expression in mouse and human myoblasts and to markedly stimulate brown adipose determination in C2C12 myoblasts and satellite cells. We characterized the effects of satellite cell-specific p53 genetic depletion on induction of brown adipocytes where we discovered satellite cells lacking p53 result in precocious brown adipose formation within regenerating skeletal muscles. Transient inhibition of p53 in regenerating fibers through Pifithrin-a likewise results in brown adipose formation as well as an increase in mitochondrial biogenesis. Mechanistically, we uncovered that p53 inhibition leads to a deficit in miR-133 processing suggesting that p53 promotes myogenesis in part through promoting microRNA processing. These results suggest cyclic Pifithrin-a and other transient p53 inhibitors may hold potential as anti-obesity compounds.

Conclusion:
The p53 axis poses a novel pathway regulating satellite cell fate choices through microRNA processing where pharmacological inhibition may pose a potential as a treatment for obesity.

104 Atypical Protein Kinase C in Post-Stroke Neurogenesis

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Background
The histone acetyltransferase, Creb-Binding Protein (CBP), has previously been shown to regulate appropriate differentiation of neural stem cells. Recent work from our lab showed that atypical PKC-mediated CBP phosphorylation at Ser436 is required for adult hippocampal neuronal differentiation and maturation in an age-dependent manner.

Objective
In the current study, we will determine the functional role of the aPKC-CBP pathway in post-stroke neurogenesis and behavioral recovery following focal ischemic stroke.

Methods
To do that, we performed endothelin-1 (ET-1) induced cortical stroke in the CBPS436A knock-in (KI) mouse strain, where the aPKC-CBP pathway is functionally deleted by replacing Ser436residue with Alanine (A).

Results
Our preliminary data showed that the percentage of BrdU labelled NeuN positive neurons in the injured cortex was significantly decreased in CBPS436A KI mice as compared to their wildtype littermates 14 days following stroke. Consistent with this, we observed that CBPS436A KI mice displayed impaired migration of DCX positive neuroblasts/immature neurons away from the subventricular zone (SVZ) towards the injured site and reduced number of DCX positive cells in the injured cortex. Interestingly, a population of locally-derived Sox2 positive neural precursors from leptomeninges was observed in the infarct core 3 days after ET-1 stroke. Our preliminary data showed that CBPS436A KI mice displayed a trend of increased number of these locally-derived

neural precursor cells.

Conclusions
These results suggested that the aPKC-CBP pathway plays an important role in mediating post-stroke neurogenesis, and may be a viable target to harness endogenous repair mechanism.

105 **ROLE OF FRIZZLED-7/ SYNDECAN-4 RECEPTOR COMPLEX IN DUCHENNE MUSCULAR DYSTROPHY**

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Background: Satellite Cells (SCs), the myogenic progenitors responsible for skeletal muscle homeostasis, reside in specialized niches supporting many aspects of stem cell identity. Interactions between SCs and their environment through Extracellular Matrix (ECM) proteins are crucial for regulating SCs niche. Recently our group discovered that Syndecan-4 (Sdc-4) and Frizzled-7 (Fzd7) form a co-receptor complex in SCs and the binding of the ECM glycoprotein Fibronectin (FN) to Sdc4 stimulates the ability of Wnt7a, Fzd7 ligand, to induce the symmetric expansion of stem SCs.

Objective: Study the synergy of the receptor complex Sdc4-Fzd7 in SCs and primary myoblast in a myopathological context, along the different steps of the regenerative process: Expansion, Migration, Myogenesis and Hypertrophy.

Methods: Experiments were performed comparing the effect of Wnt7a stimulation versus a combined treatment of Wnt7a plus Retronectin (fragment of FN containing Sdc4 binding site) in SC-derived primary myoblasts and SCs cultured on myofibers from Myf5-Cre:R26R-YFP mdx mice. Effects on SC expansion, myoblast migration, myogenesis, and hypertrophy were analyzed.

Results: We confirmed that the Sdc4-Fzd7 complex is present in mdx primary myoblasts by proximity ligation assay. Co-stimulation with Wnt7a and Retronectin increases migration and myogenesis in primary myoblasts compared with Wnt7a treatment alone. Conversely, while Wnt7a significantly increased hypertrophy of mdx myotubes, there was no synergistic effect after co-treatment with Wnt7a and Retronectin. Co-stimulation of SCs cultured on myofiber cultures resulted in an increase of the mdx stem SC population through symmetric expansion.

Conclusions: Wnt7a augments its beneficial effects synergizing the Sdc4-Fzd7 complex in mdx SCs. Interestingly, the therapeutic impact of Wnt7a is augmented upon co-treatment with a smaller FN fragment just containing the Sdc4 biding site. Currently we are examining the molecular mechanisms that regulate this process providing a rationale for the therapeutic potential of Wnt7a combined with FN to ameliorate muscular dystrophies.

106 **WN1316, a Novel Anti-oxidant Compound for the Treatment of Leber's Hereditary Optic Neuropathy**

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Background: Leber's Hereditary Optic Neuropathy (LHON) is a mitochondrial genetic neurodegenerative disorder causing blindness in the second or third decade of life. Oxidative stress is a main pathway in LHON pathology resulting in specific loss of retinal ganglion cells (RGCs) and their axons that comprise the optic nerve. WN1316 is a small molecular compound with potent anti-oxidant properties.

Hypothesis: WN1316 will be effective in the treatment of LHON because it targets the oxidative stress pathway. This hypothesis will be tested in in vitro and in vivo models of oxidative stress and LHON.

