The Ottawa Hospital RESEARCH INSTITUTE | L'Hôpital d'Ottawa INSTITUT DE RECHERCHE

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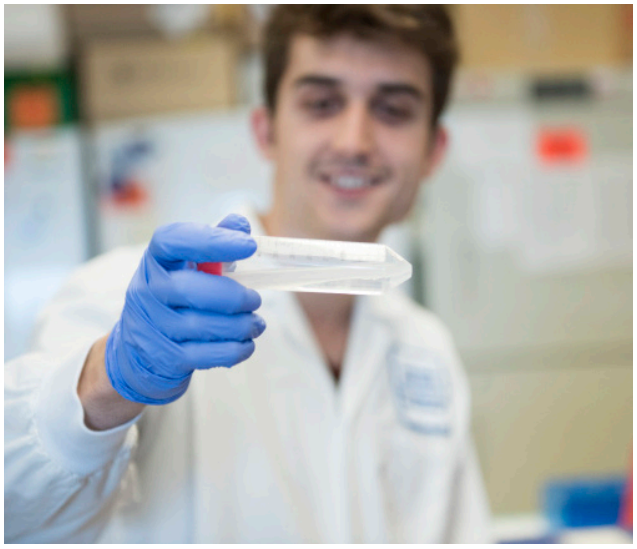
2019 RESEARCH DAY



Program and Abstracts

Thursday, November 7, 2019
7:30 a.m. – 5:00 p.m.

St. Elias Centre
750 Ridgewood Ave.
Ottawa, ON

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Your participation in Foundation events, tours and stories allows us to advance the entire research enterprise at The Ottawa Hospital through initiatives such as:

- Core Resources like the Ottawa Methods Centre, StemCore, ICES and Proteomics
- Seminars and events such as OHRI Research Day
- Matching funds for grants and salary awards such as Translational Research Grants, QEII-GSST trainee awards and TOHAMO grants

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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WELCOME TO RESEARCH DAY

Duncan Stewart, MD, FRCPC

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

Executive Vice-President, Research,
The Ottawa Hospital

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, in partnership with the University of Ottawa.

Our first guest speaker is John Chafe, who in 2001 became the second person in Canada to particpate in the Ottawa-led clinical trial of immunoablation and autotologous stem cell transplantation for MS. This procedure is now considered a standard of care for individuals with very active MS, thanks in large part to this pioneering study.

Our second guest speaker is Dr. Adrian Krainer, Biochemist and Molecular Geneticist at Cold Spring Harbor Laboratory. Dr. Krainer studies RNA splicing mechanisms and regulation, and he is one of the inventors of the RNA-targeted antisense therapeutic nusinersen (Spinraza), the first approved drug to treat the neurodegenerative disease spinal muscular atrophy.

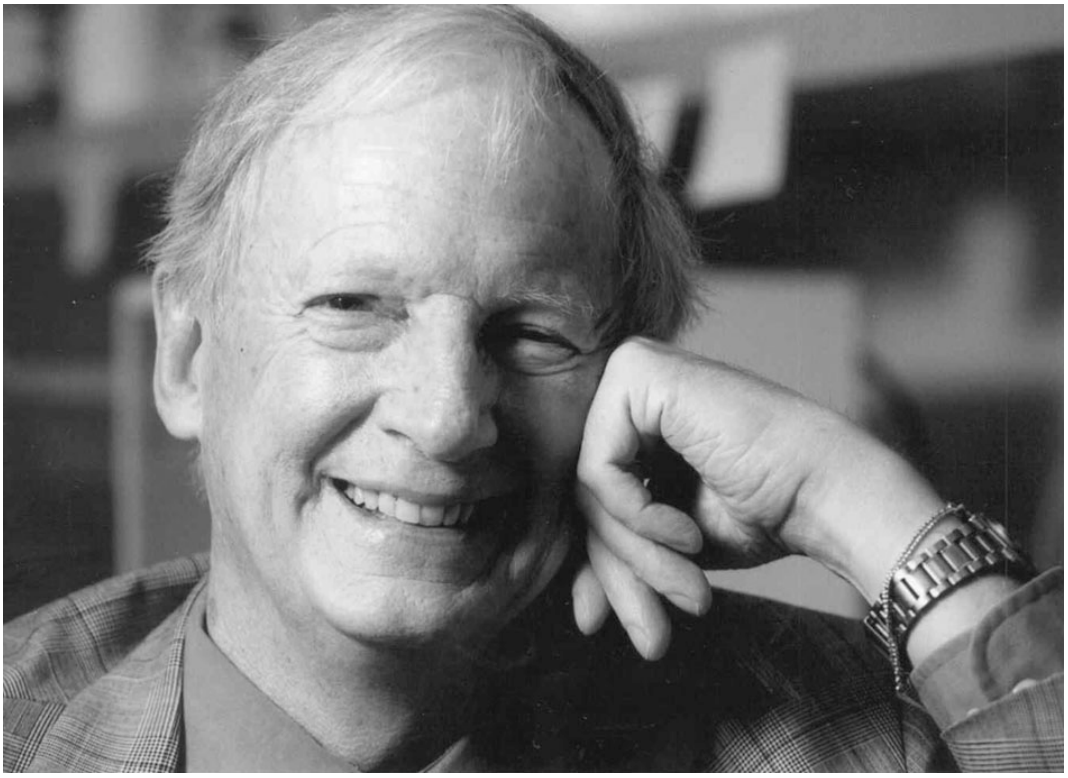
Our third guest speaker is Dr. John Marshall, Co-Director of the Critical Illness and Injury Research Centre at St. Michael's Hospital. Dr. Marshall's academic interests lie in the area of sepsis and life-threatening infection, trauma, and the host innate immune response to these. He will speak on scientific literacy and global collaboration in perilous times.

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 keynote speakers to our presenters, judges, moderators, planning committee and volunteers. I would also like to thank the sponsors for helping to make today's event possible, and I encourage you to visit their tables.

I would also like to thank Drs. Lauralyn McIntyre and Paul Albert, the Research Day co-chairs, who have worked tirelessly to make this event possible. They are always open to input on how to make this inspiring day even better.

Finally, I would like to thank everyone who has donated to The Ottawa Hospital Foundation and helped with fundraising events. Support from our Foundation is crucial for OHRI Research Day, as well as for all our shared core resources and infrastructure for research.

DR. J. DAVID GRIMES LECTURE



Dr. J. David Grimes, MD, FRSPC

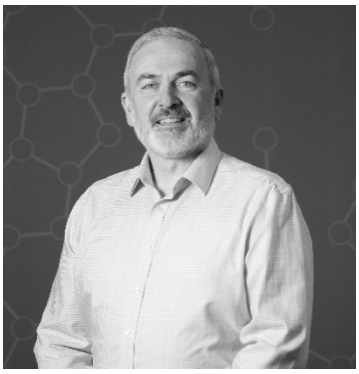
This annual lecture is named in honour of Dr. J. David Grimes, founder of the Loeb Research Institute, which was the predecessor of the Ottawa Hospital Research Institute at the Civic Campus.

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 Research Career Achievement Award, which is awarded annually at The Ottawa Hospital Gala.

DR. J. DAVID GRIMES LECTURE

Dr. Adrian Krainer



Dr. Adrian R. Krainer, Ph.D. is the St Giles Foundation Professor at Cold Spring Harbor Laboratory. He studies RNA splicing mechanisms and regulation, and he is one of the inventors of the RNA-targeted antisense therapeutic nusinersen (Spinraza), the first approved drug to treat the neurodegenerative disease spinal muscular atrophy. He has published over 200 peer-reviewed articles and holds seven U.S. patents and 83 foreign patents that have been licensed or sublicensed to three companies. Krainer is a co-founder of Stoke Therapeutics. He is a fellow of the American Academy of Arts & Sciences, the National Academy of Inventors (USA), and the Royal Society of Medicine (U.K.).

KEYNOTE LECTURE

Dr. John Marshall

Dr. John Marshall is a Professor of Surgery at the University of Toronto, a trauma surgeon and critical care physician at St. Michael's Hospital, and a Senior Investigator in the Keenan Research Centre for Biomedical Science. His academic interests lie in the area of sepsis and life-threatening infection, trauma, and the host innate immune response to these. His CIHR-funded laboratory studies the cellular mechanisms that prolong neutrophil survival in critical illness. He has an active clinical research interest in sepsis and ICU-acquired infection, and in the design of clinical trials and outcome measures.

PATIENT GUEST SPEAKER

John Chafe

John Chafe was very active growing up and had just started a career with a major bank when he was diagnosed with multiple sclerosis in 1994. His MS progressed rapidly, and he was relying on a cane just a few years later. Then, in 2001, John became the 2nd person in Canada to participate in a clinical trial of immunoablation and stem cell transplantation for MS, led by Dr. Harold Atkins and Mark Freedman at The Ottawa Hospital. The groundbreaking results were published in The Lancet in 2016 and the procedure is now considered a standart therapy for people with very active MS. John is happily married and has a 9-year-old daughter.

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 Allison Tscherner and Sara Timpano for their excellent job running the summer student program this year.

Winner of the Dr. Goodman Cohen Summer Student Award

Grace Fox (supervised by Dean Fergusson and Manoj Lalu)

" Building a platform for meaningful patient partnership to accelerate "bench-to-bedside" translation of promising new therapies"

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 in Nova Scotia. The youngest of seven siblings, Dr. Cohen was the only one in this family to attend university. 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.

RESEARCH TRAINEE SALARY AWARDS

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

Rana Abdelhalim
Nouf Alluqmani
Meshach Asare Werehene
Katherine Atkinson
Russell Barkley
Donald Bastin
Alexandra Beaudry-Richard
Rania Berjawi
Laura Boland
Emily Brown
Valerie Cardin
Jessica Chan
Loucia Chehade
William Chen
Justin Chitpin
Nicholas Cober
David Cook
Mathieu Crupi
Alison Cudmore
Sarah Cummings
Chanele Cyr
Kyle Daines
David Datzkiw
Annemarie Dedek
Jessie Duong
Daniel El Kodsi
Peter Feige
Kristianne Galpin
Emma Grigor
Emily Hladkowicz
Jonathan Hodgins
Taylor Jamieson
Samantha Kornfeld
Radmila Kovac
Chloé Landry
Julia Lauzon
Flore Lesage
Michael Leung
Heidi Li
Juan Li
Alex Lin
Michaela Lunn
David Massicotte-Azarniouch
Graeme McDowell
Ivana Mizikova
Ivana Nad
Javad Niazmand
Nathaniel Noblett
Daniel Oliver Read
Heather O'Reilly
Adam Pietrobon
Eyal Podolsky
Aoife Reilly
Daniel Robinson
Galaxia Rodriguez
Chris Rousso
John Saber
Ryan Sandarage
Daniel Serrano
Julie Shaw
Abera Surendran
Keara Sutherland
Zaid Taha
Alvin Tieu
Sarah Tucker

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. Paul Albert (Co-chair)
Dr. Lauralyn McIntyre (Co-chair)
Dr. Anouk Fortin
Dr. Tim Ramsay
Dr. Jay Baltz
Dr. Ketul Chaudhary
Jennifer Ganton
Dr. Luc Sabourin
Amelia Buchanan
Dr. Angela Crawley
Dr. Ian Lorimer
Dr. Duncan Stewart
Jennifer Valentino

Volunteers

Greg Canham
Heidi Hickey
Natasha Hollywood
Jaahnavi Dave
Melanie Genereaux
Adrienne Szalamin
Wayne Lowe
Terri Van Gulik

OHRI RESEARCH DAY PROGRAM

7:30 AM **REGISTRATION / POSTER SETUP / CONTINENTAL BREAKFAST**
Sponsored by 10x Genomics

8:15 AM **OPENING REMARKS** (Lauralyn McIntyre, Paul Albert, Duncan Stewart, Jocelyn Côté)

8:30 AM **Revealing cell fate determinants in health and disease** (50 Minutes)
(Talks: 9 minutes plus 3 minutes discussion)
Moderators: Jood Madani and Adam Pietrobon

- **David Cook** (Barbara Vanderhyden Group) *Revealing context-specificity of the epithelial-mesenchymal transition using highly multiplexed single-cell RNA sequencing*
- **Ivana Mižíková** (Bernard Thébaud Group) *Single cell RNA analysis of cellular niche in normal and impaired postnatal lung development*
- **Daniel Robinson** (Jeffrey Dilworth Group) *Promoter-proximal stalling of RNA-Polymerase II is required to regulate myoblast fate determination during myogenesis*
- **Alex Lin** (Michael Rudnicki Group) *Single Cell Analyses Identify a Novel Muscle Stem Cell Subpopulation*

9:20 AM **New insights into neurological mechanisms** (50 Minutes)
(Talks: 9 minutes plus 3 minutes discussion)
Moderators: Hesham Ismail and Eyal Podolsky

- **Julie Ouellette** (Baptiste Lacoste Group) *Endothelial cell-autonomous contribution to 16p11.2 deletion autism syndrome*
- **Irina Alecu** (Steffany Bennett Group) *Disruptions in Lipid Metabolism and Associated Motor Impairments in an iPLA2ß Knock-out Mouse Model of Neuroaxonal Dystrophy and Parkinsonism*
- **Hannah Gillis** (David Picketts Group) *Investigating the Role of the Neuropeptide VGF in Post-stroke Recovery and Repair*
- **Ronda Lun** (Dariush Dowlatshahi Group) *Calculation of Multiple Intracerebral Hemorrhage Scores Using Delayed Imaging Outperform Baseline in Acute Intracerebral Hemorrhage*

10:10 AM **REFRESHMENT BREAK** (15 minutes)
Sponsored by MJS BioLynx

10:25 AM **POSTER SESSION 1** (60 minutes)
For presenters last names starting A to K

11:25 AM **PATIENT GUEST SPEAKER** (10 minutes)
Stubborn
John Chafe, participant in the Ottawa-led clinical trial of stem cell transplantation for MS
Introduction: **Marjorie Bowman**

11:35 AM **DR. J. DAVID GRIMES LECTURE** (35 minutes plus 10 minutes discussion)
Adrian Krainer, Biochemist and Molecular Geneticist at Cold Spring Harbor Laboratory
Moderator: **Rashmi Kothary**

12:20 PM **BUFFET LUNCH/ POSTER SETUP** (50 minutes)

1:10 PM **POSTER SESSION 2 (60 minutes)**
Sponsored by Stem Cell Technologies
For presenters last names starting L to Z

2:10 PM **KEYNOTE LECTURE** (35 minutes plus 10 minutes discussion)
Scientific literacy and global collaboration in perilous times
John Marshall, Co-Director of the Critical Illness and Injury Research Centre and Trauma Surgeon and Intensivist at St. Michael's Hospital. Professor of Surgery at the University of Toronto
Moderator: **Lauralyn McIntyre**

2:55 PM **REFRESHMENT BREAK** (15 minutes)
Sponsored by Miltenyi

3:10 PM **Improving outcomes of disease treatment** (50 Minutes)
(Talks: 9 minutes plus 3 minutes discussion)
Moderators: Zachary Cantor and Kyla Young

- **André Carrington** (Doug Manuel Group) *Evaluating binary diagnostic tests and classifiers for optimal, equitable and explainable results*
- **Kelly Farrah** (Kednapa Thavorn Group) *Burden and Financial Impact of Sepsis in Ontario: A Population-based, Retrospective Cohort Study*
- **Akram Abolbaghaei** (Dylan Burger Group) *Circulating Microparticles and Pregnancy Outcomes in Type 1 Diabetes*
- **Angus Macaulay** (Jay Baltz Group) *How the oocyte detaches from the zona pellucida at ovulation*

4:00 PM **Harnessing immune mechanisms in disease** (50 Minutes)
(Talks: 9 minutes plus 3 minutes discussion)
Moderators: Abera Surendran and Daniel Serrano

- **Mathieu Crupi** (John Bell Group) *Characterization of oncolytic vaccinia viruses that secrete Bi-specific T cell Engagers*
- **Michaela Lunn** (Michael Schlossmacher Group) *Elucidating the role of lrrk2 kinase activity in the innate immune system*
- **Marisa Market** (Rebecca Auer Group) *Downregulated Activating Receptors are Responsible for Suppressed IFN? Production in Postoperative Natural Killer Cells*
- **Emily Hladkowicz** (Daniel McIsaac Group) *PRedICT: A patient-oriented risk communication tool to improve patient experience, knowledge and outcomes after elective surgery*

4:50 PM **RECEPTION AND CASH BAR** (15 Minutes)
Sponsored by Borden Ladner Gervais LLP

5:05 PM **AWARDS AND CLOSING REMARKS**
Award presentation sponsored by BioRad, CCRM, ThermoFisher Scientific, and VWR, part of Avantor
Moderators: Duncan Stewart, Lauralyn McIntyre and Paul Albert

ORAL PRESENTATIONS:

Revealing cell fate determinants in health and disease (8:30 – 9:20)

Moderators: **Jood Madani and Adam Pietrobon**

1-1

Revealing context-specificity of the epithelial-mesenchymal transition using highly multiplexed single-cell RNA sequencing

David P. Cook[1,2], Barbara C. Vanderhyden[1,2]

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

Background: Epithelial-mesenchymal (E/M) heterogeneity is ubiquitous within all epithelial tissues and the reversible transition between these two states provides cells with plasticity that contributes to a variety of biological processes, including tumour progression. While the epithelial-mesenchymal transition (EMT) has been extensively studied, a variety of EMT responses have been reported and no common, EMT-defining gene expression program has been identified

Objective: Here, we leverage highly multiplexed single-cell RNA sequencing (scRNA-seq) to interrogate EMT variability and determine if a common EMT expression program exists, assessing 103,999 cells from 960 samples, comprising 12 EMT time course experiments and 16 independent kinase inhibitor screens.

Results: Here, we leverage highly multiplexed single-cell RNA sequencing (scRNA-seq) to interrogate this variation and determine if a common EMT expression program exists, assessing 103,999 cells from 960 samples, comprising 12 EMT time course experiments and 16 independent kinase inhibitor screens. We demonstrate that the vast majority of transcriptional changes are context-specific, regardless of the cell type or factor used to induce the transition. Further, we show that the EMT response is not simply a linear transition between opposing E/M expression programs. While many canonical EMT genes were poor markers of the transition in our models, we identified 86 mesenchymal genes conserved across most conditions whose expression is also well-correlated in a variety of mouse and human epithelial and carcinoma tissues. Transcription factor activity was also largely context-specific, with only several transcription factors, including SOX4 and RELB, showing consistent activity across many conditions. Finally, kinase inhibitor screens revealed multiple paracrine dependencies, including a novel association between TGFB1 and the TNF-associated RIPK1.

Conclusion: Together, these results suggest that the EMT is not a single cellular process, but rather, is a collection of disparate responses poorly conserved across contexts. As E/M heterogeneity is an intrinsic property of tumours, revising our conceptual model of the EMT to accommodate this variability is essential to understand and develop targeted therapies for metastatic disease.

1-2

Single cell RNA analysis of cellular niche in normal and impaired postnatal lung development

Ivana Mižiková [1,2], Maria Hurskainen [1,3], David Cook [2,4], Chanele Cyr-Depauw [1,2], Flore Lesage [1,2], Barbara Vanderhyden [2,4,6], Bernard Thébaud [1,2,5]

[1] Sinclair Centre for Regenerative Medicine, Ottawa Hospital Research Institute, Ottawa, ON, Canada
[2] Department of Cellular and Molecular Medicine, University of Ottawa, Ottawa, Ontario, Canada
[3] Department of Pediatric Cardiology, Children's Hospital, Helsinki University Hospital and University of Helsinki, Helsinki, Finland
[4] Cancer Therapeutics Program, Ottawa Hospital Research Institute, Ottawa, Ontario, Canada
[6] Department of Pediatrics, Children's Hospital of Eastern Ontario, University of Ottawa, Ottawa, Ontario, Canada
[7] Department of Obstetrics and Gynecology, University of Ottawa/The Ottawa Hospital, Ottawa, Ontario, Canada

Background: Extreme preterm birth is the primary cause of death in children below 5 years. The chronic lung disease bronchopulmonary dysplasia (BPD) is the most common complication in preterm infants. It is characterized by alveolar hypoplasia and dysmorphic vasculature. Changes in cellular composition and gene expression underlying the development of BPD are not fully understood.
Objective: To create a detailed temporal map of normal and aberrant lung development using single-cell RNA sequencing.
Methods: We performed scRNA-seq on lung cells from 36 mice at postnatal days 3, 7 and 14. BPD was mimicked by exposing mice to hyperoxia (85% O_2). We used a high number of biological and technical replicates in combination with the MULTI-seq sample labeling to minimize experimental bias and increase reproducibility.
Results: A total of 66.200 cells were analyzed throughout three crucial time points of postnatal lung development, across which we identified 1 mesothelial, 7 epithelial, 12 stromal, 8 endothelial, 15 myeloid and 11 lymphoid cell clusters.
Among all cell populations, immune cells showed the most dynamic changes during normal development and after hyperoxic exposure. Normal development was characterized by the lymphoid cells expansion, while myeloid cells remained the major immune cell type after hyperoxia exposure. The 15 clusters of myeloid cells included macrophages, monocytes, neutrophils, dendritic cells, mast cells and basophils. Hyperoxia impaired the composition and developmental trajectories particularly amongst the macrophage populations. Specifically, hyperoxia i) reduced the homeostatic alveolar macrophage population and ii) almost entirely abolished the proliferating, self-renewing alveolar macrophages. In addition, two hyperoxia-specific populations involved in innate immune response, inflammation and regeneration were identified. Hyperoxia further enhanced the M1 signature and induced a new population characterized by both M1 and M2 genes. Finally, we observed an up-regulation of Csf1r and Ccr2 in hyperoxia-induced macrophages and increased Ccr2 expression in monocytes. Moreover, the expression of Csf1r and Ccr2 ligands was also increased in hyperoxia-specific neutrophil and macrophage clusters. Consistent with previous findings, our data shows that hyperoxia alters lung macrophage populations through the CSF1-CSF1R and CCL2-CCR2 axis.
Conclusions: We present, for the first time, a detailed cell and molecular atlas of the postnatal developing lung. We demonstrated extensive changes in cellular composition caused by hyperoxia and identified the effector cells of the innate immune response, pivotal in processes of lung injury. Understanding the temporal changes on transcriptional level provides new means to study the pathogenesis and novel therapies for BPD.