Methods: SH-SY5Y human neuroblastoma-derived cells and 661W mouse retinal photoreceptor-derived cells were used in the in vitro studies. To test the efficacy of WN1316 against cell death induced by menadione and H2O2, SH-SY5Y and 661W cells were pretreated with WN1316 followed by exposure to the oxidative stressors. The potential synergistic effects between WN1316 and the X-linked Inhibitor of Apoptosis (XIAP) were tested in the 661W cell line. Possible toxic effects of WN1316 were also assessed. For the in vivo studies, an LHON mouse model was generated by intravitreal injections of adeno-associated virus (AAV) serotype 2 expressing the mutant form of human mitochondrial ND4 (mND4) gene in one eye. The second eye received AAV-GFP injection as a control. Mice were treated daily for 5 months with 100ug/kg WN1316 by oral gavage. Retinal ganglion cell function was assessed using the Scotopic Threshold Response (STR). Histological studies are underway on retinal cryosections and whole mounts to

assess retinal structure, nerve fibre layer (NFL) thickness and RGC numbers. Axon counts will be conducted on optic nerves sections.

Results: Treatment of SH-SY5Y cells with WN1316 potently suppresses cell death induced by the oxidative-stressor menadione. WN1316 protects SH-SY5Y cells in a dose-dependent manner and is not toxic even at high concentrations. In addition, WN1316 clearly protects 661W cells from both H2O2 and menadione. In the menadione assay, combination therapy with WN1316 and XIAP gave the best neuroprotection. In vivo, STR analysis suggests that WN1316 can significantly protect RGC function. Histological analyses are underway.

Conclusion: Leber's hereditary optic neuropathy (LHON) is a devastating disease without cure to date. WN1316 is a novel small molecular compound with potent anti-oxidant properties. It targets the main pathological cause in LHON. WN1316 shows potent neuroprotective properties in our in vitro studies, and we believe that it will be equally effective in vivo in the treatment of LHON.

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107 **Stable Revascularization of Lung Scaffold Enhanced by Combined Delivery of Induced Pluripotent Stem Cell Derived Endothelial Cells and Smooth Muscle Cells**

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Background: Bioartificial lungs represent a novel alternative for organ transplantation; however, inadequate revascularization leading to thrombosis-induced organ failure has limited in vivo function and survival of recellularized lung scaffolds.

Methods: In this study, endothelial (ECs), derived either from induced pluripotent stem cell (iPSC-ECs) or GFP-labelled HUVECs, were co-cultured with smooth muscle cells (iPSC-SMCs) to improve network formation as assessed using a Matrigel assay. iPSC derived ECs and SMCs were initially characterized using flow cytometric analysis and immunostaining respectively.

Results: iPSC-ECs were found by flow cytometric analysis to demonstrated immunophenotype characteristic of ECs as evident by high expression of CD31,CD34, CD144 and VEGFR. Similarly iPSC-SMCs stained positive for SMC markers Calponin and alpha-SMA whilst being negative for pluripotency markers Nanog and Tra-1-60. Both red fluorescent CellMask stained iPSC-SMCs and native pulmonary artery derived SMCs (PASMC) aligned spontaneously with ECs to form networks that recapitulated ontogeny of endogenous vessels formation. Networks formed by HUVECs alone were transient, degenerating by 48hrs (branching length (BL) 13.3+0.06 vs. 9.0+0.07 ?m, p=0.02; and nodes: 37+6.5 vs 17+1, p=0.22, at 24 and 48h, respectively). Co-culture of HUVEC with iPSC-SMC in a 3:1 ratio resulted in a marked increase in the durability of in vitro vascular networks (BL 17.5+0.45 vs 14.4+1.2, p=0.15 and nodes 57+2.2 vs 38+2.5, p=0.007; 24 and 48h, respectively). Interestingly, the synergistic effect demonstrated by co-culture of iPSC-SMCs with ECs in terms of network persistence, number of nodes formed and total branching length was not observed upon co-culture with PASMC. The effect of co-culture on EC expression of selected endothelial, angiogenic and matrix related genes was assessed after MACS separation at different time-points (24-72h). IPSC-SMCs induced a gene expression profile consistent with vascular stabilization with progressive increases in TIE2, KLF2 and CD34, collagen 1 and laminin-a1, as well as increase in ratio of Angpt1:Angpt2. This gene profile was similar when ECs were co-cultured with PASMCs. Intriguingly, increase in expression of selected endothelial genes such as eNOS, TIE2 and KLF2 was more pronounced in ECs co-cultured with iPSC-SMCs compared to PASMCs. Subsequently, decellularized rat lung scaffolds were seeded with iPSC derived ECs and SMCs at a 3:1 ratio. H&E staining demonstrated a high density of cell engraftment including structures resembling mature blood vessels as early as three days post-seeding.

Conclusion: iPSC-SMCs improved the stability of vascular network formation potentially through the upregulation of endothelial and matrix-related genes as well as providing structural support.

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108 **Differentiation of Human Induced Pluripotent Stem Cells into Definitive Endoderm for Re-cellularization of Lung Scaffolds**

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Background: The increasing prevalence of serious respiratory diseases in Canada outmatches the availability of donor-compatible lung transplantations and chronic rejection limits lung graft survival which is among the worst of all organs. Induced pluripotent stem cells (iPSCs) have been a major advance in stem cell biology, heralding a new era in regenerative medicine. Specifically, harnessing their differentiation potential for patient-specific tissue repair and regeneration makes them attractive candidates to be exploited for clinical therapies.

Objective: We aim to generate de novo lung tissue, in particular lung epithelium and other airway components, by retracing the

pattern of lung embryogenesis and recapitulate definitive endoderm (DE) in vitro.

Method: Human iPSCs were cultured onto matrigel-coated wells as individual colonies prior to DE induction. Results: Cells displaying a flattened and cuboidal morphology were observed to egress from the peripheral edges, forming a uniform monolayer by day 2 post-differentiation. Quantitative reverse transcription - polymerase chain reaction (qRT-PCR) was performed to verify the identity of the differentiated cells. Our results indicate that the hallmarks of pluripotency were significantly downregulated by approximately 10% for both OCT4 and NANOG, and 70% for SOX2 gene expression. Conversely, positive endodermal markers (CXCR4, SOX17, FOXA2) were concomitantly augmented significantly by at least 4-fold, 50-fold, and 950-fold, respectively.

Conclusion: Morphological and gene expression changes are consistent with successful transition from a pluripotent state to committed DE. In future, we will determine if DE or their downstream derivatives are able to repopulate a de-cellularized lung matrix and establish the various cell populations residing in adult lung.

109 ATRX prevents degradation of stalled replication forks to facilitate progenitor cell expansion and upper layer neuron production

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Background
Expansive growth of neural progenitor cells (NPCs) is a prerequisite to the temporal waves of neuronal differentiation that generate the six-layered neocortex. NPC expansion places a heavy burden on proteins that regulate chromatin packaging and genome integrity, which is further reflected by the growing number of developmental disorders caused by mutations in chromatin regulators. Accordingly, mutations in ATRX, a chromatin remodelling protein required for heterochromatin maintenance at telomeres and simple repeats, cause the ATR-X syndrome.

Results
Here, we demonstrate that ATRX-null cells are sensitive to hydroxyurea-induced replication stress and accumulate DNA damage that compromises the generation of upper layer neurons. Specifically, reduced RAD51/BRCA1 foci and PARP1 hyperactivation indicated that stalled forks are not efficiently protected. DNA fibre assays confirmed that ATRX was required for protection while the MRE11 inhibitor mirin could prevent degradation.

Conclusion
Thus, ATRX is required to limit replication stress during NPC expansion to facilitate late-born neuron production.

110 Non-cell autonomous Atrx activity promotes retinal neuroprotection

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

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

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

Results: Amacrine and horizontal cell loss occurs when Atrx is deleted in multipotent progenitor cells early in retinal development, but not upon Atrx gene inactivation in lineage-restricted, post-mitotic amacrine and horizontal precursor cells. Selective 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 survival of the synaptically connected retinal inhibitory interneurons. Further analysis reveals misexpression of bipolar cell subtype-specific proteins and alterations in neuronal morphology that may underlie defects in retinal synaptic communication. Gene expression changes reflect an excitotoxic environment that may be contributing to the cell death. Transgenic mice harbouring a mutation mimicking ATR-X syndrome patients exhibit phenotypic features similar to the conditional knockout mice, suggesting common mechanisms of visual dysfunction.

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

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111 **Neural Progenitor Cell (NPC) Function Is Perturbed in Bronchopulmonary Dysplasia (BPD) Model**

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Background: BPD, a chronic lung disease, is the most common complication of prematurity. The disease results from the oxygen therapy and ventilator support given to extremely premature infants for acute respiratory failure. BPD is an independent risk factor for adverse neurodevelopmental outcomes. The cellular mechanisms leading to brain injury remain unclear.

We hypothesized that in experimental BPD, NPC self-renewal and differentiation are impaired.

Objective: To determine whether hyperoxia exposure in mice impairs NPC function.

Methods: Newborn mice were exposed to room air (21% oxygen) or 80% oxygen (BPD model) for 10 days. NPCs were harvested from the subventricular zone (SVZ) and hippocampus. Neurosphere assays were used to assess self-renewal and compare neurosphere size. Spontaneous differentiation was examined through morphological observation and immunocytochemistry.

Results: SVZ NPCs from BPD mice formed considerably fewer primary and secondary neurospheres than those isolated from normoxia controls, demonstrating a marked decrease in self-renewal ability. NPCs from the hippocampus showed no difference between groups in terms of self-renewal. During the first passage, NPCs isolated from BPD mice, from both brain regions, formed larger neurospheres than NPCs isolated from controls. By the second passage, hyperoxia NPCs from both brain regions demonstrated greater spontaneous differentiation than normoxia NPCs.

Conclusions: Decreased self-renewal capacity and increased spontaneous differentiation demonstrate impairments in the functional capabilities of hyperoxia-exposed NPCs. These results provide insight into the possible mechanism of BPD-associated brain damage. This study will aid in the development of future treatments to protect the brains of infants with BPD.

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112 **Investigating Intrinsic Defects of Skeletal Muscle Regeneration in a Mouse Model of Spinal Muscular Atrophy**

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Background: Accumulating evidence indicates that abnormalities in muscles can occur independent of motor neuron deterioration in Spinal Muscular Atrophy (SMA). Muscle weakness occurs prior to detectable deficits in neuromuscular integrity, implying muscle intrinsic defects other than from neurogenic atrophy. Our group and others have reported mis-regulated myogenic gene expression in pre-symptomatic stages of multiple SMA mouse models and in SMA patients, strongly suggesting a defect in myogenesis and muscle repair in SMA. Here, we aim to explore muscle satellite cell function and muscle regeneration in SMA.

Results: Using the Smn2B/- mouse model of SMA, we are assessing the regenerative capacity of muscle satellite cells in vitro and in vivo. We are addressing the following two points: 1. Do Smn2B/- mice have a comparable number of muscle satellite cells and myofibers in representative muscles during the entire disease progression as in wild type controls? We have observed significant reduction in the number of myonuclei per myofiber in extensor digitorum longus (EDL) muscles while the total number of myofibers remains the same as in wild type mice. In order to gain insight into the underlying mechanism for the reduction in myonuclei number, we are extending our analysis to different time points. 2. Does Smn deficiency cause recipients, to assess their regenerative capacity in vivo.