1-3

Promoter-proximal stalling of RNA-Polymerase II is required to regulate myoblast fate determination during myogenesis

Daniel Robinson[1,2], Karen Adelman[3], Jeffrey Dilworth[1,2]

1. Regenerative Medicine Program, Ottawa Hospital Research Institute.
2. Cell & Molecular Medicine, University of Ottawa.
3. Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School.

Background: During skeletal muscle regeneration, muscle stem cells (MuSC) undergo coordinated fate transitions as they exit quiescence and move towards terminal differentiation. Doing so requires rapid changes in gene expression, which is coordinated on many levels of epigenetic regulation. While the complete epigenetic implications in the process are not yet fully understood, the process likely culminates in transcriptional regulatory pathways which determines whether RNA Pol II will express a gene.
One potential transcriptional regulatory mechanism that could explain this process is that mediated by the Negative Elongation Factor (NELF) complex. Here, NELF becomes associated with an active RNA Pol II downstream of the transcription start site, and retains the RNA Pol II in a poised state. Release of RNA Pol II occurs upon recruitment of the positive transcription elongation factor (pTEFb), resulting in rapid downstream gene expression. The importance of NELF in transcriptional regulation has been demonstrated in development, but remains to be studied in adult stem cell populations.

Objectives: The objective of this project is to identify if NELF-mediated transcriptional regulation controls MuSC fate changes during skeletal muscle regeneration.

Methods: Insights into the implication of the NELF complex in controlling MuSC fate changes are investigated using a satellite cell specific conditional knockout of the NELFb subunit (NELFb scKO). This prevents formation of a functional NELF complex. Coupling this model with cardiotoxin-mediated injury allows to study how the NELF complex regulates MuSC fate changes during skeletal muscle regeneration.

Results: Upon depletion of the NELFb subunit, muscle regeneration is strongly impaired. This is evidenced through reduced myofiber size of regenerating muscle, and reduced populations of MuSC in the NELFb knockout populations. Muscle re-injury results in extensive fibrosis, a near-loss of skeletal muscle, and loss of the satellite cell population. Various EdU-pulsing experiments show NELFb scKO myoblasts undergo premature differentiation, but has no effect on MuSC quiescence, activation, nor myocyte fusion. Sequencing experiments further validate that NELFb scKO myoblasts undergo premature differentiation.
Interestingly, treatment of MuSC with the pTEFb inhibitor Flavopiridol favours evasion of terminal differentiation and retain a proliferative state *in vitro*. Withdrawal of Flavopiridol allows myoblasts to resume differentiation, much like untreated controls.

Conclusions: This work suggests the NELF complex plays a critical role in mediating the transition of proliferating MuSC towards terminal differentiation. Temporarily inhibiting this process with Flavopiridol supports phenotypic findings and holds therapeutic promise for myoblast population expansion.

1-4

Single Cell Analyses Identify a Novel Muscle Stem Cell Subpopulation

Alexander Lin[1,2], Morten Ritso1[1,2], Kasun Kodippili[1,2], J. Manuel Hernández-Hernández[1,2], Michael A. Rudnicki[1,2]

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

Background: Muscle stem cells provide the regenerative potential for skeletal muscle throughout adult homeostasis, while deficiencies in their maintenance may be the etiology of muscle diseases, such as Duchenne Muscular Dystrophy. Within the bulk population of Pax7+ muscle stem cells, functionally distinct subpopulations exist, suggesting a high degree of heterogeneity that may be dysregulated in disease contexts. We are interested in uncovering these subpopulations at quiescence and understanding their lineage potentials during muscle regeneration.

Objective: Uncovering subpopulations within the heterogeneous population of Pax7+ muscle stem cells and identifying their regulators.

Methods: Quiescent and activated muscle stem cells were sorted by fluorescence-activated flow cytometry (FACS) and captured with the 10X Genomics Platform. Using Seurat, a bioinformatic single cell toolkit, we found distinct populations throughout the process of muscle regeneration. Using molecular biology techniques, we validated the stemness potential and function of a new quiescent subpopulation.

Results: We have successfully captured and sequenced >1000 muscle stem cells and have begun to bioinformatically characterize and stratify the populations. We found a distinct subpopulation of quiescent muscle stem cells that clusters away from the bulk population. Interestingly, this subpopulation is reminiscent of the Pax7+/Myf5- cells that we have previously shown to have enhanced stem cell capabilities; while it can also be marked by Integrin α1 (Itga1) expression. Using FACS to isolate Itga1+ muscle stem cells, we have conducted transplantation assays as the gold standard to test for stemness potential. Itga1+ muscle stem cells show enhanced engraftment and self-renewal capacity upon serial regenerative challenges. *Itga1* is more highly expressed in quiescent muscle stem cells and is significantly downregulated in activated cells. Moreover, 21 days after muscle injury, the proportion of Itga1+ muscle stem cells increases, suggesting clonal drift towards muscle stem cells that exhibit greater regenerative potential. Altogether, our data shows that Itga1 marks a unique and functionally different muscle stem cell subpopulation at quiescence. We are currently further characterizing how Itga1 regulates quiescence, whether a similar population exists in human, and how this subpopulation changes in ageing or dystrophic contexts.

Conclusion: Within the bulk population of muscle stem cells, we have identified a novel subpopulation that has greater self-renewal capacity.

New Insights into neurological mechanisms (9:20 – 10:10)

Moderators: **Hesham Ismail and Eyal Podolsky**

2-1

Endothelial cell-autonomous contribution to 16p11.2 deletion autism syndrome

Julie Ouellette[1*], Xavier Toussay[1*], Mirabelle Ho[2], Cesar H. Comin[3], Luciano da F. Costa[4], María Lacalle-Aurioles[5], Qing Yan Liu[6,7], Sonia Leclerc[6], Youlian Pan[8], Ziying Liu[8], Jean-François Thibodeau[9], Melissa Yin[10], Micaël Carrier[11], Cameron J. Morse[1], Moises Freitas-Andrade[1], Peter Van Dyken[1], Yannick D. Benoit[7], Marie-Ève Tremblay[11], Christopher R. Kennedy[7,9], Dylan Burger[7,9], Duncan J. Stewart[2], and Baptiste Lacoste[1,7,12]

1. Neuroscience Program, Ottawa Hospital Research Institute, Ottawa, ON, Canada; 2. Regenerative Medicine Program, Ottawa Hospital Research Institute, Ottawa, ON, Canada; 3. Department of Computer Science, Federal University of São Carlos, São Carlos, Brazil; 4. São Carlos Institute of Physics, FCM-USP, University of São Paulo, Brazil; 5. Laboratory of Cerebrovascular Research, Montreal Neurological Institute, McGill University, Montréal, QC, Canada; 6. Human Health and Therapeutics, National Research Council of Canada, Ottawa, Ontario, Canada; 7. Faculty of Medicine, Department of Cellular and Molecular Medicine, University of Ottawa, Ottawa, ON, Canada; 8. Digital Technologies, National Research Council of Canada, Ottawa, Ontario, Canada; 9. Kidney Research Center, Ottawa Hospital Research Institute, Ottawa, ON, Canada
10. FUJIFILM VisualSonics, Inc., Toronto, ON, Canada; 11. Axe neurosciences, Centre de recherche du CHU de Québec-Université Laval, Québec, QC, Canada; 12. University of Ottawa Brain and Mind Research Institute, Ottawa, ON, Canada
***** *Contributed equally to the work*

Background: Although the neuronal underpinning of ASD are being unraveled, very little is known about vascular contributions of these conditions. Brain development and function rely on vascular features that ensure adequate supply of oxygen and nutrients from the blood stream. These features consist of a well-established vascular network, a functional blood-brain barrier, as well as cerebral blood flow regulation. Early life impairments in these features can lead to neurodevelopmental defects. Very few studies have considered the role of the brain vasculature to autism spectrum disorders (ASD). Only a postmortem study in young ASD brains suggested an impairment in angiogenesis, and a functional imaging study proposed a link between ASD and altered cerebral perfusion.
Objective: The objective of this study is to investigate the pathological development of the brain vasculature in a mouse model of the 16p11.2 deletion autism syndrome (*16p11.2*$^{df/+}$ mice).
Methods: A multidisciplinary approach was used in order to decipher the cerebrovascular underpinnings of ASD in a mouse model of the 16p11.2 deletion syndrome (*16p11.2*$^{df/+}$ mice). Cerebrovascular function was measured using laser Doppler Flowmetry; acoustic contrast imaging of brain blood perfusion; *ex vivo* vascular reactivity using pressure myography or wire myography; and brain electrical activity was measured with an electroencephalogram (EEG). Cerebrovascular structure was assessed using immunohistochemistry and three-dimensional image analysis of reconstructed vascular networks using computational tools. Mouse brain endothelial cell and human-induced endothelial cell health from 16p11.2 deletion carriers were used for immunocytochemistry, Matrigel-based network formation assay, cell cycle analysis and/or gene expression changes. ASD-like behaviors were assessed in mice with the beam break test, marble burying test, novel object recognition task and rotarod.
Results: 16p11.2 hemizygosity was linked to functional and structural abnormalities of brain vascular networks. Cortical microvascular density and branching were reduced at postnatal day (P)14. Baseline cerebral blood flow, neurovascular coupling and cerebrovascular reactivity were altered at P50 in *16p11.2*$^{df/+}$ mice while stimulus-evoked EEG activity was increased in *16p11.2*$^{df/+}$ mice. Network-forming defects were also identified *in vitro* using *16p11.2*$^{df/+}$ primary mouse brain endothelial cells and human iPSC-derived endothelial cells from 16p11.2 deletion carriers. Finally, mice with endothelial-specific 16p11.2 haploinsufficiency (*Ve-Cad-Cre;16p11.2*$^{flox/+}$) partially recapitulated autism-associated behavioral traits, including locomotor hyperactivity, as well as some cerebrovascular phenotypes.
Conclusion: These findings establish vascular cells as key contributors to the pathophysiology of the 16p11.2 deletion autism syndrome and provide novel insight into how the cerebral endothelium fine-tunes brain maturation.

2-2

Disruptions in Lipid Metabolism and Associated Motor Impairments in an iPLA2ß Knock-out Mouse Model of Neuroaxonal Dystrophy and Parkinsonism

Irina Alecu[1,2,3], Matthew A. Lenardis[1,2,3], Hongbin Xu[1,2,3], Graeme Taylor[1,2,3], Steffany A.L. Bennett[1,2,3]

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Background: Calcium-independent phospholipase A2 (iPLA2β) is responsible for cleaving phospholipids at the *sn-2* position, generating 1-acyl-lysophospholipids and free fatty acids. iPLA2β dysfunction has been implicated in a number of pathologies associated with cognitive and motor skill impairment, including infantile neuroaxonal dystrophy and dystonia-parkinsonism.

Objective: It is still not known how iPLA2β deficiency contributes to the pathologies described above, and the lipid changes caused by enzymatic deficiency have never been reported in mammals. Our objective was to determine the lipid changes associated with behavioural changes in an iPLA2β knock-out mouse model of neuroaxonal dystrophy and parkinsonism.

Methods: We generated an iPLA2β knock-out (KO) mouse model and evaluated motor coordination and strength, spatial learning and memory, and anxiety using several behavioural tests. We also performed lipidomic analysis of glycerophosphatidylcholines (GPC) and sphingolipids using liquid chromatography-mass spectrometry of three different brain regions.

Results: We found that iPLA2β KO mice exhibited significant impairment in motor function, including in strength and coordination, compared to wild-type controls but did not exhibit significant deficits in spatial learning and memory. As iPLA2β is involved in the Lands cycle, we expected to see a decrease in lyso-GPCs and a consequent increase in GPCs; however, levels of these lipids were unaffected in KO mice. Interestingly, there was a significant increase in the sphingolipid intermediate C16-ceramide in all three brain regions, with most ceramide species being significantly elevated in the hippocampus.

Conclusion: Our findings suggest that changes in the lipidome are specifically associated with motor impairment but not with cognitive impairment, and that dysregulation of C16-ceramide homeostasis may itself be toxic. Our study is the first to show that in mammals iPLA2β plays an as-of-yet unknown role in sphingolipid homeostasis which is associated with motor impairment. It is possible that this metabolic pathway may be affected in parkinsonism-associated neurodegeneration.

2-3

Investigating the Role of the Neuropeptide VGF in Post-stroke Recovery and Repair

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Background: The high incidence of stroke worldwide, as well as the poor efficacy of neuroprotective drugs has shifted the focus of research towards therapies targeting post-stroke recovery and rehabilitation. VGF (non acronym) is a neuropeptide processed to form several smaller peptides that are well-known mediators of brain repair and has recently been identified as a post-stroke repair molecule.

Objective: In the present study, we assess the role of VGF and its secreted peptides in the post-stroke recovery phase to determine its ability to influence brain repair and functional recovery.

Methods: Photothrombosis induced ischemic model: Using 1% Rose Bengal (photosensitive dye) and a collated beam of light, we induced stroke in the left frontal cortex of adult mice. VGF or a control vehicle was then adenovirally delivered to the mice two days post-stroke. The mice were euthanized for tissue collection or underwent behavioural analysis at various time points ranging from two days to 4 weeks post-injection.
Role of VGF in post-stroke neurogenesis: The number of newborn neurons in the periinfarct area of coronal sections were quantified using immunofluorescence. Doublecortin (DCX) labelling was used to distinguish newly generated neurons, indicative of neurogenesis.
Effects of VGF on inflammation in vivo: Neuroinflammation around the periinfarct area was assessed using immunofluorescence. Markers of gliosis such as Allograft inflammatory factor 1 (AIF-1/Iba1) and glial fibrillary acidic protein (GFAP) were used to label microglial/macrophages in the periinfarct area. Raw264.7 cells were used in a migration assay to determine the chemoattractant properties of VGF peptides.

Results: The absence of VGF prevents the migratory activity of neural stem cells post-stroke resulting in more significant behavioural deficits, that can be ameliorated upon adenoviral delivery of VGF.WT mice treated with VGF demonstrate increased migration of newborn neurons to the peri-infarct region as well as improved functional recovery post-stroke.VGF also stimulates the migration of inflammatory cells *in vitro* through C3aR and *in vivo* through adenoviral delivery. VGF treated macrophages show increased phosphorylation of ERK abundance and activation of MAPK cell survival pathway.

Conclusions: The presence or absence of VGF significantly influences the migration of newborn neurons as well as the recruitment of neuroinflammatory cells in the peri-infarct region, ultimately affecting functional recovery post-stroke. These findings suggest that VGF plays a crucial role in the post-stroke recovery phase and its repair processes.

2-4

Calculation of Multiple Intracerebral Hemorrhage Scores Using Delayed Imaging Outperform Baseline in Acute Intracerebral Hemorrhage

Ronda Lun MD[123], Vignan Yogendrakumar MD[123], Dar Dowlatshahi MD PhD[123], on behalf of the PREDICT Collaboration.

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[3]Department of Medicine, Division of Neurology, The Ottawa Hospital.

Background and Purpose:
Patients with intracerebral hemorrhage (ICH) are at risk of early hematoma expansion and development of complications such as intraventricular extension and hydrocephalus. Current prognostic scores are largely based on baseline radiographic features, which may not account for these changes. We proposed that calculation of ICH prognostic scores using delayed imaging characteristics will have better predictive value for mortality than their baseline counterparts.

Methods:
We analyzed data from 280 consecutive patients presenting with ICH within 6 hours of onset from the observational PREDICT study (Prediction of Hematoma Growth and Outcome in Patients with Intracerebral Hemorrhage Using the CT-Angiography Spot Sign). ICH Score, FUNC Score, and modified-ICH (MICH) Score were calculated using both baseline and 24-hour radiographic characteristics. The primary outcome was mortality at 90 days. We generated receiver operating characteristic (ROC) curves looking at the predictive ability of all three scores for mortality. Baseline and 24-hour curves were compared with non-parametric methods.

Results:
Two hundred eighty patients were included in analysis, with a 90-day mortality of 25.3% (71/280). All three prognostic scores calculated at 24 hours were more predictive of mortality as compared to baseline calculations (see Figure 1), with their respective p-values calculated to be 0.046, 0.0052, and 0.0008 for ICH Score, FUNC, and MICH respectively.

Conclusion:
Calculation of the ICH Score, FUNC Score and MICH Score using delayed imaging demonstrated better prognostic value in predicting 90-day mortality compared to scores calculated at presentation. This could help improve prognostication and supports delaying discussions around withdrawal of care in ICH patients.

Improving outcomes of disease treatment (3:10 -4:00)

Moderators: **Zachary Cantor and Kyla Young**

3-1

Evaluating binary diagnostic tests and classifiers for optimal, equitable and explainable results

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Background: In binary diagnostic testing and classification, the receiver-operator characteristic (ROC) plot and the area under the ROC curve (AUC) describes the trade-off between two types of error: false positives and false negatives. Different trade-offs occur at different decision thresholds. Only part of the ROC curve and AUC are informative however when they are used with imbalanced data (e.g., low or high prevalence). Alternatives to the AUC have been proposed, such as the partial AUC and the area under the precision-recall curve, however, these alternatives cannot be as fully interpreted as the AUC, in part because they ignore some information about actual negatives.
Objective: We discuss some concerns with existing measures, and we derive and propose a new concordant partial AUC, a new partial c statistic for ROC data and a concordance matrix as foundational measures and methods to help understand and explain parts of the ROC plot and AUC.
Methods: Our proposed partial measures are derived from the c statistic and AUC. They are validated as equal to each other where expected and equal in summation to whole measures where expected. We propose a concordance matrix and show equivalence between a matrix border and the empirical ROC curve. Our partial measures are tested for validity on a classic ROC example from Fawcett, a variation thereof, and two real-life benchmark data sets in breast cancer from the UCI machine learning repository: the Wisconsin and Ljubljana data sets. Interpretation of an example is then provided.
Results: Results show the expected equalities between our new partial measures and the existing whole measures. The example interpretation illustrates the need for our newly derived partial measures.
Conclusions: The concordant partial area under the ROC curve was proposed and unlike previous alternatives, it maintains the characteristics of the AUC. The first partial c statistic for ROC plots was also proposed as an unbiased interpretation for part of an ROC curve. The expected equalities among and between our newly derived partial measures and their existing full measure counterparts are confirmed. These measures may be used with any data set but this paper focuses on their utility for imbalanced data.

Acknowledgments: This work is derived from a paper submitted with the same first author and co-author above, but with additional co-authors: Paul Fieguth, Hammad Qazi, Andreas Holzinger, Helen Chen.

3-2

Burden and Financial Impact of Sepsis in Ontario: A Population-based, Retrospective Cohort Study

Kelly Farrah[1,2,3], Lauralyn McIntyre[1,2,4], Robert Talarico[3], Monica Taljaard[1,2], Dean Fergusson[1,2], Christopher J. Doig[5], Alan J. Forster[1,2], Doug Coyle[1], Kednapa Thavorn[1,2,3]

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5. Department of Community Health Sciences, University of Calgary

Background: Although the short-term mortality and healthcare costs for sepsis patients are known to be high, the long-term attributable mortality and costs of sepsis are unclear.

Objectives: To determine the attributable all-cause mortality and incremental healthcare costs of sepsis patients compared to non-sepsis hospital controls.

Methods: We conducted a population-based retrospective cohort study and included a cohort of adult sepsis patients and non-sepsis controls aged 18 years or older who were admitted to a hospital in Ontario between April 1, 2012 and March 31, 2016, with follow up to 31 March 2017. We used a validated Canadian method to define sepsis from health administrative data. Sepsis cases and hospitalized controls were matched 1:1 based on the propensity score, age, sex, type of admission, and date of admission. We used Cox proportional hazard regressions and generalized linear models to adjust for remaining confounders and determine attributable all-cause mortality and rehospitalization as well as incremental health system costs in sepsis patients compared to controls over up to 5 years follow-up.

Results: After matching, 196,922 pairs of sepsis cases and controls were included in the analysis, of which 132,718 had non-severe sepsis (infection without organ dysfunction); 64,204 had severe sepsis (infection with organ dysfunction). Median follow-up time was 2.1 years. Over the follow-up period, severe sepsis was associated with a higher risk of mortality compared to matched controls (hazard ratio 1.66, 95% confidence interval [CI]:1.63-1.68). Both severe and non-severe sepsis patients had a higher risk of rehospitalization compared to matched controls, with hazard ratios of 1.53 (95% CI: 1.50-1.55) and 1.41 (95% CI: 1.40-1.43), respectively. Incremental one-year health system costs for severe sepsis and non-severe sepsis patients were C$29,238 and C$9,475, respectively, compared to matched controls. Incremental costs remained significantly higher in sepsis patients compared to controls for up to 5 years.

Conclusions: Compared to hospitalized controls, severe sepsis patients were more likely to experience the higher risks of death and hospital readmission and incur the greater health system costs. The annual 1-year incremental costs to the Ontario health system were estimated to be $672.4 million for severe sepsis patients and $423.2 million for patients with non-severe sepsis.