Summary: Our studies will provide a better understanding of muscle satellite cell function and muscle regeneration in the context of SMA.

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113 **Dystonin loss-of-function in oligodendrocytes does not impair migration, differentiation, or myelination**

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Dystonin (also known as BPAG1) is a large cytoskeletal linker protein with both actin- and tubulin-binding domains. Loss of function of this protein results in a sensory neuropathy called Hereditary Sensory and Autonomic Neuropathy type VI (HSAN-VI), modeled in mice with dystonia musculorum (dt). The disease presents with severe joint contractures and a multitude of dysautonomias, which is ultimately fatal. While dystonin loss-of-function primarily leads to sensory neuron degeneration, it has also been shown that peripheral myelination is compromised due to Schwann cell differentiation abnormalities. Interestingly, central nervous system (CNS) neurons appear to be little affected by loss of dystonin, suggesting that they possess a compensatory mechanism not present in sensory neurons. We sought to determine if this dichotomy also exists between Schwann cells and oligodendrocytes (OLs), the CNS myelinating cells. To address this, primary OLs were isolated from a severe dt model (dt27J), and assessed for differentiation capacity as well as maturation marker expression by immunofluorescence. Deficiencies were not found in branching ability, or in the expression of myelin markers myelin-associated glycoprotein (MAG) or myelin basic protein (MBP), in dt27J OLs relative to their wild type littermates. Using a recently developed oligosphere assay, we further analysed the ability of primary oligodendrocyte progenitor cells (OPCs) to migrate and again found no difference between dt27J and wild type. Finally, in vivo analysis of OL myelination was done by electron microscopy on phenotype-stage optic nerve. While some myelin abnormalities were observed in dt27J optic nerve, G-ratio and percent myelinated axon analyses revealed no significant differences when compared to wild type. These data suggest that, like CNS neurons, OLs also possess a compensatory mechanism for overcoming the loss of dystonin, setting them apart from their Schwann cell counterparts in the periphery. Future work will aim to identify the compensatory factor, which may uncover therapeutic strategies for diseases such as HSAN-VI where neuronal and glial cytoskeletal integrity are compromised.

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114 **Effect of genetic background on the phenotype of an intermediate mouse model of spinal muscular atrophy (SMA)**

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Background:
Spinal muscular atrophy (SMA) is the leading genetic cause of mortality in infants. SMA in humans is caused by mutations in Survival Motor Neuron 1 gene (SMN1) and incomplete compensation by the nearly identical SMN2 gene. Several SMA mouse models have been generated which recapitulate different types of SMA. The genetic background of the mice has a substantial effect in the severity of the phenotype. Previously, our lab generated a SMA mouse model by introducing mutations in a splicing enhancer element within exon 7 of mouse Smn, with the new allele being referred to as Smn2B. Smn2B/- pups with a mixed genetic background show a median survival age of 28 days and a considerable fraction of them survive longer.

Objective:
To generate congenic Smn2B/- mice in both FVB and C57BL/6 mouse strains.
We hypothesize that congenic Smn2B/- mice will show a less variable phenotype than Smn2B/- mice with a mixed background.

Methods:

We generated two congenic Smn2B mouse lines by crossing mixed background Smn2B/+ mice with congenic wild typeC57BL/6 or FVB mice and repeated that for ten consequent generations. At the sixth generation (i.e. incipient congenic), Smn2B/2B mice (of C57BL/6 or FVB backgrounds) were mated with congenic Smn+/- of the relevant background. The pups (both Smn2B/+ and Smn2B/-) were used to measure daily weights, survival, hanging time, myofiber size and motor neuron number.

Results:
We found that C57 Smn2B/- pups have a relatively longer median survival than FVB Smn2B/- pups (P25 vs. P19). However, all Smn2B/- pups died before the age of P40. The growth pause in C57 Smn2B/- pups happens slightly later than FVB Smn2B/- pups (P12 vs. P11). Motor function tests are also impaired later in C57 Smn2B/- pups (P17 vs. P15).

Conclusion:
The congenic Smn2B/- pups represent an intermediate SMA phenotype and the phenotype is slightly milder in C57Smn2B/- pups than FVB Smn2B/- pups.

115 **The Ottawa Bioinformatics Core Facility**

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The Ottawa Bioinformatics Core is core facility of the University of Ottawa and the Ottawa Hospital Research Institute. We provide advice on bioinformatics research design, conduct bioinformatics analysis, provide data warehousing services, and provide support for grant proposals that involve bioinformatics (including conducting pilot studies, support/collaboration letters, methodological text, etc.)
Our areas of greatest expertise are:
> High-throughput sequencing,
> Microarrays,
> Genomic sequence/motif analysis
> Integration of multiple data sources
> Network analysis
We have experience with many other types of bioinformatics, and/or can refer you to other local experts, depending on your needs. We can also help to arrange for the analysis of your biological samples with local core facilities such as StemCore, the Proteomics Resource Centre, or the OHRI Mass Spectrometry Core Facility.

116 **TAL1 interferes with the regulatory function of the T-cell regulator BCL11B in T-ALL**

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The bHLH transcription factor TAL1 is a master regulator of hematopoiesis where it is essential for specification and self-renewal of hematopoietic stem cells and differentiation of erythroid and megakaryocytes. In contrast, TAL1 is turned off early in T-cell progenitors and the aberrant expression of TAL1 leads to development of T-cell acute lymphoblastic leukemia (T-ALL). It has been previously shown that the leukemic function of TAL1 entails inhibition of both T-cell differentiation and apoptosis. However, the molecular mechanism(s) through which TAL1 induces and maintains a leukemic phenotype is not entirely clear. While TAL1 has been shown to directly regulate the expression of genes that control proliferation, apoptosis and differentiation, other non-mutually exclusive models have been proposed to explain the oncogenic role of TAL1, including TAL1-mediated interference with critical T-cell regulators.