3-3

Circulating Microparticles and Pregnancy Outcomes in Type 1 Diabetes

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4. Department of Women and Children's Health, St Thomas' Hospital, King's College London, London, UK.
5. Department of Medicine, Sinai Health System, Toronto, ON.

Learning Objectives: Examine the association between circulating MPs and maternal and fetal outcomes in women with T1D in pregnancy.

Background: Women with diabetes are far more likely to experience adverse pregnancy outcomes than non-diabetic women, however tools to identify those at risk of complications are lacking. Microparticles (MPs) are 100-1000 nm extracellular vesicles that are released from stressed cells and accumulate in the circulation in vascular disease. Our lab has shown that MP formation is induced by high glucose exposure and that levels of circulating MPs are increased in animal models of diabetes. The purpose of the present study was to quantify circulating MPs in pregnant women with type 1 diabetes and to examine the association between MP levels and maternal and fetal outcomes.

Method/Results: We studied archived plasma samples from the Continuous Glucose Monitoring in Women with Type 1 Diabetes in Pregnancy Trial (CONCEPTT) trial. MPs were quantified by flow cytometry and endothelial MPs were identified as VE-Cadherin$^+$, platelet MPs as CD41$^+$ and leukocyte MPs as CD45$^+$ events. At enrollment, endothelial and leukocyte MPs were positively correlated with systolic blood pressure ($P<0.05$), however this association was not seen at 24 or 34 weeks of age. Logistic regression analysis showed that high levels of endothelial MPs (above median) at enrollment was associated with increased risk of neonatal intensive care unit admission (OR: 2.49, 1.13-5.49) positivity for the composite fetal outcome (pregnancy loss, birth injury, neonatal hypoglycaemia, hyperbilirubinaemia, respiratory distress, and high-level neonatal care for more than 24h) [OR: 2.12, 1.13-3.99]. Similarly, high levels of platelet MPs at enrollment was associated with increased risk of neonatal intensive care unit admission (OR: 2.09, 1.06-4.08).

Conclusion: Our results suggest that elevated endothelial and platelet-derived MPs may be associated with adverse pregnancy outcomes in women with type 1 diabetes.

3-4

How the oocyte detaches from the zona pellucida at ovulation

Angus Macaulay, Kevin Moore, Jay Baltz

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Background: Fully-grown oocytes are tightly attached to the zona pellucida (ZP). Within the first four hours after ovulation is triggered or upon oocyte isolation from the follicle, the oocyte progressively detaches from the ZP. Detachment during meiotic maturation is part of the process by which oocytes first become capable of independently regulating their cell volume yet how this occurs remains unknown.

Objective: We hypothesized that oolemma-zona release is mediated by a peptidase cleaving the ZP proteins that remain in their transmembrane form, attaching the oocyte to the ZP. We therefore sought to determine whether proteinase activity was required for oocyte-ZP detachment and to identify the proteinase.

Methodology and Results: We performed an initial bioinformatics screen using published RNAseq datasets for mouse oocytes (GSE70116) to reveal oocyte transcripts encoding peptidases in the MEROPS peptidase database. These were further refined using Gene Ontology terms indicating localization in the plasma membrane or extracellular space. This led to the identification of thirty-nine candidate extracellular peptidases in oocytes. Inhibitors matched to each class of candidates were screened for their ability to prevent oocyte detachment from the ZP using an osmotic shock assay. We found that only inhibition of the matrix metalloproteinases class of peptidases significantly inhibited oocyte-ZP detachment. Oocytes remained strongly attached to the ZP in the presence of the metalloproteinase inhibitors batimistat and marimistat for at least 24 hours, persisting even through first polar body formation. Both inhibitors suppressed volume regulation in the oocytes identified by a measurable decrease in 3H-glycine uptake. Immunofluorescence imaging using a monoclonal antibody that recognizes an epitope just distal to the putative cleavage site of the ZP3 protein showed progressive loss of transmembrane ZP3 within the first four hours of maturation, a loss that was prevented by batimistat and marimistat inhibition. Further study of the kinetics of the putative cleavage site and the role of metalloproteinases is ongoing and includes using a fluorescent ZP3 construct (ZP3::EGFP) expressed in oocytes (a gift from Jurrien Dean, NIH).

Conclusion: We have identified a family of peptidases responsible for the detachment of the oolemma and ZP following isolation from the follicle. We also show a connection bewteen attachment and oocyte volume regulation. We hope that further study will elucidate connections to other key oocyte processes contributing to oocyte quality.

Harnessing immune mechanisms in disease (4:00 – 4:50)

Moderators: **Abera Surendran and Daniel Serrano**

4-1

Characterization of oncolytic vaccinia viruses that secrete Bi-specific T cell Engagers

Mathieu Crupi[1,2], Thijs Janssen[1], Jessie Duong[1,2], Sarwat Khan[1], Camille Victoor[1], Julia Petryk[1], John Bell[1,2]

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2. Biochemistry, Microbiology & Immunology, University of Ottawa

The hypoxic nature of the tumour microenvironment impedes the initiation and/or elaboration of an effective anti-tumour immune response. We have demonstrated that T cells can remain active in hypoxic environments in the absence of cancer cells, consistent with data showing that the hypoxia inducible factor (HIF) downregulates the expression of MHC-I in cancer cells. Since MHC-I is required for T cell recognition and killing of tumour cells, this is yet another mechanism of immune escape. One strategy to circumvent this problem is the use of Bi-specific T cell Engagers (BiTEs) that are able to mediate T cell recognition and killing of tumour cells in an MHC-independent fashion. BiTEs consist of linked variable chain antibody fragments directed against the T cell antigen CD3 and a specific tumour-associated antigen. Unfortunately for many BiTEs in clinical development, there are toxicities associated with systemic administration and challenges to reaching high local concentrations to be effective in most solid cancers.

Oncolytic virotherapy has potential as an anti-tumour agent, most notably for its ability to lyse solid tumour cells and generate an anti-tumour immune response. Here, we show that the poxvirus life cycle protects vaccinia virus from attenuation in hypoxic conditions. Our data demonstrate that vaccinia virus is therefore a promising platform for BiTE production in hypoxic microenvironments. We show that murine and human versions of CEACAM5 BiTEs produced from vaccinia virus lead to cures in colorectal immune-competent murine models and xenograft models injected with human PBMCs, respectively. Using OVs as delivery vehicles to secrete BiTEs to the exact site where they are only needed at picomolar concentrations may be a very effective strategy that can lead to improved outcomes with less off-target toxicities.

4-2

Elucidating the role of LRRK2 kinase activity in the innate immune system

Michaela Lunn[1,2], Bojan Shutinoski[1], Quinton Hake-Volling[2], Mansoureh Hakimi[1], Julianna Tomlinson[1], Earl Brown[1], Michael G. Schlossmacher[1]

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Background: Mutations in LRRK2 are linked to increased risk of Parkinson disease (PD) where the most common mutations cause an increase in LRRK2 kinase activity. As such, inhibition of its kinase is being pursued as a therapeutic target. We have demonstrated that LRRK2 functions in the innate immune system and that the kinase-linked LRRK2 mutation promotes a stronger immune response. It is therefore possible that chronic kinase inhibition may put patients at increased risk of infections.

Objective: We hypothesized that LRRK2 kinase was required for its role in the innate immune system. We tested this both in vivo, using LRRK2 p.D1994S kinase-dead mice, and in vitro using bone marrow derived macrophages (BMDM) from wild-type mice with pharmacological LRRK2 kinase inhibition. For our in vivo studies, mice were infected with a neurotropic virus (reovirus-T3D) delivered either systemically or directly to the brain and we measured survival and viral load in key organs.

Results: In response to a systemic infection, p.D1994S kinase-dead mice showed increased survival from encephalitis compared to wild-type littermate controls. After direct-brain inoculation of reovirus-T3D, kinase-dead mice had the same survival and viral load as wild-type mice. We found similar results on cell viability and viral load in BMDMs when using a Lrrk2 kinase inhibitor.

Conclusion: The loss of Lrrk2 kinase activity did not confer an increased risk to reovirus-T3D infection. Our in vivo data suggest that the loss of Lrrk2 kinase function provides relative protection against encephalitis due to reovirus-T3D, and effect occurs primarily in the periphery rather than the brain.

4-3

Downregulated Activating Receptors are Responsible for Suppressed IFN? Production in Postoperative Natural Killer Cells

Market, M[1,2], Tennakoon, G[1], Leonard Angka, M.Sc.[1,2], Juliana Ng, B.Sc.[1], Marlena Scaffidi[1], Ahwon Jeong1,2, Christiano Tanese de Souza, DVM[2], Michael A. Kennedy[2], PhD, & Rebecca C. Auer, MD, M.Sc.[2,3]

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Background: Surgery, a potentially curative treatment for patients with solid malignancies, has been linked to increased cancer recurrence and metastases. The proposed mechanism is profound suppression of Natural Killer (NK) cell effector functions, including IFNγ secretion and cytotoxic activity. NK cell activity is regulated through the integration of signals via cell surface receptors. We hypothesize that **soluble, immune modulatory factors, released in response to surgical stress, impair the ability of NK cells to respond to activating stimuli by altering either receptor expression profile or downstream signaling.**

Methods: Peripheral blood samples were collected from cancer surgery patients preoperatively (baseline) and on postoperative day 1 (POD1). NK cell receptor expression (IL-2R, IL-12R, DNAM-1, NKG2D) as well as cytokine-dependent receptor activation and IFNγ production were assessed by flow cytometry as a proxy of NK cell activation. Isolated NK cells cultured in plasma derived from healthy donors or cancer patients at baseline or POD1 were similarly assessed to determine whether soluble factors contribute to the suppression of NK cells.

Results: Surgical stress is associated with a significant reduction in the expression of both IL-2 (CD132) and IL-12 (CD212) receptor subunits ($p<0.05$, $n=11$;$p<0.05$, $n=12$). In addition, NK cells produced significantly less IFNγ when stimulated with IL-2 and IL-12 ($p<0.05$, $n=13$). This reduced receptor expression was associated with a reduction in the cytokine-dependent phosphorylation of downstream signaling molecules STAT5($p<0.05$, $n=10$), S6K ($p<0.05$, $n=8$), and p38 MAPK ($p<0.05$, $n=9$). The expression of additional activating receptors NKG2D and DNAM-1 was similarly reduced postoperatively ($p<0.05$, $n=20$). Finally, this postoperative phenotype could be replicated in healthy cells after incubation with POD1 plasma but not with healthy or baseline plasma.

Conclusion: We have shown that surgically stressed NK cells have reduced receptor expression and downstream signaling activity paired with suppressed IFNγ production. We suggest that some soluble factor(s) present in POD1 plasma may be responsible. Future research will focus on identifying these factor(s) and assessing their potential as therapeutic targets.

4-4

PRedICT: A patient-oriented risk communication tool to improve patient experience, knowledge and outcomes after elective surgery

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9. Department of Anesthesia, Pain Management & Perioperative Medicine, Dalhousie University

Background: More than 15 000 people have surgery at The Ottawa Hospital (TOH) every year; 15% experience serious complications and over 1% die after surgery. Patients want to receive more information prior to surgery, yet, our review of preoperative surgical consultations at TOH found that personalized risk estimates were documented in only 20% of cases. When patients lack information about risks and benefits, their decision quality and satisfaction can be much lower.

Objective: To evaluate whether using a newly developed, patient-oriented personalized preoperative risk communication tablet application (PREDICT app) improves patient knowledge of their risk profile, and satisfaction with preoperative care.

Methods: The PREDICT app was developed with the TOH mHealth lab. It populates the National Surgical Quality Improvement Program Universal Risk Calculator with a self-reported personal health history (calibrated to TOH outcomes) and generates a personalized report of risks of mortality, serious complications, and expected length of stay. The intervention used a prospective controlled before-and-after design. Study procedures were identical in each phase except that participants in the intervention phase used the PREDICT app. The primary outcome was change in patient knowledge from before their pre-admission appointment to after. Secondary outcomes included patient experience and anxiety, and acceptability of the PREDICT app to patients and clinicians.

Results: We enrolled 183 participants. People in the intervention phase were 1.85 (95%CI 1.03-2.36) times more likely to improve their knowledge of risks and expected outcomes and 2.47 (95%CI 1.42-4.33) times more likely to report higher satisfaction than standard care patients. Anxiety levels were equal and 98% of patients said they would use the app again before a future surgery. Clinicians found the app useful, acceptable, and felt that care could be improved using the information generated.

Conclusions: Using a patient-facing personalized risk calculation and communication tablet application before surgery improves patients' knowledge of their risks and expected outcomes, improves satisfaction and is feasible and acceptable in preoperative practice. Economic evaluation of the PREDICT app is ongoing; integration of the PREDICT app into routine practice should include linkage to actionable interventions and process of care to support improvements in care and outcomes for surgical patients at TOH.

Lats1/2 Inactivation Induces High-Grade Serous Carcinoma in Mouse Ovarian Surface Epithelium

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Background: The Hippo pathway controls tumorigenesis through a kinase cascade that inactivates Yes-associated protein (YAP). YAP activation is suppressed by large tumor suppressor (LATS1 and 2) family members. It has been shown that LATS1 expression in human ovarian epithelium (OSE) is decreased in the transition to carcinoma, although the role of LATS1 during this process is unknown.
In this study, our objective was to explore the consequences of loss of LATS1/2 in OSE cells.

Results: The results indicated the OSE cell-specific deletion of *Lats1/2* leads to ovarian tumors with ascites and metastases spread throughout the peritoneal cavity by a month after intrabursal injection of adenovirus expressing Cre-recombinase (AdCre).

Histologically, tumors derived from the conditional deletion of *Lats1/2* resembled high-grade serous carcinoma (HGSC) starting from the OSE and were strongly positive for PAX8, WT1, CK19 and Ki67. Moreover, isolation of OSE cells from *Lats1/2*(flox/flox) mice and deletion of *Lats1/2* expression by AdCre induced cell proliferation, colony formation, tumorigenesis and changes in cell morphology. The increased proliferation was associated with disruption of Hippo pathway signaling, as seen by a decrease in YAP phosphorylation and an increase in YAP-TEAD transcriptional targets, including *Ccn1*,-*2*,-*5* and *Birc7* ($P<0.05$). Analysis of RNA-Seq data of AdCre treated and untreated cells revealed significant increases in AKT-PI3K, MAPK and other pathways involved in cancer.

Collectively, the data indicate that dysregulation of the Hippo signaling pathway can lead to the initiation and progression of OSE-derived ovarian cancer and loss of LATS1/2 is a potent stimulus for the growth of HGSC.

Investigating the role of SOX10 in BRAFV600E driven melanomagenesis and therapy resistance

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Introduction: BRAF is a kinase that is mutated in over 50% of melanoma cases. These mutations create a constitutively active kinase promoting tumor initiation and growth. Currently the best treatment for this form of melanoma is adjuvant and immunotherapy. Although both have shown success, melanoma is prone to resistance and tumors relapse. We believe it is important to understand what drives melanoma and how these factors may promote resistance to therapies.

Objective: Others have shown Sox10 a transcription factor, is important in melanocyte differentiation, development and benign nevi formation. Although its expression increases following melanoma development its ability to drive tumor formation and progression has yet to be assessed. We propose to study Sox10s ability to drive melanomagenesis and hope to identify how it may play a role in therapy resistance.

Methods/Results: Melanoma cell lines were tested for Sox10 expression by western and qPCR. Cells endogenously expressing or lacking Sox10 were injected into C57 mice to observe tumor growth (monitored every 3 days until endpoint). $Sox10^+$ mouse melanoma cells grew faster *in vivo* compared to $Sox10^-$ cells. Although expected, $Sox10^-$ cells grew suggesting Sox10 is not required for tumor progression. Further in-depth analysis of these cells allowed us to identify other key transcription factors that may be playing a role in melanoma tumor development. We also found that the $SOX10^+$ cells are sensitive to adjuvant therapy (vemurafenib) while the $Sox10^-$ cells had an intrinsic resistance. To create vemurafenib resistant cells our $Sox10^+$ cells were treated for 6 weeks at their IC50. Following resistance Sox10 expression was lost but the transcriptional network of the resistant clones were similar to our $Sox10^-$ cells. This suggests Sox10 may promote tumor growth but is detrimental for melanomas being treated with adjuvant therapy.

Conclusion: Sox10 promotes tumor growth but is not required. Although Sox10 promotes tumor growth, the $Sox10^-$ cells gain an advantage following adjuvant treatment. Therefore by determining the transcriptional network of melanoma cases we may be able to identify appropriate treatment methods that result in an increase in efficacy.

A Systematic Review and Network Meta-Analysis of Existing Pharmacologic Therapies in Patients with Idiopathic Sudden Sensorineural Hearing Loss

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Background: Hearing loss is one of the leading causes of disability worldwide. Idiopathic sudden sensorineural hearing loss (ISSNHL) is a common but difficult to treat condition that has a sudden onset of ≤ 72 hour associated with various etiologies, most being idiopathic. There exists a wide range of therapeutic options, however, the uncertainty surrounding their comparative efficacy and safety makes selection difficult.

Objective: To conduct a systematic review and network meta-analysis (NMA) to assess the relative effects of competing treatments for management of ISSNHL

Method: We searched MEDLINE, Embase, Cochrane Library and grey literature from inception to February 8th 2018 and screened the retrieved records in an online systematic review software program (Distiller Systematic Review (DSR) Software©) at level one (title and abstract), and level two (full text). We assessed the quality of the included studies via the Cochrane risk of bias tool for randomized clinical trials. Both screening and quality assessment were carried out independently by two reviewers, and disagreements were resolved through consensus or third-party adjudication. This review was registered with PROSPERO (CRD42017073756).

Results: We identified 1,138 bibliographic records, screened 613 after removing duplicates and included 19 studies . 12 studies were rated as unclear and 7 as high risk of bias. We identified data on several interventions for ISSNHL therapy and constructed treatment networks consisting of six intervention groups placebo; intratympanic (IT) steroid; IT plus systemic steroid; per oral (PO) steroid; intravenous (IV) steroid; and IV plus PO steroid for our NMAs).
IT plus systemic steroids demonstrated the largest difference in PTA improvement compared to placebo (25.85 dB, 95% CrI 7.18-40.58), followed by IV plus PO steroids (22.06 dB, 95% CrI 1.24-39.17), IT steroids (18.24 dB, 95% CrI 3.00-29.81).
The binary outcomes of hearing recovery demonstrated similar relative ordering of interventions. The largest OR compared to placebo was associated with IT+ systemic steroid followed by IV+PO steroids, PO steroid and IT steroid.

Conclusions: IT plus systemic steroid treatment was rated as the best among the six interventions compared, based on unclear to high risk of bias trials, and all active treatments were better than placebo in improving PTA. However, it should be noted that certain comparisons included either few studies of small sample size, and analyses were unable to control for steroid type and dosage. Given these limitations, further data originating from methodologically sound and rigorous trials with adequate reporting are needed to confirm our findings.

Interplay between Signaling of Dopamine D1 Receptor and Synapse-Associated Protein 102

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Background

Dopamine D1-class receptors (D1R and D5R) belong to G protein-coupled receptors (GPCRs) and regulate notably movement, reward, and learning. They have been implicated in multiple neurological and psychiatric disorders. Dopamine D1 receptors are concentrated in dendritic spines of neurons and are localized post-synaptically in the spine's neck/head, where it can modulate local ion channels, protein trafficking, and signaling cascades via direct protein-protein interactions. Among proteins localized in synapse, there are the postsynaptic density protein 95 (PSD-95) and the synapse-associated protein 102 (SAP102). They are scaffolding proteins belonging to membrane-associated guanylate kinases (MAGUKs). PSD-95 and SAP102 are highly enriched in synapses where they regulate receptor and channel protein localization within synapse and organize numerous signaling protein complexes required for synaptic development and plasticity.

Objectives

It has been reported that PSD-95 interacts with both dopamine D1 and D5 receptors and the co-expression of D1/PSD95 inhibit the D1-mediated cAMP level and did not alter D1 receptor binding property. In the present study, we aim to define the role of SAP102 in the regulation of dopamine D1-class receptors by characterizing the structural determinants modulating the interaction of the complex. In addition, we assessed the effect of SAP102 on D1-class receptor mediated stimulation of adenylyl cyclase (AC) and binding properties.

Methods

- Co-immunoprecipitation
- cAMP studies
- Saturation binding studies

Results

The co-transfected HEK293 cells results suggest that D1R interacts with SAP102 but not with D5R. The deletion mutants of D1R showed that intracellular loop 3 (IL3) of D1R is mediating the interaction with SAP102, and the Ser and Thr residues of IL3 of D1R may require for the interaction. However, we cannot rule out that SAP102 interaction is also modulated by other cytosolic regions of D1R. These findings were further confirmed by brain tissues and the results showed the formation of the native D1R/SAP102 complex in the hippocampus and striatum. Moreover, co-expression of SAP102 with D1 in HEK293T cells activates the D1-mediated cAMP accumulation with dopamine agonist (DA) or selective D1R agonist (SKF81297), whereas PSD-95 decrease the D1R-mediated cAMP production. Lastly, the binding properties of D1 receptor was impacted in co-transfected HEK293 with SAP102 and exhibited both lower Kd and Bmax values relative to D1 receptor. Interestingly, SAP102 does not alter neither D5 receptor-stimulated cAMP production nor D5 receptor-binding properties.

Conclusion

Overall, the results provide a novel mechanism for SAP102 where it specifically modulates the interaction and the signaling properties of D1 receptor.

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ULK1-mediated phosphorylation of ATG16L1 promotes xenophagy, but destabilizes the ATG16L1 Crohn's mutant

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Background: Autophagy is a highly regulated catabolic pathway that is potently induced by stressors including starvation and infection. An essential component of the autophagy pathway is an ATG16L1-containing E3-like enzyme, which is responsible for lipidating LC3B and driving autophagosome formation. ATG16L1 polymorphisms have been linked to the development of Crohn's disease (CD) and phosphorylation of CD-associated ATG16L1 (caATG16L1) has been hypothesized to contribute to cleavage and autophagy dysfunction.

Objective: Determine the anti-microbial functions of ULK-mediate phosphorylation of ATG16L1 under both wildtype and Crohn's disease backgrounds

Methods: In vitro kinase assay, mass spectrometry, western blot, immunofluorescence microscopy, colony forming assays

Results: Here we show that ULK1 kinase directly phosphorylates ATG16L1 in response to infection and starvation. Moreover, we show that ULK1-mediated phosphorylation drives the destabilization of caATG16L1 in response to stress. Additionally, we found that phosphorylated ATG16L1 was specifically localized to the site of internalized bacteria indicating a role for ATG16L1 in the promotion of anti-bacterial autophagy. Lastly, we show that stable cell lines harbouring a phospho-dead mutant of ATG16L1 have impaired xenophagy.

Conclusions: In summary, our results show that ATG16L1 is a novel target of ULK1 kinase and that ULK1-signalling to ATG16L1 is a double-edged sword, enhancing function of the wildtype ATG16L1, but promoting degradation of caATG16L1.

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Perioperative arginine immunonutrition prevents metastases by accelerating Natural Killer cell recovery following surgery

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Background: Primary tumour resection for curative intent is a physiologically stressful process reported to contribute to an increased occurrence of metastases. Previously, we have shown that Natural Killer (NK) cell function is profoundly suppressed after surgery and that the rapid expansion of myeloid derived suppressor cells (MDSCs) mediates this. MDSCs express the arginine-metabolising enzyme, Arginase-1, and arginine bioavailability is quickly reduced post-surgery. Numerous meta-analyses have reported that perioperative arginine supplementation in surgery patients can reduce post-operative complications and hospital length of stay, but do not make mention of how arginine is improving post-operative recovery.
Objective: Investigate the impact of perioperative arginine on NK cell function and tumour metastases following surgery.
Methods: C57Bl6 mice were fed an arginine-enriched diet (AED; 4% greater arginine content than control) or control diet ad libitum for 14 days. Lung metastases, NK function (cytotoxicity, IFN-gamma production, cell-surface markers), MDSC expansion and blood arginine levels were assessed following IV injection of B16F10LacZ melanoma cells and surgical stress (abdominal laparotomy and nephrectomy) in both groups.
Results: Surgical stress rapidly decreased blood arginine levels (180µmol to 95µmol, n=13/group) 4hrs post-operation and led to a >2-fold increase in MDSCs (CD11b+Gr1+) with increased Arginase-1 expression and activity. The AED was able to increase pre-operative arginine levels 1.7-fold higher compared to control diet (278.8µmol vs 161.2µmol, n=16/group). Following surgery, AED fed mice had a clear reduction in post-operative lung metastases compared to control diet (90 vs 220; n=24/group). Importantly, depleting NK cells (anti-NK1.1) confirmed that the effects of AED on lung metastases was dependent on NK cells. NK functional assays revealed accelerated recovery in AED mice. Cytotoxicity, IFN-gamma secretion, and activation markers (NKG2D, DNAM1) were all returned to baseline by 72hrs in AED mice, but not control mice. Lastly, pre-operative depletion of MDSCs (anti-Gr1) or inhibition of Arginase-1 (with CB1158) prevented the drop in arginine at 4hrs indicating a major role of MDSCs to post-operative arginine regulation.

Conclusion: NK cell dysfunction after surgery is intimately tied with metastatic relapse and there are currently no perioperative therapies approved to combat this. Arginine immunonutrition around the time of surgery improves immediate postoperative outcomes but the mechanism has yet to be elucidated. This study is the first to show that perioperative arginine can protect against surgery-induced metastases by accelerating NK cell recovery following surgery.

The immuno-suppressive role of plasma gelsolin in ovarian cancer chemoresistance

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Background: Ovarian Cancer (OVCA) is the leading cause of death in gynecologic cancer. Although combined surgical debulking and chemotherapy is an important treatment strategy, chemoresistance remains a major challenge. Tumor-derived soluble factors down-regulate immune cells which influence the responsiveness of cancer cells to chemotherapy. Although exosomes are involved in cell-cell communications, their role in chemoresistance in OVCA is unclear. Although we have previously shown that total gelsolin (GSN) overexpression in gynecologic cancers is significantly associated with chemoresistance, poor prognosis and cancer deaths, we have yet to demonstrate if these effects are as a result of the secreted isoform plasma gelsolin (pGSN) or the cytosolic isoform (cGSN). Here, we hypothesize that exosomal pGSN suppresses the anti-tumor functions of T-cells and M1 macrophages as well as present as biomarker for early stage detection and residual disease (RD) prediction.

Objective: To determine if and how OVCA cell-immune cell interactions regulate chemosensitivity and how deregulation of these interactions modulate TME immunity and tumor chemosensitivity.
Methods: This involved clinical and in vitro studies with primary OVCA cells and OVCA cell lines with various histologic subtypes, human peripheral CD4+ and CD8+ T-cells, OVCA patient TMA, murine ID8 cells, THP-1 monocytes and human peripheral CD14+ monocytes. T-cell/M1 macrophage and OVCA cells cultures/co-cultures, gain- and loss-in-functions studies, extracellular vesicle dynamics, apoptosis, cytokine and immune profiling and protein expression were assessed with standard molecular and cellular techniques to determine the mechanisms involved in pGSN-mediated OVCA chemoresistance and immune suppression.

Results: We have shown that pGSN secreted and transported via exosomes, up-regulates HIF-1α–mediated pGSN expression in chemoresistant OVCA cells in an autocrine manner and confers cisplatin resistance in otherwise chemosensitive OVCA cells. In chemosensitive condition, exosomal pGSN secretion is low hence allowing an optimal CD8+ T-cell function. This resulted in elevated levels of IFNγ which reduced glutathione (GSH) production and sensitized chemosensitive cells to CDDP-induced apoptosis. In the chemoresistant condition, increased exosomal pGSN secretion by OVCA cells induced caspase-8/3-dependent apoptosis in CD8+ T-cells and M1 macrophages. IFNγ secretion was therefore reduced, a response that resulted in high GSH production and CDDP resistance in OVCA cells. Pre-operative plasma levels of pGSN showed higher test accuracy compared with CA-125 as favorable biomarker for early stage detection, RD and patients' prognostification.

Conclusion: These findings suggest that pGSN may play a role in immune-modulation and chemoresistance in OVCA, providing novel insights into the coldness of OVCA and suggesting an efficient alternative therapy.

Assessing Natural Killer cytotoxicity towards human melanoma cell lines

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Background: Natural Killer (NK) cells play a significant role in anti-tumor immunity through their cytotoxic activity. Enhancing NK cell cytotoxicity is an attractive immunotherapeutic target for treating cancers. Currently, we are conducting a drug screen to identify drug candidates capable of boosting NK cell cytotoxicity. We will then validate the drug hits using multiple cancer cell lines. Here, we identify melanoma cell lines that are suitable targets for NK cell-mediated killing.

Objective: To validate whether identified drug hits will increase NK cell cytotoxicity towards melanoma cell lines.

Methods: First, the M14 melanoma cell line was transduced with a retroviral vector encoding GFP. Cytotoxicity assays were performed to compare NK cell killing of K562-GFP cells (leukemia cell line) and M14-GFP cells. NK92 cells (human NK cell line) were cultured with K562-GFP or M14-GFP cells at 1:1, 3:1, 9:1, and 27:1 effector to target (E:T) ratios for five hours. After the incubation, NK cell cytotoxicity was analyzed by flow-cytometry or a fluorescent microplate reader assay.

Results: GFP expression in M14 cells was confirmed by flow cytometry. M14-GFP cells were then sorted to obtain a pure population. To assess NK cell-directed killing of M14-GFP cells, GFP fluorescence was measured by using a fluorescent plate reader. We show that loss of GFP fluorescence is detectable at increasing E:T ratios, suggesting a decrease in target cell viability. These results were also confirmed by flow cytometry.

Conclusion: We determined by flow cytometry and a fluorescent microplate reader that loss of target cell fluorescence can be used to measure NK cell cytotoxicity. The cytotoxicity assay revealed that M14 cells are suitable NK cell targets. In the future, we will use these melanoma cell lines to validate drug-screen candidates.

The Hippo pathway is a central mediator of receptor tyrosine kinase during tumorigenesis

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Background: The Hippo pathway plays important roles in organ size control, tumorigenesis, tissue homeostasis, mechanotransduction, immunity, stem cell and differentiation. Identification of novel regulators of the Hippo pathway is one of important research areas in cancer biology. While several stimuli of the Hippo pathway have been reported, it upstream regulators are mainly unknown.

Objective: The goal of this work was to identify novel receptor tyrosine kinase regulating the Hippo pathway.

Methods: In our study, we used split luciferase complementation assay to create the first biosensor to monitor the activity of LATS kinase, a central player of the Hippo pathway. We used this LATS biosensor to perform gain of function screen for new RTKs regulating the Hippo pathway.

Results: Through our biosensor-based screening, we have identified several novel RTKs (e.g. FGFR, TAM, RET, VEGFR, etc) that regulate the Hippo pathway by downregulating LATS. Moreover, we have investigated the molecular mechanisms underlying the regulation of the Hippo pathway by these RTKs and their roles in tumorigenesis. We have further validated multiple RTKs including TAM family (Tyro3, AXL, and MERTK), RET, and FGFR and their extracellular growth factor ligands GAS6/GDNF/FGF and found that the Hippo pathway is a critical mediator of the transformed phenotypes (e.g. cell proliferation, transformation, cell motility, angiogenesis) induced by all these RTKs and their respective ligands. Also, for the first time we found tyrosine phosphorylation at different site on YAP/TAZ by RTKs, which activates YAP/TAZ during tumorigenesis.

Conclusion: In conclusion, by using our newly developed Hippo activity biosensor and a RTK library, we performed the first systematic gain-of-function screen for novel RTKs regulating the Hippo pathway. Interestingly we identified the Hippo pathway and YAP/TAZ oncoproteins as a central output of various biological processes induced by ligand activation of multiple RTKs.

Enhancing the spread of oncolytic measles virus

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Background

Immunotherapies are emerging as a promising approach to cancer treatment where the patient's own immune system is stimulated to overcome immunosuppressive barriers in the tumour microenvironment. Oncolytic measles virus (MeV) is a replication competent vaccine strain capable of generating a strong cancer-specific immune response. The focus of our laboratory is genetically engineering MeV to improve viral delivery, targeting, cytotoxicity, and immunogenicity, with the goal of combining these approaches to develop next generation vectors possessing a superior therapeutic index. It has been observed that the replication and spread of MeV through solid tumours is limited.

Objective

To address this, we are exploring methods of downregulating host antiviral restriction factors through RNA interference to boost measles oncolysis. Furthermore, we will explore methods of delivering favorable siRNAs to the tumour, as cargo in nanoparticles or by encoding therapeutic artificial microRNAs into the virus.

Methods

siRNAs designed to specifically target host antiviral genes are transfected into different cancer cell lines, followed by infection with measles virus encoding a green fluorescent marker (GFP). Fluorescence microscopy images are taken throughout the incubation to visually assess the effect of target gene knockdown on measles infection. Viral progeny is measured by TCID50, and cytotoxicity using the XTT cell viability assay.

Results

During an siRNA screen of different antiviral restriction factors, one siRNA drastically boosted measles spread and cytotoxicity in different tumour entities. This effect has been extensively characterized in cell culture, and seems to be unique to the chosen siRNA sequence and involves more than knock-down of its main target protein. Currently, we hypothesize the phenotype is caused by off-target effects whereby another gene is being downregulated unintentionally.

Conclusions

One siRNA is significantly enhancing measles fusion and spread in cancer cell monolayers *in vitro*. Future work will elucidate the mechanism, and it's relevance in enhancing the spread of oncolytic measles virus through tumours *in vivo.* Methods of delivering this siRNA to the tumour will also be explored.

Phenotype characterization of Leber's hereditary optic neuropathy mouse models

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Background: Leber's hereditary optic neuropathy (LHON) is a rare inherited disease affecting approximately 1 in 50,000 people worldwide. It is caused by one of several possible mutations in mitochondrial DNA, that produce a mutant Complex I subunit involved in oxidative phosphorylation. These mutations lead to an increase in the production of reactive oxygen species (ROS), and ultimately, to retinal ganglion cell (RGC) apoptotic death. Current LHON animal models are not without their limitations. Once disease onset is induced, histological and functional changes consistent with LHON take a long time to develop.

Objective: Our objective is to create an LHON mouse model with accelerated onset, and to expand upon the knowledge regarding the pathology of existing models.

Methods: One of the current models of LHON is generated by expressing the mutant human *ND4* gene in RGCs in wildtype mice. In order to accelerate disease onset, we will introduce the mutant *ND4* gene to RGCs in mice with a background of increased oxidative stress. We will test RGC function over time using electroretinogram (ERG) measurements and then harvest the mouse retina to test for histological differences. The age of onset in these mice will be compared to current models which harbor the *ND4* mutation on a wildtype background. Secondly, we will characterize the functional and histological phenotype of an existing mouse model carrying a mutation in the mitochondrial *ND6* gene. RGC function in these mice has not previously been tested. Finally, samples from all mice will be collected to measure ROS levels in the retina.

Results: RGC functional data shows that the LHON mice display a trend of decreased ERG responses. However, this data is not statistically significant and it remains to be seen whether this decrease is due to normal aging or the modeled disease. Additional animals are being tested to increase the sample size in order to reach significance. ROS assays are underway to determine if the retinas of the mutant mice have increased oxidative stress in comparison to wildtype retinas. These will help us form a more complete picture of what is going on in the retina on a molecular level.

Conclusions: We believe that introducing the *ND4* mutation to mice with a background of oxidative stress will accelerate the onset of an LHON phenotype. Once the LHON phenotype has been induced and characterized, we can test potential therapies for LHON in both the *ND4* and *ND6* mutant models.

Quality and safety in long-term care in Ontario: the impact of language discordance

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Background. Linguistic barriers can compromise access and quality of healthcare. In Ontario, the quality of long-term care (LTC) have improved over time. However, variations by language and other sociodemographic and health factors have not been examined. We conducted a cross-sectional analysis of the quality of LTC in Ontario in 2016 to identify disparities by language group and assess the effect of the language discordance on the quality of care.

Methods. Retrospective cohort of 47,727 nursing homes residents across Ontario. Resident assessments using the RAI-MDS from the Continuing Care Reporting System (CCRS), were used to estimate quality and safety indicators of LTC (pain, falls, physical restraints, depression and use of antipsychotics). French Designation status was used as proxy for the language of the nursing home. All measures were estimated by language group (Anglophones, Francophones) and designation status. Multivariate logistic regression models were used to assess the effect of language discordance, language of resident and language of the home (i.e. French designation status), on the quality of care.

Results. Overall, Francophones had significantly higher levels of pain (11% vs 10%) and physical restraints (7.3% vs. 5.2%), whereas a higher proportion of Anglophones experienced depression (24% vs. 23%) and had pressure ulcers (11% vs. 9%). Unadjusted rates showed worse outcomes for most quality indicators among Francophones living in non-designated homes and Anglophones in designated facilities. Francophone residents in non-designated homes had higher levels of use of antipsychotics, depression, pressure ulcers, and falls compared to Francophones in designated homes. Among Anglophones, those in designated homes had higher levels of all indicators except for pressure ulcers, which was higher in designated facilities. The fully adjusted analysis showed that Anglophones were less likely to receive antipsychotic medication (OR 0.89; 95%CI:0.77-1.02), and more likely to experience depression (OR 1.05; 95%CI:0.97-1.13), pain (OR 1.09; 95%CI:0.93-1.27), and be physically restrained (OR 1.08; 95%CI:0.82-1.41). These differences were not statistically significant. Cross-level interaction between language of resident and language of facility, showed no effect on the quality indicators, except for pain. Both language groups in non-designated homes were significantly less likely to experience pain.

Conclusions. Quality indicators outcomes tend to be worse in the language discordance context by designation status of the nursing home in Ontario. However, after adjusting for individual and facility-level characteristics there was no effect of the discordant context in the quality of care, except for the experience of pain.

Chromatin remodelling during myoblast differentiation involves the caspase-dependent removal of Satb2

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Background: Chromatin remodeling is an essential event that occurs during myoblast differentiation to allow for cell fate-specific genes to be efficiently expressed. The special AT-rich sequence-binding (Satb) protein family consists of chromatin architectural proteins that have been implicated in the regulation of stem cell differentiation.

Objective: This study examines the role of Satb2 in myoblast differentiation.

Methods: Western blot and immunofluorescent analyses assessed the expression profile of Satb2 during C2C12 differentiation. Subsequent siRNA-mediated knockdown of Satb2 probed the role of Satb2 in the differentiation process. ChIP-seq was then performed to look at the binding targets for Satb2, and this was followed by RNA-seq on Satb2-expressing and -deficient cells to assess changes in expression profiles. Treatment of *Pax7CreER/Satb2*$^{fl/fl}$ mice with tamoxifen was used to assess the in vivo effects of Satb2 loss in muscle satellite cells. A caspase cleavage assay and an in vitro inhibition of caspases with DEVD were then used to assess the potential role of caspases in regulating Satb2 levels during myoblast differentiation. Subsequent siRNA-mediated knockdown of caspase 7 was used to confirm its role in regulating Satb2 levels.

Results: Satb2 expression decreases during early myoblast differentiation and is virtually nonexistent in mature muscle fibers. The decrease in Satb2 expression during myoblast differentiation is the result of its caspase 7-dependent cleavage following the initiation of satellite cell differentiation. The premature knockdown of Satb2 led to the accelerated expression of myosin heavy chain in differentiating muscle cells. Moreover, the in vivo loss of Satb2 in muscle satellite cells led to a decrease in overall muscle fiber size and a reduction in the number of Pax7-expressing progenitor cells.

Conclusion: This study suggests that the temporal control of caspase 7-dependent cleavage of Satb2 in skeletal muscle satellite cells is critical for the proper execution of the myogenic differentiation program.

Feasibility Study of Narrative Exposure Therapy in Homeless Individuals with Post-Traumatic Stress Disorder

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Background: Mental health disorders, including post-traumatic stress disorder (PTSD), are common among individuals who are homeless. Homeless individuals have higher rates of premature mortality especially from suicide, violence, and accidents, and attend emergency departments more often than non-homeless individuals. These issues result in a population with complex needs that traditional mental health care is poorly equipped to serve.

Objective: The aim of this pilot study is to assess the feasibility and acceptability of providing Narrative Exposure Therapy, which attempts to place the trauma within a narrative of the individual's life, within the shelter system to homeless individuals who suffer from PTSD.

Methods: We conducted a parallel group randomized controlled trial (RCT), allocating participants into either receiving individual Narrative Exposure Therapy or individual Narrative Exposure therapy plus the option of having a genealogy report. Our primary outcome of interest is the acceptability and feasibility of Narrative Exposure Therapy, while other secondary outcomes include acceptability and feasibility of collecting outcome data, PTSD symptoms, housing status, quality of life, and substance use.

Results: To date, a total of 12/24 (50%) of participants have been enrolled in the study, including 7 males and 5 females with a mean (SD) age of 43 (7.4) years. We will present preliminary baseline and follow-up data including a preliminary assessment of feasibility and acceptability of the study intervention. The intervention will be deemed to be acceptable to participants if 50% of individuals approached to participate are enrolled in the study. Similarly, conducting a large-scale RCT will be considered feasible if we could recruit the target sample size within a six-month period. We will also present health outcome data using descriptive statistics for the appropriate rating scale outcome with 95% confidence intervals.

Conclusion: There is a clear gap in the provision of services that address the experience of trauma in homeless individuals. The results of this study will help design a full-scale RCT and hopefully inform the treatment of homeless individuals who struggle with PTSD.

Effects of Resistant Starches on Inflammatory Bowel Disease: Preclinical and Clinical Systematic Reviews

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Background: Resistant starches (RS) may represent a novel treatment for inflammatory bowel disease (IBD), a debilitating chronic disease with limited treatment options.[1] However, questions about the efficacy and safety of RS as an IBD treatment remain unanswered. In preparation of an early phase efficacy trial to be undertaken, we performed two systematic reviews of RS use for IBD.

Objective*:* Evaluate the effects and safety of resistant starch as a therapy within populations of inflammatory bowel disease.

Methods: Two literature searches were performed on Medline, EMBASE, and the Cochrane Central Register of Controlled Trials. In the clinical review, studies of RS therapy in IBD participants were included. In the preclinical review, studies of RS therapy using *in vivo* animal models of IBD were included. The primary outcomes were: clinical remission (clinical review) and histological evaluation of bowel (preclinical review). A random effects model was used in the meta-analysis.

Results: Seven clinical and 20 preclinical studies met eligibility. Clinically, 80% of studies reporting clinical remission demonstrated significant improvement in the RS groups. No increase in adverse events was seen. Preclinically, RS was associated with a significant reduction in histological damage compared to control (standardized mean difference -1.62, 95% confidence interval -2.23 to -1.02). No preclinical studies reported adverse events. In both reviews, studies were at a high or unclear risk of bias.

Conclusion: We demonstrated that RS was associated with reduced histology damage in animal studies, and improvements in clinical remission in IBD patients. However, the methodological quality of studies was poor, and high-quality randomized trials are needed.