To better understand the oncogenic role of TAL1, we performed a proteomic screen for TAL1-interacting proteins in the Jurkat T-ALL cell line. This approach identified the transcription factor BCL11B, a major T-cell regulator necessary for lineage commitment, survival and differentiation of early T-cell progenitors, as a TAL1 interacting partner. The TAL1-BCL11B interaction was then confirmed by reciprocal immunoprecipitation and by in vitro interaction assays with purified proteins. To determine whether TAL1 and BCL11B interact at gene regulatory regions, we analyzed TAL1 and BCL11B binding genome-wide using ChIP-sequencing in patient-derived T-ALL primary blasts. Gene ontology analysis of BCL11B target genes identified enriched categories related to T-cell differentiation and apoptosis, which is consistent with the role of BCL11B as a major regulator of the T-cell lineage. Strikingly, over 60% of BCL11B target sites are also bound by TAL1, suggesting that each factor influences the function of the other. To gain further insight into this process, we analyzed changes in gene expression profiles by RNA-sequencing upon knockdown of TAL1 or BCL11B. This experiment revealed that while BCL11B regulates some of its target genes independently, TAL1 interferes with the expression of specific subsets of BCL11B targets. The finding that TAL1 attenuates (but does not completely eliminates) the transcriptional regulatory function of BCL11B is consistent with previous studies showing that BCL11B is a haploinsufficient tumor

suppressor due to heterozygous inactivating mutations and deletions occurring in a subset of T-ALLs. Furthermore, it suggests that in T-ALL, a decreased activity of BCL11B is sufficient to permit cell survival but not to promote differentiation,highlighting an important contribution of the transcriptional interference model in the overall mechanism of TAL1-mediated leukemogenesis.

117 Delineating the caspase-dependent targets and signal pathways that promote pathological cardiac hypertrophy

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Background: Cardiac hypertrophy occurs when the heart size increases to maintain cardiac output at times of stress. Interestingly, this pathological process is characterized by cell behaviours which are typically associated with apoptosis. We previously demonstrated the essential role of the intrinsic cell death pathway during cardiac hypertrophy; however, the caspase-dependent pathways and cleavage targets remain elusive. It is proposed that cardiac hypertrophy is mediated by the activation of caspase-dependent pathways which activate hypertrophic transcription/gene expression programs and induce cytoskeletal remodelling which originate in part by caspase-mediated cleavage. Here, the myocyte enhancer factor 2 (MEF2) transcription factor inhibitor histone deacetylase 3 (HDAC3) and gelsolin (GSN), an actin-binding protein, were evaluated as potential caspase cleavage substrates.

Objective: The importance of substrate caspase-mediated cleavage in the induction and/or maintenance of cardiomyocyte hypertrophy was investigated.

Methods: In vitro cleavage assays were completed with effector caspase 3/7 and HDAC3 or GSN recombinant protein. Analysis was completed by both western blot and silver nitrate staining followed by mass spectrometry analysis of the potential caspase-dependent cleavage fragments. Additionally, HDAC3 and GSN levels were analyzed in primary rat cardiomyocytes treated with hypertrophy agonist phenylephrine (PE) compared to control serum-free media treatment. Cardiomyocytes were also transfected with luciferase reporter plasmids for hypertrophic markers and reporter activity was measured.

Results: HDAC3 cleavage was observed during early stages of hypertrophy and reduced in the presence of a caspase inhibitor. Luciferase assays demonstrated that the transcriptional activity of MEF2 is dependent on intact caspase function suggesting caspase-directed HDAC3 cleavage may serve as a novel regulatory mechanism to alleviate MEF2 suppression to engage the hypertrophy gene expression program. Caspase mediated GSN cleavage occurs at latter stages and is coincident with the cytoskeletal alterations that occur during this process. We have generated adenoviral vectors containing caspase cleavage mutants and cleaved forms of GSN and will discuss the impact of these modified substrates on the hypertrophy process in vitro and in vivo.

Conclusions: Collectively, this work suggests that caspase signalling acts to engage both the transcriptional program and cytoskeletal accommodations that characterize cardiac hypertrophy. Importantly, these observations suggest that identification of inhibitors that suppress caspase activity and/or activity of its cognate substrates may offer novel therapeutic targets to limit the development of pathological hypertrophy.

118 BIDCHIPS: bias decomposition and removal from ChIP-seq data clarifies true binding signal and its functional correlates

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Background
Unraveling transcriptional regulatory networks is a central problem in molecular biology and, in this quest, chromatin immunoprecipitation and sequencing (ChIP-seq) technology has given us the unprecedented ability to identify sites of protein-DNA binding and histone modification genome wide. However, multiple systemic and procedural biases hinder harnessing the full potential of this technology.

Results
Here, we present a novel framework where the genome-wide ChIP-seq signal is viewed as being quantifiably influenced by

different, measurable sources of bias, which can then be computationally subtracted away. We use a compendium of 123 human ENCODE ChIP-seq datasets to build regression models that tell us how much of a ChIP-seq signal can be attributed to mappability, GC-content, chromatin accessibility, and factors represented in input DNA and IgG controls. We use this model to separate out the non-binding influences from the ChIP-seq signal, thereby obtain a purified signal that associates better to TF-DNA-binding motifs than do other measures of peak significance. We also carry out a multiscale analysis that reveals how ChIP-seq signal biases differ across different scales. Finally, we investigate previously reported associations between gene expression and ChIP-seq signals at transcription start sites, and show that we can discriminate ChIP-seq signals that are truly related to gene expression from those that are merely correlated by virtue of bias.