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Identification and Evaluation of Novel Rhabdomyosarcoma Antigens for Use in Oncolytic Vaccines

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Background: Cancer immunotherapies focused on tumor-specific T cell response are promising therapeutic alternatives for cancer because in addition to eliminating cancer cells, they can also establish an active and long-term surveillance against relapsing tumours. The efficacy of such therapies depends primarily on the immunogenicity of the tumours. However, the lack of targetable and known specific antigens for rhabdomyosarcoma (RMS) poses as a limitation for the development of active immunotherapies against this cancer. Thus, identification of immunogenic antigens is a critical step. One such methodology for the identification of cancer antigen involves peptide elution from MHC I derived from the tissue of interest. Mass spectrometry is then performed to identify the amino acid sequence of the eluted peptides and validated for immunogenicity.

Objective: The goal of this project is to identify potential RMS-associated or RMS specific antigens that can be employed in a prime-boost oncolytic vaccine.

Methods: The method for identifying RMS antigens involves first the elution of ligands from MHC I isolated from a RMS (76-9 cell line) bearing and oncolytic virus treated mouse. Mass spectrometry is then used to identify the amino acid sequence of the eluted peptides. The resulting MHC I ligands are then screened using an optimized approach involving prediction software by Dr. Shashi Gujjar (University of Dalhousie, Halifax) in order to predict the immunoprecipitates most likely to mount anti-tumor specific CD8+ T cell responses. Ligands that produce potent CD8+ T cell responses *in vitro* will then be used to immunize mice and assessed for their potential as peptide and oncolytic vaccines.

Results: The initial MHC I ligand screening from 76-9 tumour tissue and spleen led to the identification of 107 unique peptides not present in naïve spleen. Of these, up to 23 peptides were identified as having immunogenic potential based on their ability to induce cytokine production from CD8+ T cells isolated from 76-9 tumour bearing spleen.

Conclusion: Identification of peptides by way of peptide elution from MHC I and verification of their immunogenic potential through *in vitro* methods is a promising approach to discover sarcoma specific antigens. This study serves as a pipeline for the discovery of sarcoma antigens and their incorporation into T cell based immunotherapies such as oncolytic vaccines.

Intact viral sensing mechanisms may correlate to efficacy of autologous infected cell vaccines in models of murine leukemia

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Background

Acute leukemia is an aggressive disease with a poor 5-year prognostic. Although recent advances such as CAR-T therapy or checkpoint blockade are promising, they vary in their efficacy between patients, are associated with significant toxicity and may not elicit long term protection. Our group has previously shown that a vaccination strategy using autologous leukemia cells infected with an oncolytic virus ex-vivo (infected cell vaccine) can elicit a robust and durable anti-tumor response in a model of Acute Lymphoblastic Leukemia (L1210) but not in an Acute Myeloid Leukemia model (C1498). One major difference between these models is the expression of ssRNA viral sensors (i.e. TLR7).

Objectives

To determine the differences in intrinsic antiviral responses between L1210 and C1498 cell lines and whether these contribute to the efficacy of infected cell vaccines.

Methods

Total cellular RNA or total protein and cell culture supernatants were collected from L1210 and C1498 cells after infection with MG1-GFP or VSV-GFP virus (MOI:10). TLR7-KO L1210-Cas9 cells were generated using CRISPR. Following the isolation and expansion of single clones, genome editing results were analyzed through Synthego's online CRISPR analysis tool *ICE*.

Results

Here we report a robust response by the L1210 cell line following viral infection, characterized by production of IFNβ, RANTES and IL-6 as well as an upregulation of CD40. This response was not observed in the C1498 cell line, which additionally succumbs faster to oncolytic viral infection. Interestingly, while the antiviral mechanisms were still intact in L1210 cells, we observed no phosphorylation of IRF3 in C1498 which suggests a defect in their ssRNA viral sensing mechanisms. Therefore, we assessed the importance of viral sensing mechanisms in L1210 cells by using a TLR7-KO L1210 cell line. Our TLR7-KO L1210 cells produced less IFNβ compared to wildtype, however, there was still IFNβ production higher than mock infected cells. A L1210 model fully deficient in type I interferon production is currently being developed to address whether interferon production by the infected cell vaccine is necessary to generate a robust anti-tumor response.

Conclusion

In consideration of these findings, we hypothesize that intact viral sensing mechanisms are important for mounting a robust immune response to autologous infected cell vaccines. Therefore, these findings can be leveraged to create a more immunogenic infected cell vaccine.

Implementation of a Collagenase-Encoding Oncolytic Vaccinia Virus for the Treatment of Solid Tumors

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Oncolytic viruses (OVs) are promising cancer-targeting therapeutics due to their preferential tropism for cancer cells and the ability to encode transgenes that enhance their cytotoxicity. However, the infiltration of OVs into solid tumors is inhibited by the dense extracellular matrix (ECM) that composes the local tumor microenvironment. Collagen is a major component of the ECM and an important determinant of cancer metastasis. It has also been identified as a barrier that prevents the distribution of OVs within tumors. Here, we implement a transgenic oncolytic Vaccinia virus expressing collagenase from *Clostridium histolyticum* to selectively degrade collagen in the tumor microenvironment. Preliminary results from *in vitro and in vivo* experiments will be presented.

Palliative Bowel Surgery: Are we helping patients?

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BACKGROUND: Malignant bowel obstruction (MBO) is a frequent complication in the metastatic cancer population with a reported incidence of 28-51% and high rates of morbidity and mortality. Selecting appropriate management, either surgical or medical, is crucial in prolonging survival and maximizing remaining quality of life; however, two Cochrane reviews published in 2000 and 2015 demonstrated no consensus. A prediction model to identify patients who would most benefit from palliative bowel surgery is needed.

OBJECTIVES: The primary objective of this study is to develop a prediction model to predict successful surgical palliation, defined as survival of at least 3 months post-operation. The secondary objectives are to evaluate and compare the demographics, clinical characteristics and outcomes ofsurgically vs. medically managed patients presenting with MBO.

METHODS: A retrospective single-center study examined demographic, clinical and surgical variables/outcomes for patients managed medically or surgically, following presentation of malignant bowel obstruction (MBO) between 2008-2017. Mann-Whitney-U test was used to compare continuous variables; Fischer's-exact test for dichotomous variables; Log-rank for survival curve comparison. In the surgical group, a bivariate and multivariate logistic regression analysis identified predictors of death at 3 months following MBO surgery.

RESULTS: Among 402 patients, 144(36%) were surgically managed and 258(64%) were medically managed. Significant differences($p<0.05$) between surgical/medical groups: survival, ascites, lymph nodes/soft tissue metastases, active treatment at acute presentation, discharge location. As expected given the selection bias for healthier surgical patients, the surgical group had higher survival at 3 months [68.4%(95%CI:60.6-76.2) vs 34.9%(95%CI:28.8-41.0)], at 24 months [31.8%(95%CI:23.6-40.0) vs 8.7%(95%CI:4.78-12.6)($p< 0.001$) and longermedian survival [8 months(0-70) vs 1 month(0-87)($p<0.001$)]. In the surgical group, low albumin, diversion surgery, ascites and >/= 2 metastatic sites were combined to form a multivariate prediction model (R^2:0.532) of dying within the first three months.

CONCLUSIONS: Predictors of death at 3 months following MBO surgery were combined to form a strong prediction model. From this, we hope to develop, internally and externally validate a simple clinical risk score to appropriately select patients for palliative surgery, with the goal of increasing quality of life, reducing morbidity and maximizing potential to continue palliative treatment.

Do patients with prostate cancer benefit from peri-operative hormonal therapy? A systematic review and meta-analysis

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Background:
Thousands of men with non-metastatic prostate cancer are treated by radical prostatectomy in Canada each year. While some men are cured with surgery alone, a substantial proportion will experience cancer recurrence. Androgen deprivation therapy (ADT), a hormonal treatment that interferes with prostate cancer growth, is proven to be effective for patients treated with radiation. ADT may also reduce the risk of recurrence in surgically treated patients, but the effect of ADT in this setting is not well established.

Objective:
To review the evidence evaluating peri-operative ADT for patients with prostate cancer.

Methods:
MEDLINE, EMBASE and the Cochrane Library were searched from inception to June 2018. Randomized control trials comparing ADT with radical prostatectomy versus prostatectomy alone in patients with clinically localized prostate cancer were included. Screening, full-text selection, data extraction, and risk of bias assessments were performed in duplicate by two independent reviewers. The primary outcomes were cancer recurrence and overall survival. Secondary outcomes included pathologic outcomes in patients treated with ADT prior to surgery.

Results:
Overall, 15 randomized trials involving 7520 patients evaluated at least one of the primary or secondary outcomes. Eleven studies evaluated the effects of neoadjuvant (prior to surgery) ADT (n=2,322) and four studies evaluated the effects of adjuvant (after surgery) ADT (n=5,198). Neoadjuvant studies were limited because the treatments were only administered for 3 months. Adjuvant studies were also limited because they only evaluated weak anti-androgens. Administration of ADT for 3 months prior to surgery significantly decreased the rate of positive surgical margins (pooled RR 0.48, 95%CI 0.41-0.56) and extraprostatic tumour extension (pooled RR 0.75, 95%CI 0.64-0.89). However, no differences were observed for overall or progression free survival (pooled RR 1.32, 95%CI 0.82-2.13; pooled RR 0.99, 95%CI 0.73-1.33, respectively). Similarly, administration of a weak anti-androgen following surgery demonstrated no difference in overall or progression free survival in the 3 trials that presented these data (pooled RR 1.02, 95%CI 0.92-1.13; pooled RR 0.77, 95%CI 0.58-1.01, respectively).

Conclusion:
There is limited evidence to assess the effect of peri-operative ADT. Based on the available literature, short-term neoadjuvant ADT causes a pathologic downstaging of prostate tumours, but does not reduce the risk of prostate cancer recurrence and does not extend survival. No study evaluates the effect of longer durations of ADT. Given the strong rationale for peri-operative ADT, further trials are needed to evaluate the benefits and harms of this treatment.

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Getting patients to scopes in the north: a review of endoscopy cancellations in remote northern Canada

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Background: Colonoscopy is a critical diagnostic and therapeutic tool and access to these specialized medical services is challenging in rural northern Canada. Colonoscopy is important for managing gastrointestinal disease including colorectal cancer which may be diagnosed, and in some cases removed, endoscopically. The importance of timely access to colonoscopy is therefore reflected in Canadian CRC screening guidelines, which recommend patients wait no longer than 60 days after positive FIT(Sey et al., 2012). Patients in northern Canada face challenges in accessing timely colonoscopy, in part, due to frequent cancellations, for which, the reasons remain poorly understood.

Objective: We sought to better understand the trends and reasons for colonoscopy cancellation at a territory hospital in the Northwest Territories.

Methods: We conducted a retrospective review of all colonoscopies which had been cancelled between January 1 2018 – January 1 2019 at the Medical Daycare Unit(MDCU) at the Stanton Territorial Hospital (Yellowknife,NWT) as identified by the MDCU cancellation logs. Community of residence, timing of cancellation, and reason for cancellation were captured as recorded by clerical staff. A thematic analysis was conducted to group the reasons for cancellation. Descriptive statistics were generated using Microsoft Excel V16.16.13.

Results: We found 373(20.4%) of scheduled colonoscopies were cancelled during the 12 month period. 54(14.5%) of cancellations attributed to patient “no shows”, and occured 28 days after booking. Reasons for cancellation were grouped into 20 themes, covering a range of personal, social, systematic, and geographic factors. The most frequently cited theme was work or family commitments (indicated by 70(78.02%)). Others frequently cited include being away from home, unwell, and physician-initiated for medical reasons. Analysis of distance traveled among cancelled patients is in progress.

Conclusion: Patients need support from employers and family in order to travel for medical procedures in the NWT. Further analysis of the root cause for these cancellations is needed to mitigate delays in access and inefficiencies in colonoscopy delivery. Collaborative efforts may be needed between employers, medical travel agencies, and homecare supports to reduce cancellations is this region.

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METABOLIC IMPAIRMENT IN MALADAPTATIVE RIGHT VENTRICULAR REMODELING SECONDARY TO PULMONARY ARTERIAL HYPERTENSION

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Background: Altered right ventricle (RV)energy metabolism has been previously reported in pulmonary arterial hypertension (PAH);however, the mechanisms underlying the transition to right heart failure inPAH remain unclear. Therefore, we explored the role of mitochondria function and energy metabolism alterations in a model of maladaptive RV remodeling secondary to severe PAH.

Methods/Results: Severe PAHwas induced by single subcutaneous injection of SU5416 in Sprague-Dawley (SD) or Fischer rats (150-250g), followed by 3-weeks of chronic hypoxia (SUHx). Fischer rats exhibited significantly higher right ventricular systolic pressure (RVSP) than SD rats (111±4 mmHg vs. 81±2, respectively; $p<0.001$) in response to SUHx. Despite a similar degree of RV hypertrophy, Fischer rats showed progressive RV failure with 100%mortality by 6 weeks whereas SD exhibited excellent survival to 7 weeks. Mitochondria function, measured 4 weeks post SUHx in permeabilized cardiac fibres isolated from the RV was similar between strains,and not significantly altered by SUHx, whereas mitochondrial ROS production was decreased similarly in both strains, consistent with depressed mitochondrial oxidative capacity.Interestingly, RV cardiomyocytes from Fisher, but not SD, SUHx rats showed a significant decrease in tricarboxilic acid cycle activity (Citrate synthase: 0.98 vs. 1.82 mU/mg protein) and palmitoyl-CoA supported respiration, despite similar mitochondrial content, suggestive of strain-dependent differences in bioenergetics and fatty acid metabolism in severe PAH. Moreover, increasing glucose oxidation by administration of dichloroacetate (75 mg/kg/day) to Fischer ratsf ailed to prevent the development of PAH or RV maladaptationin the SUHx model.

Conclusion: Our data suggest that loss of mitochondria oxidative capacity and impairment of fatty acid oxidation, rather than glucose oxidation, play an important role in RV maladaptation in response to pressure overload.

A Mental Health Needs Assessment of Ottawa's First Responders: A preliminary review of interviews with the Ottawa Fire Service and Ottawa Police Service

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Background

Due to the nature of their employment, First Responders (including firefighters, paramedics and police officers) regularly encounter emergency situations, and are frequently exposed to critical stress incidents. Routine exposure to these dangerous, often life-threatening and potentially traumatizing incidents, places First Responders at increased risk of developing mental disorders such as: post-traumatic stress disorder (PTSD), depression, generalized anxiety disorder, alcohol use disorder, and the development of suicidal thoughts. The most significant barriers to providing mental health care to First Responders are: 1) how to ensure that it is provided in a safe and acceptable fashion; and 2) knowing how to prevent mental disorders within this population. To evaluate these barriers and inform a First Responders treatment strategy, we are conducting a Needs Assessment with active First Responders of the City of Ottawa's Tri-Services and other key stakeholders.

Objective

The primary objective is to explore First Responder treatment preferences and access to care, in addition to possible resiliency factors, treatment location and hours, inclusion of family in treatment, and current available treatment of Post-Traumatic Stress Injury (PTSI) and other mental disorders.

Method

Semi-structured qualitative interviews will be used to assess preferences for treatment and access to care among current Ottawa First Responders, including frontline workers, First Responder's who have personally dealt with mental illness, senior management, the services' respective Peer Support Groups, and representatives for their unions and the Workplace Safety Insurance Board. The needs assessment interviews were guided by Theoretical Domains Framework (TDF) and the Consolidated Framework for Implementation Research (CFIR). Interviews will be conducted until data saturation is reached, anticipated to be approximately 40 interviews. NVivo Qualitative Analysis Software will be used for coding and data analysis.

Anticipated Results

Preliminary results from interviews conducted with individuals from the Ottawa Fire Service and Ottawa Police Service will be presented. We anticipate several common themes to emerge from these interviews including anonymity, stigma, and organizational culture. We also expect to see some key differences in the responses between members from different services and between members of the same service with varying seniority.

Conclusion

Both the common and divergent themes will inform the development and implementation of a specialized clinic for First Responders and how best to engage with each service. This clinic will provide additional mental health resources for a high need population and has the potential to improve mental health outcomes, including a reduction in suicide rates, for First Responders.

Defining the role of excitatory/inhibitory imbalance to the phenotype of forebrain-specific conditional Atrx KO mice

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Background

The ability of a neuron to respond to external cues is dependent on its capacity to remodel chromatin, and this is reflected in the large number of intellectual disability syndromes caused by mutations in epigenetic regulators. Indeed, mutations in the chromatin remodeler *ATRX* cause the ATR-X syndrome, a neurodevelopmental disorder affecting males. Biochemical studies and characterization of mice deleted for *Atrx* has demonstrated that it is critical for the expansion of the progenitor pool as mice die shortly after birth. However, studies in the retina have indicated that *Atrx* also has important roles in post-mitotic neurons including axonal targeting and non-cell-autonomous effects on connected neighbouring cells. To investigate the mechanisms by which Atrx contributes to cognition, we have generated Atrx-Emx1-Cre (Emx1KO) mice, which are viable into adulthood and lack expression in the cortex and hippocampus. Here, we propose to dissect the function of *Atrx* in the developing neuronal circuitry towards a better understanding of how this causes intellectual disability. We hypothesize that *Atrx* dysfunction alters the balance between inhibitory and excitatory neuronal signaling in the forebrain. To address our hypothesis, we propose the following experimental aims:

Objective

1. To characterize the development of the neocortex in *Emx1KO* mice.
2. To determine if altering the excitatory/inhibitory neuron balance can recapitulate the phenotype observed in *Emx1KO* mice.

Methods

We generated ViaatKO and Vglut2KO mice to conditionally knockout *Atrx* in inhibitory and excitatory neurons respectively. We characterize the three mouse models for morphological and cellular defects in neurogenesis using a combination of immunofluorescence and birth dating experiments.

Results

Emx1KO mice had a normal lifespan, reduced body weight, and cortical thickness. Examination of the hippocampus at P0 showed a disorganization of the neuronal layer in all the subregions including the dentate gyrus, with an increase in Ki67-positive cells. Unlike the Emx1KO, both ViaatKO and Vglut2KO mice died at birth. Surprisingly, both mouse lines had no body dysmorphia and did not exhibit any developmental delay. Further investigation also showed normal corticogenesis and minor hippocampal defects.

Conclusions

Emx1KO mice had hippocampal neurogenesis impairments that were not recapitulated in the ViaatKO/Vglut2KO mice. Instead, preliminary investigation of these mice showed no neurogenesis defects. Further investigation will be done to characterize all three mouse lines to tease out the role of the excitatory and inhibitory system in the pathogenesis of ATR-X syndrome.

Transcriptional and functional dynamics of FGL2 in murine reproductive success

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Background: Fibrinogen-like protein 2 (FGL2) is a known immunomodulator and prothrombinase, previously suggested to be involved in the immune balance of the maternal-fetal interface that is crucial to reproductive success. Murine reproductive tissues are the site of several key events that require careful endocrine and immunological regulation, from ovulation to placentation.

Objective: We aim to map spatial and temporal dynamics of FGL2 expression through murine reproductive tissues, and to determine the effect of loss or excess of FGL2 on reproductive success.

Methods: Immunostaining determined localization and intensity of expression of FGL2 through the murine reproductive tract. Tissue-specific functional assays were used to determine the effect of loss or excess of *Fgl2*, and single-cell RNA sequencing was used to explore transcriptional networks of *Fgl2* activity.

Results: In the ovary, FGL2 is expressed in the stroma and theca cell layer of follicles, and intensity of expression peaks 8 hours after hCG injection in a superovulation cycle. Strong expression is acquired by some cumulus granulosa cells shortly before ovulation and persisting in cumulus-oocyte complexes (COCs) found in the oviduct, suggesting a role in ovulation and in luteinization. *Fgl2* knockout (KO) or overexpressing (tg) animals however had a normal ovulation efficiency, as measured by the number of COCs retrieved after superovulation. *Fgl2* KO and wild-type (WT) ovaries showed equivalent numbers of functional corpora lutea, with similar extent of angiogenesis and number of M2-polarized macrophages, demonstrating normal luteinization. In the oviduct, FGL2 is expressed in the secretory cells of the epithelium, whose frequency increase from the fimbrial to the isthmal end. FGL2 was detected in the culture medium of the OVE4 cell line, confirming its secretion into oviductal fluid. Estradiol (E2) suppressed this secretion, while progesterone (P4) increased it. *Fgl2* WT, KO and tg had similar conceptus morphology at e7.5, suggesting its immunomodulating function isn't critical to early embryo and placental development. *Fgl2* tg pups were however notably smaller than their WT or KO counterparts, at birth and at weaning, indicating a probable defect in placental development. Single-cell RNA sequencing of the ovary and oviduct at different timepoints after superovulation will allow us to identify compensation mechanisms allowing for normal reproductive function in *Fgl2* KO animals.

Conclusions: Overall, these results form a comprehensive map of FGL2 expression and function through murine reproductive tissues and processes.

A critical loss of lung microvasculature triggers a feed-forward mechanism of sustained flow-mediated endothelial injury leading to the progression of severe pulmonary arterial hypertension in the rat SU5416-hypoxia model

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Introduction: Endothelial cell (EC) apoptosis is a central trigger for pulmonary arterial hypertension (PAH); however, it is not clear how a discreet EC injury, for example induced by a single injection of the VEGFR2 antagonist, SU5416 (SU), can lead to protracted and progressive PAH and arterial remodeling.

Hypothesis: EC apoptosis leads to the direct loss of microvasculature through drop-out of distal arterioles. If sufficiently extensive, this microvascular loss will result in a critical increase endothelial shear stress in the remaining microcirculation, perpetuating EC injury and setting up a feed-forward mechanism driving progression of vascular damage and remodeling in PAH.

Methods and Results: Sprague Dawley rats were treated with a single subcutaneous injection of SU (20 mg/kg) or vehicle followed by a 3-week exposure to chronic hypoxia (Hx; 10% O_2; SUHx model). Increase in lung vascular EC apoptosis was evident as early as 3 days, and persisted for at least 7 weeks, post SU injection. The increases in right ventricular systolic pressure (RVSP) were first observed at day 7 post-SU (31 ± 3 vs 26 ± 1 mmHg, $p<0.01$) and progressively increased to reach a plateau at 4 weeks (84 ± 15 mmHg) with a further non-significant progression at 7 weeks (94 ± 8 mmHg). Lung microvascular volume, assessed by microCT (SkyScan 1272, Bruker, Belgium), was reduced by ~ 50% at 4 weeks post SU compared to control rats (2.1 ± 4.2x10^{10} μ^3 and 4.2 ± 5.4x10^{10} μ^3, respectively; $p<0.001$) with further decrease at 7 weeks post SU (1.6 ± 0.18x10^{10}). Interestingly, there was no increase in RVSP in the SUHx model until a threshold of ~80% microvascular loss was reached, after which a steep increase in pressure correlated closely with loss of microvascular volume ($r=0.66$; $p<0.02$). Moreover, occlusive arteriopathy, which was not evident at 4 weeks, was widespread at 7 weeks post SU.

Conclusions: In the SUHx model, persistent lung EC apoptosis is only evident after lung microvascular loss reaches a threshold of ~80%, and ongoing EC injury and apoptosis, sustained by hemodynamic abnormalities, drives the development of protracted PAH and occlusive arterial remodeling.

Necroptosis and neuroinflammation in the pathogenesis of spinal muscular atrophy

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Background: Spinal muscular atrophy (SMA) is a neuromuscular disease characterised by motor neuron loss, leading to muscle weakness and decreased mobility. Neuroinflammation has been established as a contributor in many neurodegenerative disorders and is a key player in the pathogenesis of amyotrophic lateral sclerosis (ALS). Given that ALS and SMA share many clinical features, it is possible that neuroinflammation may be contributory to motor neuron cell death and shortened lifespan. Recent findings have exposed necroptosis as a major contributor to motor neuron death in ALS. Necroptosis is mediated by receptor-interacting protein kinase 1 (RIPK1) and RIPK3. When RIPK3 is phosphorylated, inflammasome activation occurs which associates with caspase-1, ultimately causing secretion of pro-inflammatory cytokines. Interestingly, in-vivo Rip3 knockdown increased motor neuron survival in mice exposed to toxic ALS mutant astrocytes.

Objective: To explore the pathogenic significance of this molecular death pathway in SMA with the hypothesis that necroptosis mediated neuroinflammation is the basis of cell death in the spinal cord.

Methods: To gain insight into the role of caspase-1 and RIPK3 in SMA, we've generated a triple knockout (TKO) mouse model: Smn2B/-, Caspase1-/-, RIPK3-/-.

Results: Our preliminary data shows increased survival and mobility in the TKO mice compared to Smn2B/- mice. Motor neuron counts, neuromuscular junction pathology and myofiber size is currently being investigated in the TKO.

Conclusions: Understanding the implications of the necroptosis pathway in SMA pathogenesis may suggest the need to target Caspase-1 and RIPK3 to inhibit multiple cell death pathways and ameliorate neuroinflammation in the disease context.

High-content Analysis Identifies KDR to Modulate Muscle Stem Cell Self-Renewal

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Introduction

Skeletal muscle satellite stem cells in adult muscle facilitate postnatal growth and regeneration. A subset of satellite cells has been shown to recapitulate the muscle stem cell reservoir by symmetric expansion and asymmetric self-renewal via cell polarity pathways such as Wnt, JAK/Stat and p38α pathways. In Duchenne Muscular Dystrophy (DMD), dystrophin-deficient satellite cells are unable to maintain cell polarity during asymmetric self-renewal, resulting in increased mitotic errors and decreased regeneration potential. The importance of asymmetric self-renewal has also been demonstrated in aging muscle, where the decline in stem cell self-renewal leads to a reduced capability for muscle regeneration. Despite the critical role of stem cell self-renewal in the maintenance of the stem cell pool, the molecular mechanisms governing these processes are not yet well characterized. There are likely additional undiscovered cell polarity pathways that regulate satellite stem cell self-renewal, pathways that may be perturbed in mdx mice. We hypothesize that the restoration of satellite cell asymmetric self-renewal and cell polarity pathways will restore the muscle regeneration capacity in mdx mice.

Objective

1. Identify novel modulators of muscle satellite stem cell self-renewal
2. Elucidate how KDR affects muscle stem cell regeneration
3. Determine whether the role of KDR is perturbed in a mouse model for Duchenne's (MDX).

Methods

We have developed a novel high-content analysis platform using myofibers derived from *Myf5-Cre:R26YFP* mice to screen small molecule compounds previously characterized in clinical trials.

Results

We provide evidence that Kinase Domain Receptor (KDR) requires the formation of the dystroglycan complex to modulate satellite stem cell asymmetric division. Knockdown of KDR by siRNA decreases asymmetric division, while stimulation of KDR by ligand treatment increases asymmetric division. KDR stimulation was not able to rescue asymmetric division in MDX mice, and proximity ligation assay results show a direct interaction between KDR and the dystroglycan complex.

Conclusions

We provide evidence that KDR requires the formation of the dystroglycan complex to modulate asymmetric division in muscle satellite stem cells. Understanding the different pathways that modulate muscle stem cell self-renewal will provide further insight on additional therapeutic targets in the treatment of DMD.

A Bayesian probabilistic classifier for targeted lipidomics identification

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Background: Targeted lipidomics monitors a diagnostic product ion to reduce matrix complexity and maximize quantitation in the context of liquid chromatography coupled to tandem mass spectrometry via electrospray ionization (LC-ESI-MS/MS). Commonly, these approaches are used to target lipid analytes but not to unbiasedly identify lipid species. Here, we ask whether alternative bioinformatic approaches can improve detection, discovery, and quantification of low and high abundance lipid species.

Objective: We present a novel bioinformatics method for discovery-driven "targeted" lipidomics that combines precursor ion scan (PIS), multiple reaction monitoring (MRM), and information dependent acquisition-enhanced product ion (IDA-EPI) scans. Our method is capable of assigning lipid identities to MRM peaks and confirming these labels using biophysical rules that are empirically validated to influence elution across multiple LC gradients.

Methods: Using a gold standard library of matrix-specific PIS, MRM, and IDA-EPI data, our Bayesian probabilistic classifier computes the posterior probability of correctly identifying a lipid species conditioned on the observed peak retention time, relative retention time, area, height, tailing factor, asymmetry factor, full width half max, and precursor and product ion mass. Lipid labels are assigned using maximum weighted bipartite matching which considers the joint probability of correctly identifying all lipid peaks within a given transition. Validating these assignments is achieved by decomposing each lipid species into their molecular constituents and modelling the influence of individual chemical groups, modifications, and linkages on retention time.

Results: We show that Bayesian lipid identification combined with biophysical retention time modelling successfully identifies over 90% of sphingolipid species profiled within blood and brain matrices in both humans and mice. Our approach is computationally efficient, interpretable, and adaptable to any targeted LC-MS platform.

Conclusion: Using our algorithm, we demonstrate an automated method for effective lipid identification and enhanced accuracy of targeted lipidomics data.

Mesenchymal stromal cell-derived small extracellular vesicles as a potential therapeutic for treatment of pulmonary arterial hypertension

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Background: Mesenchymal stromal cells (MSCs) have shown promise as therapy for treatment of cardiovascular disease. The therapeutic effect of MSCs is predominately paracrine mediated; where the release of small (<150nm) and large (150-1000nm) extracellular vesicles (EV) plays an important part in cell-cell communication. EVs are packaged with important cargo, including proteins, mRNA and microRNA that have therapeutic potential.

Objective: Our objective is to assess the potency of MSC derived EVs as a therapeutic for pulmonary arterial hypertension (PAH). We hypothesized that small EVs released from MSCs would promote vascular regeneration in PAH, leading to improvements in pulmonary hemodynamics.

Methods: To test this, MSC derived small EVs were collected during 24h of serum-free culture and purified using sequential ultracentrifugation. EVs were characterized using ZetaView nanoparticle tracking for size distribution and western blot analysis to confirm presence of canonical EV proteins. To study the therapeutic efficacy the monocrotaline (MCT) model of PAH was used, which involved a single injection of MCT to induce PAH in Sprague Dawley rats. Three days post MCT, MSC derived EVs were delivered at 15ug protein by jugular vein. End study was performed at three weeks post MCT and right ventricular systolic pressure (RVSP) and right ventricular hypertrophy (RVH) were measured to assess severity of PAH.

Results: Characterization of size by ZetaView nanoparticle tracking revealed a population of small EVs having a median size of 108±53 nm. Western blot analysis confirmed the presence of canonical EV proteins including the tetraspanin CD63. Administration of MSC-derived small EVs lead to significant reductions in RVSP compared to vehicle control (51±3 mmHg vs 68±0.2 mmHg, respectively), and a trend towards improvement in RVH (0.4±0.02 vs 0.47vs0.06, respectively).

Conclusions: These data suggest that MSC derived EVs have therapeutic potential in the prevention of severe PAH and further experiments are warranted to optimize the dose and delivery strategies.

Proteomic Profiling of Exosome-Derived Biomarkers for Spinal Muscular Atrophy

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Background: Spinal muscular atrophy (SMA) is a neurodegenerative disease characterized by progressive skeletal muscle atrophy and death in severe cases. The genetic disorder is caused by deletion or mutation of the SMN1 gene leading to depletion of survival motor neuron (SMN) protein in cells and subsequent loss of *α*-motor neuron function. Current SMA treatments are limited, enticing the development of new approaches. However, the SMA field lacks effective biomarkers that can be used to rapidly gauge the efficacy of potential therapeutics. Our lab has recently shown that the SMN protein is naturally released from cells in extracellular vesicles (EV), and that the amount of SMN protein in the EV correlates with the disease state. Our observation suggests that EV may act as an effective biomarker for SMA. To build upon this work, we are examining the complement of proteins released in EV from tissue culture models and human patients with SMA, which may identify additional biomarkers of the disease as well as provide novel insight into the disease state.

Objectives: Determine the complement of proteins released in extracellular vesicles from tissue culture models and human patients with SMA.

Methods: EV were isolated from tissue culture models of SMA and control normal cell lines using differential centrifugation. EV were isolated from plasma from patients with SMA or normal controls using Exoquick reagent. The protein content of both samples of EV were analyzed by mass spectrometry, and bioinformatic and statistical analysis was used to determine the proteins that were differentially present in the samples. Immunoblot was used to validate the results.

Results: Analysis of the protein content of EV isolated from patient-derived fibroblast cell lines identified 134 unique proteins that showed differential abundance, 27 of which were significantly elevated and 107 reduced. We are currently validating these “hits” and examining whether these same proteins are differentially abundant in EV isolated from plasma from patients with SMA compared to normal controls.

Conclusions: We have identified a number of proteins that are differentially released in EV isolated from tissue culture models of SMA, which may act as protein biomarkers of the disease.

Building a cell atlas of human sarcoma through single-cell RNA-sequencing

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Background

Sarcomas include many cancer types that arise from mesenchymal tissue. Standard treatment for sarcomas includes surgery, radiation and chemotherapy. Unfortunately, patients with high-grade tumors have over 50% risk of recurrence after initial treatment. Immunotherapy encompasses various treatment strategies that aim to use the immune system for therapeutic gain. To date, current immunotherapies have not been successful in treating sarcomas even though immune cells are present in these tumors. Further research is needed to understand why these tumors respond poorly to immunotherapies. Here, we aim to generate a cell atlas and expression profile of immune cells in human sarcomas using single-cell RNA-sequencing.

Objective

To generate cellular and gene expression profiles of the immune cell types found in human sarcomas.

Methods and Results

Tumor samples were stored overnight in tissue preservation media and then dissociated mechanically and enzymatically. The cellular suspensions then underwent dead cell removal by magnetic separation, debris removal by centrifugation and immune cell isolation by magnetic separation. Samples were either prepared for analysis by flow cytometry or for single-cell RNA-sequencing by the 10X Genomic Platform. Our preliminary results show that our sample processing workflow produces quality samples of high viability and CD45+ cell purity while maintaining immune cell proportions.

Conclusion

Conducting single-cell RNA sequencing on immune cells from human sarcomas will aid us in better understanding the immune population present in these tumors. These findings will help in designing new immunotherapies to specifically target this cancer.

Understanding the Role of Alpha-Synuclein in Oligodendrocyte Development and Central Nervous System Myelination

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Background

Multiple system atrophy is a neurodegenerative disease hallmarked by the accumulation of alpha-synuclein in oligodendrocytes (OLs), resulting in demyelination and subsequent degeneration of neurons. Despite this known pathological hallmark, little is known about the source of alpha-synuclein or the mechanism driving its dysregulation. Additionally, functions of endogenous alpha-synuclein are slowly being elucidated for other cell types, but the role for alpha-synuclein in a healthy OL is not yet clear.

Objective

The primary aim of this study is to investigate the role of alpha-synuclein in OL differentiation and developmental myelination.

Methods

We take advantage of techniques developed in our lab to allow the establishment of a murine OL primary culture system. With this, we are able to compare the OL differentiation program from OLs derived from alpha-synuclein knockout mice, to those derived from wildtype animals. Additionally, *in vivo* assessments of these mice are included in this work.

Results

Our data clearly demonstrates endogenous expression of alpha-synuclein in primary OLs- a point that has been highly contested in the literature thus far. However, preliminary data suggests that OLs are fully capable of differentiation, despite the loss of alpha-synuclein, as assessed by a number of *in vitro* assays. Additionally, preliminary *in vivo* exploration does not highlight any overt defects in myelination.

Conclusions and Future Directions

Future experiments for this project will include RNA-sequencing of primary OL cultures derived from WT or alpha-synuclein knockout mice. These results will provide us with an indication of the role that alpha-synuclein plays in OL function. Given the findings in other groups regarding a role for alpha-synuclein in an immunological context, this avenue will also be explored, including assessment of the expression levels of alpha-synuclein following exposure to certain cytokines as well as the ability of alpha-synuclein KO OLs to tolerate the exposure of such cytokines relative to WT OLs. Altogether, results discerning the functional role of alpha-synuclein in CNS myelination are vital to provide a better understanding of how manipulation of alpha-synuclein in a disease context may affect the CNS as a whole.

Therapeutic potential of MSCs derived from multiple preterm umbilical cords in neonatal lung injury.

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Background: The chronic lung disease bronchopulmonary dysplasia (BPD) is the most severe complication of prematurity. BPD results in impairment of both lung vascular and alveolar development. Currently, there is no treatment for BPD. Mesenchymal stromal cells (MSCs) improve lung structure and function in experimental BPD, and early phase clinical trials are underway. However, the optimal source of MSCs remains to be determined. Umbilical cord MSCs (UC-MSCs) seem to exhibit superior proliferation and differentiation capacity compared to bone marrow MSCs, the most widely used source of MSCs. The primitive characteristics of UC-MSCs isolated from preterm birth (pUC-MSCs) might provide even better repair capabilities.

Objective: To determine the effect of pUC-MSCs in hyperoxia-induced experimental BPD.

Methods: Rat pups were exposed to 85% oxygen from postnatal day 0 (P0) to P14. At P4, pUC-MSCs derived from a donor without other perinatal complications (p-MSCs), and from donors exposed to preeclampsia (pPE-MSCs) and chorioamnionitis (pCH-MSCs) were administered intratracheally. Survival and body weight were recorded throughout the study. At P21, alveolar development was assessed by lung function (Flexivent) and lung structure (mean linear intercept), while pulmonary hypertension was evaluated by right ventricular hypertrophy (Fulton index) and echocardiography (PAAT/PAET). Lung vasculature volume was assessed by microCT at P35.

Results: Treatment with UC-MSCs from all donors increased survival and body weight compared to hyperoxic control pups. In addition, p-MSCs and pCH-MSCs significantly improved lung compliance, lung growth and attenuated pulmonary hypertension when compared to control hyperoxic pups.

Conclusion: pUC-MSCs provide therapeutic benefits in a rat BPD model. In the future, understanding the differences at the transcriptomic level between UC-MSCs derived from various deliveries might have the potential to provide proof of concept for superior repair capacity of preterm UC-MSCs and uncover new potential therapeutic approaches. This could lead to next generation MSC products for the treatment of complications of extreme prematurity, the number one killer of infants below the age of 5.

Preliminary Evaluation of a Modular Microprocessor-Controlled Stance-Control Knee-Ankle-Foot Orthosis

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Background:
Knee ankle foot orthoses are full leg braces for people with knee extensor weakness. Stance-control KAFO (SCKAFO) permit free knee motion during swing and knee flexion resistance during stance, making them an improvement on locked KAFO designs. Previous SCKAFO designs use mechanical or electrical control to engage flexion resistance, with some requiring full leg extension to engage (i.e., no support at other knee angles which could affect sitting, stair descent, etc.). Next generation SCKAFO will benefit from microprocessor-control by using electronic sensors and control algorithms to dictate when knee flexion resistance engages.

Objective:
The purpose of this study was to perform a preliminary biomechanical evaluation of a novel variable knee-flexion resistance microprocessor SCKAFO (VSCKAFO) that was designed to address the limitations of previous SCKAFO while maintaining stance-control functionality across various gait modes.

Methods:
Five able-bodied male participants were fit with the VSCKAFO and device settings were adjusted to each participant's preference during an accommodation period. A lower body, six degree-of-freedom marker set (30 markers) was affixed to each participant. 3D kinematic data were collected for stand-to-sit and stair descent in a motion lab with a 9-camera Vicon system. Kinetic data were recorded for stand-to-sit by using two force plates. Inertial measurement unit data were also recorded from sensors on the instrumented orthosis.

Results:
For stair descent, the maximum knee flexion achieved was similar to normal, meaning the VSCKAFO would also work for step-over-step stair descent. Most participants had slower flexion velocities than normal due to the flexion resistance. During sitting, participants with the lowest yield settings also had the greatest angular velocities, while the participant with the greatest resistance setting had the slowest angular velocity. Custom resistance settings were able to appropriately control knee angular velocity during sitting.

Conclusions:
The novel VSCKAFO appropriately resisted knee flexion during weight-bearing stair descent and stand-to-sit activities. The device was customizable to each individual, which enabled different sitting strategies for different participants. Successful biomechanical analysis with able-bodied individuals supported further testing with persons with knee-extensor weakness.

Investigating the Regulation of Muscle Stem Cell Polarity During Muscle Regeneration

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Wnt7a/Fzd7 signaling drives the symmetric division of satellite stem cells through the planar cell polarity (PCP) pathway, stimulating skeletal muscle repair and growth. Moreover, stimulating Wnt7a/Fzd7 signaling through Wnt7a treatment has been shown to ameliorate symptoms of Duchenne Muscular Dystrophy (DMD), a severely debilitating degenerative disease marked by muscle weakness and wasting. Wnt7a thus serves as a promising therapeutic for DMD; however, little is known concerning the signaling effectors downstream of Wnt7a/Fzd7 that impinge on the PCP pathway to mediate symmetric satellite stem cell division. Recently, it was demonstrated by our lab that p38γ MAP Kinase is required for symmetric satellite cell divisions. In this study we attempt to determine whether the Wnt7a/Fzd7-PCP signalling axis acts through p38γ, and identify other key transducers of Wnt7a signaling. Using proximity ligation assay we have demonstrated that Dvl2, an integral transducer of Wnt7a signaling, interacts with NuMA1 (required for spindle orientation) in satellite cells. Furthermore, we show that p38γ MAP-Kinase phosphorylates Dvl2 in vitro. Through LC-mass spectrometry we found that Dvl2 is phosphorylated at S358 by p38γ. In-progress work includes investigating the effects of silencing p38γ on Wnt7a-mediated symmetric stem cell expansion as well as the ability of Dvl2 to interact with NuMA1. Additionally, in vitro studies to examine the effects of ablating Dvl2 phosphorylation at S358 in satellite cells will determine the functional consequences of Dvl2 phosphorylation at this site. The findings from this study will yield critical information regarding Wnt7a's mechanism of action and aid in the development of Wnt7a-based therapeutics.

Emergency Department Quality Improvement In Early Pregnancy Complications: A Retrospective Analysis

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Background: Across many healthcare settings, Health Quality Ontario (HQO) Quality Standards outline specific goals and outcomes for care. In February 2019, HQO updated their Quality Standards on Early Pregnancy Complications (EPC) and Loss to better match evidence-based recommendations.

Objective: To identify possible care gaps between recent HQO Quality Standards and care provided in the emergency department (ED) at The Ottawa Hospital (TOH) and to develop clinical-care pathways to address any identified gaps.

Methods: Retrospective data collection and chart reviews were performed on 2018 ED EPC encounters at TOH. 50 encounters were screened for EPC, then were included for preliminary data analysis. Care outcomes were compared to the HQO Quality Standards: EPC and Loss. The Early Pregnancy Bleed pathway was developed by an interdisciplinary team, involving nursing educators, social workers, patient advocates, emergency physicians and obstetrician/gynecologists. The package includes a care-path, a physician order recommendation, allied-health consultation and patient information, where all patient education materials were reviewed and revised to complement the pathway.