Conclusions
Our study provides new insights into the behavior of ChIP-seq signal biases and proposes a novel mitigation framework that improves results compared to existing techniques. The study also emphasizes that properly accounting for confounders in ChIP-seq data is of paramount importance for obtaining biologically accurate insights into the workings of the complex regulatory mechanisms in living organisms. R and MATLAB packages implementing the framework can be obtained from http://www.perkinslab.ca/Software.html.

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119 **MYST1 acetyltransferase and SIRT1 deactylase interact with PAX7 in satellite cells and SIRT1 impairs PAX7 transcriptional activity.**

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Background
PAX7 is essential for the function of muscle satellite cells. It was previously determined that PAX7 methylation is important for its transcriptional activity and function in satellite cells. By mass spectrometry using immunoprecipitated FLAG-PAX7, we identified two lysine residues (K105 and K193) within the PAX7 protein that are acetylated both residues are conserved between species. To test whether these modifications have an influence on PAX7 transcriptional activity, we created a set of PAX7 mutants where the acetylated lysine residues are replaced by arginine. PAX7 transcriptional activity was monitored using a luciferase reporter under the control of Myf5, a Pax7 target gene. Treatment with trichostatin A (TSA), a histone deactylase inhibitor, increased significantly luciferase activity, but this activity was progressively lost when the mutants were introduced. This suggests that acetylation plays a role in PAX7 transcriptional activity. MYST1 and SIRT1 are both expressed in muscle satellite cells. SIRT1 is known to deacetylate myogenic transcription factor MyoD. MYST1 is known for its interaction with Wdr5, a known PAX7 partner. SIRT1 and MYST1 both share histone and non-histone targets.

Objective
Finding Pax7 acetylase and deacetylase capable of modulating its transcriptional activity.

Methods
Using co-Immunoprecipitation in both fibroblasts was well as primary cells we can detect an interaction between different proteins and PAX7. We used RT-qPCR to measure the levels Pax7 and it's downstream partners mRNA levels.

Results
We detected an interaction between PAX7 and MYST1 as well as PAX7 and SIRT1 by co-immunoprecipitation in fibroblasts and in primary myoblasts. SIRT1 interaction with Pax7 was also demonstrated by proximity ligation assay specifically in satellite cells. Moreover, expression of SIRT1 significantly reduces Luc-Myf5 reporter activity as well as siRNA against SIRT1 seems to have a modest effect on Pax7 downstream targets suggesting that SIRT1 regulates PAX7 transcriptional activity. Furthermore, PAX7 acetylation does not seem to be crucial for its interaction with known partners (Carm1, WDR5 and Ash2L), since the K/R mutations do not affect the interactions.

Conclusions
Through co-IP and RT-qPCR we determined that MYST1 and SIRT1 are good candidates for modulating post-translational modification on PAX7.

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120 **Predicting the number of latently infected cells in HIV-1 patients using viral blips**

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The major barrier for curing HIV-1 infections are latently infected cells. These cells are immune to anti-retroviral therapies and do not actively produce viral particles, but can activate in the presence of their corresponding antigens. Current therpaies are focused on activating these latent cells, which allows anti-retroviral therpies to target them. Determining the number of latently infected cells is crucial to determine drug doses required to eliminate them, while minimizing possible side effects. This number of latently infected cells can be inferred from a patient's viral load. However, patients undergoing treatments often have viral loads below the detection limit of qPCR. To avoid this problem we use bayesian analysis techniques and we exploit the fact that some times the viral load is detected, the so-called viral blips, due artifacts and inhomogeneous density fluctuations in the blood, to infer the average viral load, and thus the number of latently infected cells.

121 **Impaired transcriptional regulation of gene expression in satellite cells from dystrophic mice**

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Background: Duchenne muscular dystrophy is characterized by the lack of dystrophin, a protein linking myofibers to the extracellular matrix. We recently determined that dystrophin is not only expressed in myofibers but also in satellite cells (SC), the adult stem cells responsible for muscle regeneration.

Objective: In order to determine if SC biology was impaired in dystrophin-deficient (mdx) mice, both WT and mdx SC were prospectively isolated from uninjured muscles to analyze gene expression by RNA-sequencing.

Methods and Results: Surprisingly, we found a large proportion of genes that were differentially expressed in mdx satellite cells, a majority of which being up-regulated. As dystrophic muscles undergo multiple cycles of degeneration and regeneration, mdx mice have a high proportion of activated satellite cells, which might account for the differences observed in RNA-sequencing. However, we performed RNA-sequencing in activated WT and mdx satellite cells isolated from injured muscle following cardiotoxin injection, and a similar global increase was observed in gene expression of dystrophin-deficient SC. Those genes were analyzed for enrichment of transcription factor binding. Using publicly available ChIP-sequencing data, we determined that a significant proportion of Fosl1 and Zbed6 target genes are differentially expressed in mdx satellite cells. The expression levels of Fosl1, Zbed6 and their target genes were confirmed in SC and primary myoblasts by RT-qPCR. Fosl1 expression is increased in mdx SC, whereas the expression of all the other members of the Jun/Fos transcription factor family is decreased. Zbed6 RNA expression is not changed in mdx cells, but the expression of its target genes is impaired. Thus, we are investigating whether Zbed6 protein expression or activity is impaired in mdx cells.

Conclusion: The increased expression of the transcriptional activator Fosl1, together with a deregulation of the Zbed6 repressor expression/function, could be responsible for the global up-regulation in gene expression observed in mdx satellite cells.

122 **The Role of c-MYC in Chronic Myelogenous Leukemia**

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The protooncogene c-MYC is aberrantly expressed in many human cancers including chronic myelogenous leukemia (CML). CML is a clonal myeloproliferative disease that results from the malignant transformation of a hematopoietic stem cell by the BCR-ABL oncogene. Current treatments are successful at eliminating the bulk of CML cells, however CML initiating cells that drive disease relapse are refractory to treatment. c-MYC is a transcription factor that drives biomass accumulation and cellular proliferation. It is also necessary in conjunction with BCR-ABL to induce a CML like disease in mice. c-MYC protein levels are tightly regulated during the cell cycle by phosphorylation at specific residues Thr58 and Ser62. Phosphorylation at Thr58 is a signal to degrade c-MYC. However glycosylation of c-MYC by the enzyme OGT, at the Thr58 can compete with phosphorylation and potentially stabilize c-MYC. By inhibiting OGT, and thereby destabilizing c-MYC it may be possible to reduce levels of c-MYC protein and to target CML cells that are resistant to current therapies.

123 **Increased pulmonary vascular and systemic response to chronic hypoxia in Sirtuin 1 mutant mice: a role for hypoxia-inducible factor-3alpha?**

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Background: Pulmonary hypertension (PH) is caused by occlusive remodelling of pulmonary arterioles leading to increased pulmonary vascular resistance, right ventricle hypertrophy and failure. Sirtuin-1 (Sirt1) is an NAD+-dependent deacetylase that has been strongly implicated in endothelium homeostasis in systemic vessels, but little is known about its role in hypoxia sensing. Objective: Understand the mechanisms by which Sirt1 regulates the pulmonary vascular and systemic response to chronic hypoxia (CH)-induced PH.

Methods: Mice lacking Sirt1 catalytic activity (sirt1Y/Y, H355Y) and their wild type (WT) littermates were exposed to chronic hypoxia (10% O2) for 1, 7 or 21 days.

Results: Sirt1Y/Y mice exhibited an exaggerated increase in right ventricle systolic pressure, apparent within the first week of hypoxic exposure, which progressively increased over the 3 week CH exposure period (42±2 sirt1Y/Y vs. 30±1 WT n=27/group, p< 0.001). Right ventricular hypertrophy, assessed by the RV/LV+S weight ratio, was also increased (Day 21: 0.56±0.01 sirt1Y/Y vs. 0.43±0.01 WT, n=27/group; p< 0.001). Hemtaocrit levels were similar in Sirt1Y/Y and WT mice at baseline; however, there was a delayed increase after three weeks of CH in Sirt1Y/Y mice relative to WT mice (71±2% sirt1Y/Y vs. 63±1% WT, n=17/group; p<0.001). Erythropoietin (EPO) plasma levels were markedly increased at Day 7 in Sirt1Y/Y vs. WT mice (492±240 vs. 81±16pg/mL, respectively; n=7-9, p< 0.05) but normalized at later time points (Day 21: 75±9 sirt1Y/Y vs. 96±12pg/mL WT; n=4/group). Surprisingly, expression of both HIF1a and 2a mRNA was decreased in mutant mice lungs during chronic hypoxia at all time points, as was the expression of several HIF1/2a responsive target genes. However, there was a dramatic increase in HIF3a in Sirt1 mutant vs. WT lungs, which has been implicated both in repression of other HIFs as well as in directly mediating hypoxic signaling.

Conclusions: Loss of Sirt-1 deacetylation activity led to an exaggerated pulmonary and systemic response to hypoxia, consistent with a role for Sirt1 in hypoxia sensing and/or signaling. As well, our data suggest that HIF3a is a novel downstream target for Sirt1 in the regulation of the response to chronic hypoxia.

124 **Detecting clusters of differentially expressed exons between NGS data sets**

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Genes are transcribed into messenger RNA (mRNA), which can then be spliced into many possible transcripts before being translated into proteins. This allows a mere 22,000 human protein coding genes to produce more than 150,000 known protein coding transcripts. Differential splicing plays important roles during organism development and aberrant splicing has been noted in some disease states. Unfortunately, the software tools that quantify gene expression from Next Generation RNA Sequencing (NGS) data have limitations in terms of differential splicing identification and quantification. To develop better tools to identify differential splicing in NGS data, our strategy is to focus on detecting differential exon expression, which results from differential splicing. To accomplish our goal we identify exons within each gene that have similar expression changes and combine them into a single "exon cluster". Exon clusters are then compared to one another within each gene to identify significantly up and down regulated clusters. This analysis will help discover a greater number of differentially expressed exons than is currently possible, and by association a greater number of genes, which are alternatively spliced between NGS data sets. Ultimately, our differentially expressed exon clusters can be correlated to protein domains and transcripts, relating our results to a biologically relevant context.

125 **Polarity Regulation of Asymmetric Muscle Stem Cell Divisions**

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Background: Satellite cells are adult stem cells residing between skeletal muscle fibers and the myofiber basal lamina. In response to injury, it is necessary for satellite cells to proliferate and differentiate for the efficient repair of myofibers. Our lab has identified a subset of stem cells within the satellite cell population that undergo symmetric and asymmetric divisions to self-renew the satellite cell pool, thus maintain the regenerative capacity of muscle. Symmetric and asymmetric divisions are aligned in planar and apicobasal orientations in respect to the host myofiber and are subject to extrinsic regulation. Therefore, understanding the molecular determination of asymmetric divisions will offer important insight into the homeostatic control of muscle stem cell numbers.