Results: Forty-four (88%) patients with EPC presented with vaginal bleeding. Four patients were offered initial pain management, and no patients received screening for sexually transmitted infections or intimate partner violence. Forty-one (82%) patients received an appropriate medical interview, physical exam, and laboratory and imaging assessment. Rhesus (Rh) status was assessed on all patients who presented with bleeding, and prophylaxis was appropriately administered. In the setting of an ectopic pregnancy, a gynecology referral was requested, but psychosocial support referrals were not. If a non-viable intrauterine pregnancy (IUP) was confirmed, appropriate medical or surgical management and referral to psychosocial supports was offered to patients. For all viable IUPs, follow-up was appropriately scheduled or confirmed.

Conclusions: Preliminary analysis highlights care gaps between HQO Quality Standards and TOH ED EPC care. Initial pain management was not administered to 92% of patients. Neither sexually transmitted infection nor intimate partner violence screening were performed. No psychosocial supports were offered to patients where an ectopic pregnancy was either suspected or confirmed. Further work will aim to confirm the care gaps, especially regarding initial pain management, STI/IPV screening, and psychosocial support, in a larger patient cohort to optimize the EPC care algorithm in the ED at TOH. Staff education of the clinical pathway, especially pertaining to pathway content and utilization, as well as implementation and maintenance of the pathway with feedback, will be critical to ensure outcome measures are reviewed.

Sex-specific mechanisms of spinal hyperexcitability in the human and rat superficial dorsal horn

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Background: Clinical and epidemiological evidence suggests a sex difference in the mechanisms underlying chronic pain, with female chronic pain patients outnumbering their male counterparts by 2:1. Despite this, the neurobiological underpinnings of sexually dimorphic pain signalling remain unclear in rodents and are virtually unexplored in human preclinical models. Within the nociceptive system, the superficial dorsal horn (SDH) is a critical site of pain modulation. Pathological pain arises when there is an imbalance between excitation and inhibition in neurons of the SDH. We have recently characterized a pathway in male rat and human SDH neurons where brain-derived neurotrophic factor (BDNF) mediates a loss of inhibition through the downregulation of chloride co-transporter 2 (KCC2), which drives a downregulation of the phosphatase $STEP_{61}$ and a subsequent potentiation of GluN2B-containing NMDA receptors by activated Fyn kinase (Dedek, Xu et al, 2019). This pathological coupling mediates pain hypersensitivity in male rodent models of inflammatory and neuropathic pain (Hildebrand et al, 2016; Dedek, Xu et al, 2019).

Objective: Here, we investigate whether molecular mechanisms of spinal hyperexcitability and pathological pain are conserved between sexes.

Methods: To address the translational divide between basic science rodent studies and new clinical treatments, we have collected human spinal cord samples from male and female organ donors 1-3 hours post-aortic cross-clamping. Pathological pain was modelled using the ex vivo BDNF treatment model in both rats and humans, and inflammatory pain was modelled in rats using Freund's adjuvant (CFA). Tissue was examined using whole-cell electrophysiology, western blot analysis, and confocal microscopy.

Results: In contrast to male rodents, we find that NMDAR responses at lamina I SDH synapses are not robustly potentiated in a female *ex vivo* BDNF model of pathological pain nor in an *in vivo* CFA rat model of inflammatory pain. Surprisingly, our preliminary biochemical evidence suggests that active $STEP_{61}$ is not downregulated and active Fyn and GluN2B are not upregulated in female rodent (CFA inflammatory pain) as well as human (ex vivo BDNF) models of pathological pain.

Conclusions: We therefore conclude that neuronal mechanisms SDH hyperexcitability are sexually dimorphic in both rats and humans, with a BDNF/$STEP_{61}$/Fyn-dependent potentiation of GluN2B NMDARs in males but not females. This sexual divergence in underpinning neurobiological mechanisms of chronic pain has profound implications for the development of novel pain therapeutics.

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Characterization of Parkin as an Anti-Oxidant in Mammalian Brain Informs the Creation of New Mouse Models

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Objectives: Recessive mutations in the PARK2 gene cause early-onset Parkinson disease. The mechanism(s) by which Parkin confers protection of dopamine cells in the adult brainstem remains elusive. Our studies build on the previous finding by our team that Parkin protects against oxidative stress, and findings by Pawlyk et al., of age-dependent changes in Parkin extractability in human cortex. We have validated these findings, and found by mass spectroscopy of human brain-derived Parkin, that its solubility correlates with its oxidation. We hypothesize that Parkin functions in the human brain an anti- oxidant, acting through thiol-dependent, redox-related mechanisms.

Methods: We tested our hypothesis using both in vitro and ex vivo models, including human brain tissue, and through the creation of complex mouse models. We monitored Parkin metabolism, ROS levels, markers of oxidative stress, and performed mass spectrometry of murine parkin.

Results: Parkin oxidation was found to be partially reversible, thiol-group dependent and linked to its insolubility: Parkin's cysteine residues are oxidized in recombinant Parkin exposed to H2O2 and in murine brain following exposure to MPTP, as monitored by mass spectrometry. Cells over-expressing Parkin showed fewer ROS after exposure to pro-oxidants. Through further exploration of markers, we discovered more ROS-induced damage in brains and hearts from park2-null mice. We validated these findings in a bi-genic model of EOPD by combining parkin deficiency with sod2 haploinsufficiency. Absence of parkin elevated cytosolic H2O2 in the brain and heart of heterozygous sod2+/- animals.

Conclusion: Parkin confers protection against oxidative stress-related damage in high energy-producing organs and neurons. This effect is mediated through oxidation of its thiol groups, which correlates with its own insolubility.

Review of hemoglobinopathy testing results at The Ottawa Hospital

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Background: Hemoglobin genetics are the most reliable tool for diagnosing hemoglobinopathies; however, these tests are expensive and require specimen transport to a central lab in Western Ontario. The lack of a rational and evidence-based hemoglobinopathy diagnostic algorithm for TOH has led to confusion and inconsistencies among hematologists in determining which samples should be sent for genetics. Further, unnecessary testing ultimately leads to increased healthcare spending as well as delays in patient diagnosis.

Objectives: We aimed to determine how often our in-house diagnosis, made using CBC results, high-pressure liquid chromatography (HPLC) and/or hemoglobin electrophoresis (HbEl) matched the genetic diagnosis. We plan to use our results to decrease the amount of unnecessary testing occurring at TOH.

Methods: This study is a retrospective chart review study of patients over the age of 18 at TOH who underwent HbEl and/or HPLC and diagnosis by genotyping between November 2017-January 2019.

Results: Determining the frequency with which our diagnostic testing correlates with genetic testing is an important quality indicator of the diagnostic capabilities of the lab physician interpreting these results. In an era where patients have access to their test results, often before their physician, attention to the post-analytic phase of testing is more important than ever. Our results are currently in progress and are being analyzed to determine if there are statistically significant correlations between any of our in-house tests, such as CBC, ferritin, HbEl and/or HPLC and the presence of abnormalities on genetic testing. This information will, in turn, be used to develop an algorithm that may enable minimization of unnecessary testing at TOH. We also plan to conduct a survey of TOH hematologists to assess the clinical reliability of this algorithm.

Conclusion: Determination of diagnostic accuracy by TOH physicians will enable measures to improve physician skills. As hemoglobinopathy tests are known to have good internal quality control, attention to the post-analytical phase is crucial. This study provides an innovative method to assess the quality of physician test interpretation, which will enable improved reporting to ordering clinicians and patients themselves. Further, in a health system constrained by fiscal insufficiencies, decreasing unnecessary investigations is an important goal for all health care providers. Where our statistical analysis identifies findings which indicate that a hemoglobinopathy is not likely to be present or that genetic analysis is not required, then these results will aid in developing an algorithm to help eliminate the use of these unnecessary tests.

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Identification and characterization of stem cells in skeletal tissues

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Background: More than half of the Canadian population suffers from pathologies in bones, cartilage or joints throughout their life. Athletes who endured repeated injuries, as well as aging populations have a much higher risk of being affected by bone complications. While bone tissue has a high regenerative capability, tendons, cartilages and ligaments tend to have a slower and blunted healing process, notably in the elder generation. It is known that stem cells play a role in skeletal tissue regeneration, but it is debated what type of stem cells (skeletal stem cells, mesenchymal stem/stromal cells, pericytes, etc.) has the highest contribution, and why skeletal tissues lose this regenerative capacity with age.

Objective: Our purpose is to identify and localize stem cells in skeletal tissues and monitor their contribution to various tissues during homeostasis and tissue repair, as well as charactrize them.

Methods: Here, we combined classical lineage tracing approaches (using various inducible Cre-driver mouse lines) with whole-organ, 3D multicolor imaging cytometry to identify and localize these stem cells in the skeletal tissues. We also used standard cellular and molecular assays, including single cell RNAseq, for their characterization.

Results: So far, our data suggests that Sox9+ cells are bona fide skeletal stem cells giving rise to all bone and cartilage tissues in postnatal animals. These cells are not derived from marrow stroma or pericytes and do not contribute to these lineages in homeostasis.

Conclusion: Taken together, our results will help design more effective regenerative therapies for patients by providing a better fundamental understanding of the stem cells in skeletal tissues.

Mapping physiological post-translational modifications of Parkin in human brain

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Background: Recessive mutations in PARK2, the gene coding for Parkin, cause early-onset Parkinson's disease (PD). This results from the progressive and selective loss of dopamine-producing neurons in the substantia nigra and locus coeruleus. The disease relevant benefit that Parkin confers to these vulnerable neuronal populations has remained elusive. These two neuronal populations are unique in their high oxidative demand, resulting in relatively high levels of reactive oxygen species and reactive dopamine metabolites. In light of this, we have found that Parkin can act as a redox-active molecule. Parkin has a high number of cysteine residues (35; 7.5%), and we have demonstrated that through the oxidation of these cysteines, Parkin is able to reduce reactive oxygen species.

Objectives: We hypothesize that through the direct oxidation of Parkin's cysteine thiol groups, or by forming covalent adducts with reactive cellular metabolites such as dopamine, Parkin can help to reduce the levels of harmful species in the cell. We postulate that the loss of this anti-oxidant function results in the selective degeneration of oxidatively demanding, dopamine-producing neurons in PD. This work seeks to further explore and characterize Parkin's redox function and the neuroprotective benefit this confers to vulnerable neurons.

Methods: Liquid chromatography-based mass spectrometry is used to map cysteine modifications on recombinant and cellular-expressed human Parkin exposed to pro-oxidant conditions as well as Parkin derived from post-mortem human brain. The samples are sequentially incubated with iodoacetamide, dithiothreitol, and n-ethylmaleimide to differentially and irreversibly label oxidized vs. reduced (non-oxidized) cysteine residues.

Results: Our results reveal progressive oxidation of recombinant human Parkin cysteines by hydrogen peroxide. The number of oxidized cysteine residues correlates with Parkin's solubility. In parallel experiments, adducts of aminochrome, an oxidized metabolite of dopamine, are mapped at eight of the 35 available cysteines. Ongoing work aims to further map the cysteines that are most vulnerable to oxidation and/or dopamine adduct formation, and to compare these findings to Parkin purified from human brain.

Conclusion: By mapping the oxidation patterns of Parkin's cysteine residues we will gain better insight in its physiological role as a redox-active molecule in human brain.

Molecular regulation of brown adipogenic lineage specification from muscle stem cells

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Brown adipose converts excess energy to heat, and thus plays a central role in thermoregulation. The stimulation of brown adipogenesis has been suggested to be a potential treatment for obesity. During embryonic development, brown adipose and skeletal muscle progenitors are derived from a Pax7-expressing muscle stem cell. In adult muscle, Pax7+ muscle stem cells (satellite cells) similarly give rise to both lineages. We discovered a role for microRNA-133 (miR-133) as a lineage switch in muscle stem cells controlling the switch between myogenic versus brown adipogenic specification. Antagonizing miR-133 during muscle regeneration leads to de novo brown adipocyte generation from muscle stem cells, 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. We found Pifithrin-□ (p53 transactivation inhibitor) to be a potent inhibitor of miR-133 expression in mouse and human myoblasts and to stimulate brown adipose determination in primary 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-□ 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-□ and other transient p53 inhibitors may hold potential as anti-obesity compounds.

Long-Term Progression and Prognosis in Different Subtypes of Parkinson's Disease: Validation of a new multi-domain subtyping method

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Background: Parkinson's disease (PD) varies in clinical manifestation and course of progression from person to person. Identification of distinct PD subtypes is of great priority to enlighten underlying pathophysiology, predict progression and develop more efficient personalized care approaches. There is currently no clear way to define subtypes in PD with global consensus. The National Institutes of Health established subtype-identification as one of the top three clinical research priorities in PD

Objective: We aim to compare long-term progression and prognosis between different PD subtypes using a new multi-domain subtyping method based on initial motor and non-motor manifestations at the drug-naïve early stage.

Methods:Data on 421 individuals with *de novo* early-onset PD was retrieved from Parkinson's Progression Markers Initiative (PPMI). Using a newly developed multi-domain subtyping method (based on motor phenotype, REM sleep behavior disorder (RBD), autonomic disturbance, and early cognitive deficit), we divided PD population into three subtypes at baseline: subtype I (n=223, "*mild motor-predominant*"): composite motor score and all non-motor summary scores (NMS) were below the 75th percentile; subtype III (n=52, "*Diffuse malignant*"): (EITHER (1) composite motor score >75th percentile and >1 of 3 non-motor scores >75th percentile OR (2) all three non-motor scores >75th percentile); and subtype II (n=146, "*Intermediate*"): those not meeting criteria for subtype I or II. Rate of global progression (mixed motor and non-motor features), Schwab and England activities of daily living (SE-ADL) and developing dementia were compared between the subtypes. At the time of data retrieval (January 2019), the median follow-up time was 6 (range: 1-8) yrs.

Results: Patients with "*diffuse malignant*" PD at baseline, experienced 0.5 z-score further worsening of global composite outcome (p=0.017), 2.2 further decline in MOCA score (p=0.001) and 5.3% further decline in SE-ADL (p=0.010) after 6-years of follow-up. Hazard for MCI/dementia was significantly higher in "*diffuse malignant*" (HR=3.2, p<0.001) and "intermediate" (HR=1.8, p<0.001) subtypes compared to those subtyped as "*mild motor-predominant*". Individuals with "*diffuse malignant*" PD had the lowest level of CSF amyloid-beta (p=0.006) and SPECT striatal binding ratio (p=0.001).

Conclusions: This innovative multi-domain subtyping, which is based on initial motor phenotype and three key non-motor features (i.e. RBD, autonomic disturbance, early cognitive deficit), is a valid method to predict subgroups of PD with distinct pattern of long-term progression. This subtyping can now be applied to individual patients in real-life clinical practice, at drug-naïve early stage, using baseline motor and non-motor information to predict course of PD progression.

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Taking the first step towards building meaningful relationships between patients and researchers: Feedback from the 2018 BioCanRx Patient-Researcher Roundtable Lunch

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Background: BioCanRx's Cancer Stakeholder Alliance (CSA, a consortium of patient-centered cancer research and advocacy organizations) identified patient involvement during the development of early phase clinical trials as a priority area. Our team facilitated a BioCanRx roundtable discussion as a first step towards building meaningful relationships between patient scholars and BioCanRx investigators. The roundtable event also brought these stakeholder groups together in hopes of identifying ways patients could become involved in the BioCanRx investigators' research as well as potential barriers.
Objective: Our team aimed to capture perspectives on patient-researcher partnerships from both stakeholder groups, before and after the roundtable.
Methods: BioCanRx researchers and patient scholars were invited to attend the event. Pre-roundtable, patient scholars attended educational webinars. At the roundtable, participants were divided into groups and a facilitator guided discussions. Attendees answered pre- and post-event surveys (online and in-person, respectively) to assess patient and researcher views and perceived barriers to patient-researcher partnerships.
Results: Topics discussed included trial logistics, the importance of evaluating quality of life, accessible consent procedures, policy and funding implications for clinical trials, benefits of collaboration, and wanting to understand more about the patient experience and research process. The surveys were answered by patient scholars (n=9 pre, 10 post) and researchers (n=14 pre, 15 post). A greater proportion of patient scholars indicated they felt they could be involved in cancer immunotherapy research in the post-survey sample (Agreed or Strongly Agreed= 90% versus 63%). A greater proportion of patients also indicated they felt they could be involved in specific research activities such as identifying priorities alongside the research team (Yes= 70% versus 38%), contributing to the development of the ethics application (Yes= 50% versus 0%) and co-developing patient-facing project materials (Yes= 90% versus 50%). Similarly, a greater proportion of post-survey researchers indicated desire to engage patients in their research (Agreed or Strongly Agreed= 100% versus 75%) and feeling comfortable identifying (50% versus 17%), setting-up (57% versus 17%), building their team to involve (79% versus 50%) and maintaining a patient partnership (71% versus 42%). Top barriers for both groups were knowing how to identify partners and initiate partnerships. The short duration of the session limited discussions on how patient scholars could specifically contribute to planned studies.
Conclusions: The Patient-Researcher Roundtable was an important first step towards involving patient scholars in BioCanRx translational research. Identified barriers should be considered when planning future patient-researcher engagement events.

Building a platform for meaningful patient partnership to accelerate "bench-to-bedside" translation of promising new therapies.

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Background: The importance of 'patient engagement' in the development and delivery of clinical trials is increasingly recognized by researchers and governmental funding agencies. Improved study design and relevance of research findings are just a few of the suggested important benefits of patient engagement. Despite widespread interest, a recent systematic review found that only 23 out of 371,159 clinical trials conducted between 2011-2016 reported engaging patients. Here we describe our efforts to engage patients in an early phase clinical trial.

Objective: Our primary aim is to establish a platform for meaningful patient engagement by partnering with a patient panel to obtain their perspectives throughout the development and conduct of an upcoming early-phase clinical trial of CAR-T cell therapy.

Methods: We recruited four patient partners to our research group through referrals from our professional network and the Leukemia and Lymphoma Society of Canada. Patient partners attended team meetings on a monthly basis and provided feedback on projects informing the development of the trial protocol (systematic review, interview and survey study, early economic analysis), and documents to improve the informed consent process. Our patient partners will also be involved in the development of a peer support panel and a policy brief.

Results: Our patient partners provided valuable feedback, which led to the identification of patient-centered outcomes that have been assessed to date in CAR-T trials. Additionally, patient partners incorporated patient and caregiver expenses in our early economic analysis model. Our patient partners highlighted the important role that family and friends play in supporting trial participants and how a common understanding of the trial logistics and procedures can become lost in the scientific technicalities of the informed consent document. This has led to the development of the following patient resources; a one-page non-technical summary of the informed consent document, visual consent aids, and a glossary of common scientific terms (e.g. adverse events). These documents are structured in a way to effectively communicate the contents of the informed consent document to trial participants and members of their support system. Additionally, our patient partners have helped to identify existing peer support infrastructures, which will provide helpful models as we co-develop an electronic forum that will be used to provide support to trial participants.

Conclusion: We have found that our patient partners provide uniquely important contributions that helped to refine the clinical trial deliverables in ways that we could not have had they not been involved.

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Differences in human and animal primary neural stem cell responses to inflammatory and regenerative cues: impact on the successful translation of therapies to humans

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Background: In animal models of spinal cord injury, inflammation post-trauma activates neural stem and progenitor cells (NSPCs) which differentiate into glial scar astrocytes. To direct NSPC fate and promote regeneration instead, NSPCs can be targeted using growth factors. However, the mechanisms regulating human spinal cord NSPC pathophysiology and regeneration are not known.

Objective: To improve the translation of animal therapies for spinal cord injury, we assessed the effect of inflammatory and regenerative factors on primary NSPCs in a small (rat) and large (pig) animal model in comparison to NSPCs from humans.

Methods: Primary spinal cord NSPCs from adult humans (n=8), pigs (n=5) and rats (n=6) were cultured using the neurosphere assay. To mimic post-injury inflammation, primary-derived NSPCs were treated with pro-inflammatory factors interleukin-6 (IL-6), tumor necrosis factor-α (TNFα), or transforming growth factor-β (TGFβ). To direct regeneration, NSPCs were treated with retinoic acid (RA), platelet-derived growth factor (PDGFα), or bone morphogenic protein-(BMP4) to induce neurons, oligodendrocytes or astrocytes, respectively. Cultures were treated for 7 or 14 days, fixed, and characterized by immunocytochemistry (GFAP, β-iii tubulin, O4, and BrdU). To track proliferation, BrdU was added 24 hours prior to fixation.

Results: IL-6, TNFα, and TGFβ induced astrogenesis of rat NSPCs (3.9±0.7, 5.0±0.9, and 4.0±0.6 fold, respectively) after 7 days concomitant with reduced neurogenesis (0.14±0.90, 0.07±0.04, 0.07±0.05 fold, respectively). Pig NSPCs similarly increased astrogenesis (1.38±0.04, 1.26±0.05, and 1.45±0.04 fold, respectively) but after 14 days of treatment. On the contrary, human NSPCs had reduced astrogenesis (0.14±0.07, 0.6±0.2, and 0.12±0.07 fold, respectively) over the course of 14 days, but generated more neurons (1.23±0.05 and 1.34±0.04 fold, respectively) with IL-6 and TGFβ treatments. With regenerative factor treatment, RA increased neuron differentiation of both human and rat NSPCs, PDGFα increased oligodendrocyte differentiation of only rat NSPCs, and BMP4 increased astrocyte differentiation of human and rat NSPCs at low (40ng/mL) and high (100ng/mL) concentrations, respectively.

Conclusion: For the first time, we have directly compared human, pig, and rat spinal cord NSPC response to pathophysiological and regenerative factors and determined cell-intrinsic differences in behaviour. Improved understanding of these differences between human and animal models will be important for the successful translation of regenerative therapies to humans.