Objective: To study the extrinsic and intrinsic signaling that determines asymmetric divisions in muscle stem cells.
Methods: We have developed a in-niche high throughput screening platform to examine the proliferation kinetics of satellite stem

cells on ex-vivo cultured muscle fibers.

Results: Screening against well-characterized library of compounds that have defined therapeutic targets (>600 compounds), we have identified the EGFR and Aurora kinase A as a novel modulators of asymmetric satellite stem cell divisions. In resting tissue, we found EGFR polarized to the basal surface of satellite cells. Corresponding to the peak of asymmetric divisions, EGFR activation and Aurora kinase A expression were observed 3 days after cardiotoxin-induced muscle injury. Stimulation of cultured myofibers with recombinant EGF led to the apicobasal alignment of Aurora kinase A at the mitotic centrosomes and a 100% increase in asymmetric muscle stem cell divisions. Furthermore, perturbation of either EGFR or Aurora kinase A by chemical inhibitors or by siRNA knockdown reduces the capacity of satellite stem cells to perform asymmetric divisions. Importantly, inhibition of Aurora kinase A defaults muscle stem cells to symmetric divisions, which increases stem cell numbers. Similar to in vitro results, pharmacological inhibition of Aurora kinase A in vivo increases the muscle stem cell pool and produces a 50% increase in the number of Pax7+ satellite cells. Moreover, the increase in the muscle stem cell compartment led to an overall increase in the number of myogenic progenitors, accelerating the regeneration process.

Conclusions: We identify EGFR-Aurora kinase A pathway as a regulator of asymmetric muscle stem cell divisions. With muscle injury, EGF release stimulates the rapid production of myogenic progenitors. Pharmacological inhibition of this pathway could be used to therapeutically modulate stem cell numbers and enhance muscle regeneration.

126 **The regulation of endothelial nitric oxide synthase by extracellular matrix in human late outgrowth endothelial progenitor cells**

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Background: There is a need for a readily available source of functional autologous endothelial cells to cover blood contacting surfaces in order to induce and maintain homeostatic environment. Human late outgrowth endothelial progenitor cells (LEPCs), derived from peripheral blood mononuclear cells are proliferative and express endothelial protein profiles (i.e. CD31, 144, 146, KDR, and vWF). Endothelial nitric oxide synthase (eNOS) is an important factor that regulates homeostasis at the blood contacting surface and loss of eNOS activity is a hallmark of endothelial dysfunction.

Objective: Here, we examined the expression and activity of eNOS and elucidated the cause of differential regulation of eNOS in LEPCs compared to mature endothelial cells.

Methods and Results: We found that LEPCs express markedly lower eNOS protein (0.34 ± 0.13, Western blot), mRNA (0.29 ± 0.17, qRT-PCR) as well as activity levels (0.49 ± 0.18, Nitrite analysis) when compared to Human Umbilical Vein Endothelial cells (HUVECs) or Human Aortic Endothelial Cells (HAECs). When grown on fibronectin (FN), type I collagen (Col. I), and laminin (LAMA) we found significantly decreased eNOS protein in HUVECs (0.52 ± 0.08 fold change on FN, 0.49 ± 0.07 on Col. I and 0.53 ± 0.07 on LAMA) compared to cells on polystyrene, these findings were associated with decreased eNOS mRNA. The matrix mediated downregulation was blocked by ß1 integrin siRNA and focal adhesion kinase siRNA transfection. In addition, rho-associated protein kinase (ROCK) inhibitors including fasudil and Y27632 blocked the effect of ECM on eNOS downregulation in HUVECs. Interestingly, when cultured on polystyrene, LEPCs express significantly more focal adhesion sites, extracellular matrix (ECM) proteins especially Col. I and lower MMP-2 activity compared to HUVECs and HAECs. Blocking Col. I synthesis (siRNA) or treatment with Y27632 were found significantly enhanced eNOS expression in LEPCs (1.77 ± 0.41 and 1.39 ± 0.11 fold increase for Col. I siRNA and Y27632, respectively). Increased eNOS level further improved cell survival in LEPCs after serum deprivation.

Conclusions: Taken together, our results suggest LEPCs contain lower eNOS level compared to mature endothelial cells due to their higher ECM (e.g. Col. 1) deposition and demonstrate that blockage of ECM synthesis or cell-ECM interactions could help to develop functional endothelialized surfaces.

Evaluation Instructions

Once again , the Ottawa Hospital Research Institute is offering prizes for the best trainee presentations at Research Day. Participants will be judged in the following categories:

Best Poster ($500 for 1st, $250 for 2nd and $100 for 3rd in each category)

- Master's
- PhD
- Postdoctoral

Best Oral Presentation ($500 for 1st, $250 for 2nd and $100 for 3rd)

The following criteria have been given to all the evaluators to guide their judging.

All presentations will be evaluated with a score from zero to 100 for each of the following categories:

- Introduction (clearly presented rationale and hypothesis)
- Methodology (sufficiently clear with appropriate details)
- Results (quality and clearly explained)
- Discussion (summary, interpretation and relevance)
- Visual appearance of poster/slides
- Ability to answer questions (Not leaving enough time for questions will result in a mark of zero for this section)

The scale should be applied as follows:

0	Unable to assess
50 – 59	Below average: unclear methodology and results
60 – 69	Average: many presentations will fall into this category
70 – 79	Good: most presentations will fall into this category
80 – 89	Very good: clearly above average; only a few fall into this category
90 – 100	Excellent: Best possible!! Wow!! Top 5%.

Some of our research trainees who hold salary awards

For a full list of trainees with salary awards, please see page 5.

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