The role of Sox10 in the progression and drug resistance of HER-2 positive breast cancers

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Background:

HER-2 is an epidermal growth factor receptor that is overexpressed in approximately 30% of human breast cancers. At present, the most effective form of treatment for HER-2 positive breast cancers is a combination therapy of chemotherapy paired with Herceptin. However, approximately 70% of patients develop a resistance to Herceptin. Therefore, the identification of downstream effectors in HER-2 mediated tumorigenesis is necessary in the development of novel therapeutics.

Objective:

We have previously shown that SLK deletion induces the upregulation of Sox10 in Neu-NDL expressing cell lines and hyperplasia's. We believe that the elevated expression of Sox10 is a compensatory response to SLK deletion. We propose to further investigate the role of Sox10 in the development and progression of Neu-induced mammary tumor growth.

Methods and Results:

in vivo: We have previously shown that SLK deletion in a Neu-induced mouse mammary tumor model results in a faster tumor onset and a quicker endpoint. We intend to further investigate the phenotypic effects of ablation of both SLK and Sox10 on tumor onset and endpoint in Neu-induced mouse mammary tumor models.

in vitro: We have previously shown that the expression of Sox10 is dependent on the direct binding of active Sox9 to the *Sox10* promoter. We believe that Sox9 achieves this through a shared action with a secondary binding partner. We intend to investigate this relationship with two potential targets, p300 and 14-3-3, through immunoprecipitation assays, ChIP and using specific inhibitors of both p300 and 14-3-3.

Conclusions:

By investigating the transcriptional control of *Sox10* and its effects on Neu-induced mammary tumorigenesis, we can develop a better understanding of the downstream effectors that mediate HER-2 positive tumorigenesis and thus the identification of novel therapeutic targets in HER-2 based therapies.

Elucidating the pathogenic role of nuclear alpha-synuclein in a novel mouse model of Parkinson's disease

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Background: Parkinson's disease (PD) is a neurodegenerative disorder characterized by the degeneration of dopaminergic neurons in the substantia nigra and the accumulation of alpha-synuclein (a-syn) throughout the brain. Although a-syn is found in both the cytoplasm, typically at the synapse, as well as in the nucleus, previous studies from our lab and others have shown that its nuclear accumulation is toxic and has been shown to increase in the brain of PD patients. However, whether the nuclear targeting of endogenous a-syn is sufficient to cause toxicity remains elusive.

Objective: Perform in-depth characterization of the toxic effects of nuclear a-syn to gain better insight into its mode of toxicity.

Methods: We have created a novel mouse line ($Snca^{NLS\text{-}Flag}$) in which endogenous flag-tagged a-syn is localized to the nucleus via a nuclear localization signal (NLS) tag. I will characterize this mouse line on the behavioural, histological, and biochemical level. Our behaviour testing looks at both motor and non-motor function of the mice through 10 different assays. Histology and biochemistry will explore the levels of toxic a-syn phosphorylation (pSer129) and aggregation as well as determining the extent of cell stress. Analyses will be performed at three timepoints to track the progression of disease in young (2-3 months), mid-aged (8-9 months), and aged (18-19 months) mice.

Results: The homozygous ($Snca^{NLS\text{-}Flag/NLS\text{-}Flag}$) mice show a significant motor deficit in Adhesive Removal, Rotarod, and DigiGait tests at the 9-month timepoint that were not observed at the young, 3-month timepoint. Histological and biochemical analyses of the young, 2-month cohort showed no significant neurodegeneration nor toxic a-syn formations. However, toxic forms of phosphorylated a-syn were observed in the brains of 7-month-old mice. The emergence of these phenotypes at the mid-aged timepoint support the hypothesis that these mice will display a late-onset progression of disease resembling that of PD in humans.

Conclusions: At our mid-aged timepoint, $Snca^{NLS\text{-}Flag}$ mice exhibit clear histological and behavioural deficits. Further characterizing the effects of nuclear a-syn at an aged timepoint will be critical in chronicling the consequence of a-syn accumulation with age. Moreover, this study will not only provide a new and useful pre-clinical model of PD, but also yield important insight into mechanisms of a-syn-mediated toxicity, thereby identifying new opportunities for therapeutic intervention in PD.

Physicians' Knowledge, Attitudes and Beliefs about a Hospital Wide Cluster Cross Over Pragmatic Fluid Administration Trial: An Electronic Survey

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Background: FLUID is a hospital wide pragmatic cluster cross over randomized controlled trial developed to test whether Ringers Lactate reduces death and readmission to hospital when compared to Normal Saline. The recently completed four-centre FLUID pilot trial demonstrated feasibility for the conduct of the large FLUID trial.

Objectives: Due to FLUID's pragmatic and novel design (hospital wide intervention, waiver of consent, requirement to implement an automatic fluid substitution), we aimed to survey physicians at FLUID pilot participating centres to understand the effectiveness of FLUID communication strategies, as well as their knowledge, attitudes, and beliefs about FLUID's pragmatic trial design.

Methods: A web-based survey was developed targeting physicians at all participating FLUID pilot centres using mass email distribution lists upon completion of the pilot trial. The survey was developed through consensus with the FLUID scientific team. The survey was designed to take no more than 5 minutes to complete to maximize response rate. Survey domains included physician demographics, FLUID communication strategies, and knowledge, attitudes and beliefs about FLUID's pragmatic design. Completion was voluntary. Communication effectiveness and knowledge, attitudes and belief questions about FLUID were answered using a 5-point Likert scale response.

Results: A total of 254 physicians from four pilot centres responded to the survey, of whom 206 (81.1%) worked at academic centres. Thirty-six percent (92/254) of respondents had been in practice for greater than ten years. The most common area of practice was internal medicine (26%, 65/254). The top four reported communication strategies were email, posters, word of mouth, and study coordinator (very effective/effective: 87% (220/254), 63% (161/254), 62% (158/254), and 31% (80/254), respectively). Eighty-four percent (214/254) of physicians reported feeling comfortable (agreed or strongly agreed) having their patients participate in the study, while 5% (13/254) did not (disagreed or strongly disagreed). Most physicians (76%, 194/254) agreed or strongly agreed that waiver of informed consent was appropriate; 5% (12/254) disagreed or strongly disagreed with the appropriateness of waiver of consent. Thirty-three percent (85/254, agreed/strongly agreed) of physicians reported a strong fluid preference between the two resuscitation fluids under evaluation. However, only 10% (25/254) of physicians reported that they agreed or strongly agreed that FLUID would demonstrate a significant mortality difference between the two study fluids.

Conclusions: Among responding physicians working in tertiary care centres and one community hospital, a large degree of comfort was reported with respect to the aims, purpose, and execution of the FLUID pilot study.

Parental understanding of research consent forms in the pediatric intensive care unit: a pilot study

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Background: Informed consent is an ethical requirement for research involving human participants. In the pediatric intensive care unit (PICU), many critically ill children lack the capacity to provide informed consent for themselves, so consent is instead sought from their legal guardians. However, the stressful nature of the PICU has evoked questions regarding the validity of consent to research obtained from legal guardians in this setting, especially shortly after their child's PICU admission.

Objectives: To describe legal guardians' understanding of key concepts in a research consent form presented within 24 hours of their child's admission to the PICU, and to explore legal guardians' opinions of the format (language, length) of the consent form and the overall consent process.

Methods: The STRIPES Questionnaire was developed to test understanding of the six most important concepts in the consent form from a previous PICU trial (STRIPES, NCT02044159), as identified by a variety of stakeholders. The STRIPES consent form was explained to eligible legal guardians within 24 hours of their child's admission to the PICU at a tertiary care centre in Canada, and their understanding was evaluated using the STRIPES Questionnaire the following day. Demographic information was collected via a survey, and opinions were collected verbally and via a survey.

Results: This study included 41 legal guardians of 31 patients. Most participants were aged 30-39, and 59% had a post-secondary degree. The median number of questions answered incorrectly on the STRIPES Questionnaire was 3 out of 7 (IQR = 2-4). Participants best understood the topic of the study (5% incorrect), but 80% of participants were unable to recall a single risk. The median rating of the language in the form was 5 out of 5 (very easy to understand; IQR = 4-5) and 88% of participants said it was a reasonable length. Thirteen participants stated that their current state of mind would impede their understanding and questionnaire performance, and one mother said she would prefer that researchers use deferred consent in emergency settings.

Conclusion: Despite positive opinions of the consent form, most legal guardians did not understand all key components of the consent information provided to them orally and in writing within 24 hours of their child's PICU admission. Future studies are required to determine barriers to understanding and explore alternative approaches to obtaining consent in this setting.

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Expectations of Benefits with Deep Brain Stimulation in Parkinson's Disease Patients

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Background: It is standard of care to have a comprehensive discussion about outcomes and realistic expectations with Parkinson's disease (PD) patients living with medically-refractory and disabling motor complications before undertaking deep brain stimulation (DBS) surgery. Preliminary observations suggest that patients may feel the outcome of DBS does not meet their needs.

Objective: To characterize the phenomenon of expectations of motor and/or non-motor benefits and improvement in quality of life for PD patients undergoing DBS (primary outcome); to explore the association between patient perceived outcome and a change in efficacy outcome measures from pre-operative to post-operative assessment (secondary outcome).

Methods: We performed a retrospective and prospective cohort assessment at latest follow-up of PD patients enrolled in the Ottawa DBS program since 2014 and with a minimum of 3 months follow-up to assess possible factors contributing to their degree of satisfaction. Patients were assessed pre-operatively and at latest follow-up with the following rating scales: MDS-UPDRS part I, II, III and IV, HAM-D, MoCA, FrSBe Apathy scale and PHQ9. On follow-up, we administered a patient-centred semi-structured questionnaire on satisfaction and expectations.

Results: We included 28 patients (60.7% male) with a median disease duration of 11.6 years (IQR 7.9-15.3) at the time of DBS surgery. At latest follow-up (median 2.4; IQR 1.7-3.2 years), 75.0% (21/28) were overall satisfied. All clinical parameters and standard outcome metrics were comparable in both the satisfied and unsatisfied groups. Interestingly, the satisfied group had more frequent ongoing expectations of improvement in the cognitive and somatic categories (respectively 11.6% and 10.0% vs 0% and 0% of reported expectations).

Conclusion: Using a novel patient-centered approach, PD patients who underwent DBS identified expectations that have not been consistently observed to improve with DBS. There is a gap between patient-reported expectations and pre-operative counselling. We hypothesize that a patient counselling tool could meet this need.

The Use of Correct Propensity Score Methodology in Contemporary High-Impact Surgical Literature

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Background: Propensity score (PS) analysis is a statistical method commonly used in observational trials to account for confounding. Improper use of PS analysis can bias the effect estimate.

Objective: The aim of this study is to review the use and reporting of PS methods in high impact surgical journals with a focus on propensity score matching (PSM). We also investigated the impact of appropriate methodology on study conclusion.

Methods: The 10 surgical journals with the highest impact factor were searched to identify studies utilizing PS analysis from January 1st, 2016 to December 14th, 2018. We selected evaluation criteria for the proper conduct of PS analysis based on previous reports. Two authors systematically appraised the quality of reporting of PS analyses. Univariate and multivariate regression was performed to determine the relationship between appropriate use of PS analysis and study conclusion.

Results: PS analysis was employed in 303 surgical studies. Ninety one percent (n=275) of studies included the covariates used to generate the PS and 79% (n=239) included the type of regression model used. Ninety percent (n=272) of studies did not justify the covariates included in their PS. Eighty three percent of studies used PSM (n=254), with 48% (n=156) failing to assess covariate balance between groups. We found that justification of the selection of covariates included in the PS and the characterization of unmatched patients were both associated with lower odds of the study finding a significant result (OR 0.32, 95%CI 0.16-0.88, p=0.02, and OR=0.35, 95%CI 0.17-0.75, p=0.007 respectively at multivariate logistic regression).

Conclusion: This study demonstrates that even in research published in high-quality surgical journals, several studies report their PS methodology inadequately. The inadequate conduct of PS analysis may impact a study's conclusion. Our work identifies the need for more rigorous reporting of PS methodology and proposes reporting recommendations for authors using PS analysis.

Marker-less stride analysis in a hallway using depth sensors

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Background:

Motion capture technologies used for analyzing human gait can be classified into marker-based and marker-less categories. In marker-based systems, such as optical motion capture, the person wears either passive markers that reflect light or active markers that produce light. Marker-less systems capture human movement directly from camera images or sensors. The ability of depth sensors to deliver 3D information in real-time makes marker-less motion capture more open to many interesting applications. One of the most researched topics with depth sensors is human motion capture and gait analysis.

Objective:

The objective of this research is to create and validate a high-speed depth sensing system to track the foot while walking to analyze stride parameters and gait events, while a person walks along an institutional hallway.

Methods:

Twenty-two able-bodied volunteers were recruited from students and staff at the University of Ottawa. Reflective passive markers were attached to the participant's lower body and then the participant walked 12 times along a walkway of realistic hallway dimensions. Data were captured from a 1.5m length using both a Vicon system and the new marker-less system with six Intel Realsense D415 depth sensors.

Depth camera data were fed to an algorithm developed in this research, to analyze the data and automatically locate the foot. Based on toe and heel locations, the algorithm will output stride parameters such as walking speed, stride length, stride time, step length, step width, foot clearance, foot angle, etc. The same parameters were calculated with Vicon data.

Results:

Marker-less outcomes were in agreement with the Vicon system for the temporal stride parameters and spatial stride parameters related to anterior-posterior foot location. Errors were high for mediolateral spatial stride parameters such as step width, foot angle, and foot clearance.

Conclusions:

This low-cost, non-invasive, marker-less system is convenient to use and has the ability to capture a person's stride parameters with accurate results for anterior posterior and temporal measures. Mediolateral parameters errors were due to depth image noise during the foot contact phases.

Machine Learning Model Development for Prediction of Long Term Mortality and Functional Outcomes in the Rapid Response Activations

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Background: Rapid Response Teams (RRT) respond quickly when urgent medical support is required in hospital. Prognostication during the RRT encounter is challenging given the acuity of the encounter, lack of previous knowledge of the patient, and the subsequent need for rapid decision-making. Very few tools are available to support RRT prognostication during an intervention, resulting in many admissions to the intensive care unit (ICU), although not always required or beneficial for the patient. Advances in technological and digital strategies (ie. Machine Learning Mortality Prediction Models (MLM)) will drive innovation to enhance patient safety; the MLM may be an important tool in enhanced RRT prognostication accuracy.

Objective: The study will re-design and validate an enhanced MLM to support rapid decision-making by the RRT by including both medical history data and laboratory results into the model to predict a primary outcome of short- (6-month) and long- (12-month) term functional outcome: this is the first MLM designed specifically for the RRT setting to examine functional outcomes.

Methods: The study will include recipients of RRT care over 18 years of age at the General and Civic campuses of the Ottawa Hospital (TOH). Ottawa Hospital Data Warehouse data will be analyzed and later connected to the ICES database to obtain outcome data at one-year. Prediction models will be developed and clinically validated for predictive ability, followed by development of a point-of-care clinical decision support application for mortality and functional outcome prediction by physicians.

Results: Our group has assessed the impact of incorporating laboratory data into a previously designed MLM (Shappel et al, 2018) resulting in improved prognostication and significantly improved in-patient mortality predictions after RRT intervention. We anticipate this information would afford physicians the functionality component currently lacking in RRT prognostication.

Conclusions: The novel focus on functional outcomes may enrich discussion between the medical team and the patient/family in terms of long-term prognosis, potentially altering the patients' goals of care decisions. Future directions include pilot testing of the enhanced MLM in the RRT setting at TOH with subsequent RRT implementation, and evaluations in ICU cost-effectiveness and physician, patient, and family satisfaction.

NK cells acquire PD-1 expression through trogocytosis

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Background: Natural killer (NK) cells are innate lymphoid cells that play and important role during the immune response to viral infection and tumor formation. Recently, it was demonstrated that NK cell suppression through the PD-1/PD-L1 inhibitory axis is a key mechanism of tumor cell immune evasion. NK cells gain expression of PD-1 which results in reduced NK cell tumor directed cytotoxicity. Trogocytosis is a mechanism of membrane exchange between immune cells and other immune or target cells mediated by cell to cell contact.

Objective: In this study, we aim to elucidate the mechanisms of acquired PD-1 expression on NK cells and determine the impact of trogocytosed PD-1 on NK function.

Methods: Using the mouse T cell lymphoma cell line RMA, we generated RMA cells expressing the congenic marker Thy1.1 either expressing or knockout for PD-1. Employing these cells in co-culture assays with primary mouse NK cells isolated from *Pdcd1*$^{-/-}$ mice we assessed through flow cytometry the acquisition of PD-1 on the NK cells. *In vivo* tumor studies were done through injection of the RMA cells subcutaneously in to wild-type or *Pdcd1*$^{-/-}$ mice. Tumors were collected and flow cytometry was done to assess PD-1 expression on NK cells.

Results: Here we demonstrate that NK cells acquire the expression of PD-1 from PD-1 expressing tumor cells through trogocytosis. NK cells collected from PD1$^{-/-}$ C57bl/6J mice were demonstrated to acquire PD-1 expression following co-culture with RMA cells. This acquisition of PD-1 did not occur on PD-1$^{-/-}$ NK cell when cultured using a transwell to prevent cell-cell contact. Additionally, the NK cells isolated from the tumors of PD-1$^{-/-}$ mice injected with PD-1 expressing RMA cells acquired PD-1 expression.

Conclusion: This data suggests that the trogocytic transfer of PD-1 from tumor cells to NK cells may play an important role in supressing NK cell tumor directed cytotoxicity. This represents a novel mechanism that can be exploited by tumors to evade immune cell killing.

EEG Markers of Cognitive Engagement in Concussion

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Background: The ability to focus on cognitive tasks impacts everything from our social interactions to our success in the classroom or workplace. Concussion negatively impacts the ability to focus and causes patients to experience signs of mental fatigue more quickly than those without concussion. The mechanisms behind these changes are still not well understood.

Objective: Therefore, the purpose of this study was to use portable electroencephalography and qualitative assessments to characterize cognitive changes associated with the perceived increase in mental load and to identify markers of mental fatigue in these individuals.

Methods: Fifteen concussion patients and fifteen age-matched controls were recruited to participate in this study. Participants performed two, thirty-minute testing sessions spaced 1 month apart. In each session, participants performed 8 cognitive tasks eliciting varying levels of cognitive activity. The cognitive activity was quantitatively assessed using a MUSE and EMOTIV non-invasive EEG headset. These data were compared to a perceived level of cognitive engagement determined by the individual using the Klepsh et al (2017) cognitive engagement scale and mental fatigue assessed by the Mental Fatigue Scale (Johansson, 2014).

Results: The results demonstrate that frequency-based EEG changes correlated with decreased ability to focus on the cognitive task and with perceived cognitive fatigue in both concussion patients and healthy controls.

Conclusions: Future studies should utilize the same methods to monitor cognitive activity differences during daily functional living.

Studying the Effects of SUMOylation on CaMKIIa within the Context of Synaptic Plasticity

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Background: Calcium-calmodulin dependent kinase II alpha (CaMKIIa) is a highly expressed protein in the dendritic spines of glutamatergic neurons within the adult brain and is critically involved in synaptic plasticity. During long-term potentiation (LTP) at glutamatergic synapses, CaMKIIa concentration increases in the spines and binds Ca2+/CaM to become active. Once active, CaMKIIa phosphorylates AMPA receptors to promote their trafficking to the postsynaptic density; thus, strengthening synaptic transmission. Recently, we have identified that all four CaMKII isoforms are modified by Small Ubiquitin-like Modifiers (SUMOs); specifically by SUMO2, and have confirmed the SUMOylation event of CaMKIIa. Additionally, it has been found that activation of glutamate receptors promotes increases in SUMOylation within dendritic spines.

Objective: As a result, we propose that SUMOylation is an important regulator of CaMKIIa that contributes to learning and memory formation upon NMDA receptor activation. Interestingly, mutations within the strongest CaMKIIa SUMOylation site have recently been linked to Autism Spectrum Disorder, further supporting the importance of SUMOylation on proper neurodevelopment.

Methods: In order to investigate the mechanisms by which SUMOylation effects CaMKIIa function, I will knock-out Ubc9 (a required mediator of SUMOylation), SUMO1, and SUMO2 in mouse primary cortical neurons using CRISPR/Cas9 technology. From this, I will analyze the effects on CaMKIIa using genetic, biochemical, and microscopy based techniques. Additionally, I will investigate the cellular consequence of CaMKIIa SUMOylation using glycine-induced LTP in mouse primary cortical neurons.

Conclusion: This work will uncover the mechanism by which CaMKIIa is regulated by SUMO2, as well as elucidate the role of SUMOylation in synaptic plasticity.

Systematic Review of Economic Evaluation of Chimeric Antigen Receptor T-cell (CAR-T) Therapies for Patient with Hematologic or Solid Malignancies

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Background: Chimeric Antigen Receptor T-cell (CAR-T) therapy is a new class of cancer immunotherapies that genetically engineer patient's T cells to target the disease. Although the therapy is promising, a careful investigation of its cost-effectiveness is required due to its high costs.

Objectives: This study aims to systematically review the cost-effectiveness of CAR-T therapy for patients with hematologic or solid malignancies.

Methods: We systematically searched electronic databases, such as MEDLINE and Embase, and gray literature. Search strategies were developed by an experienced librarian and the research team. Eligibility criteria included systematic reviews, health technology assessments or economic evaluations comparing costs and effects of CAR-T therapy in patients diagnosed with hematologic cancers or solid tumors. Two reviews will independently screen studies using predefined inclusion criteria, extract data, and assess methodological quality of the included studies using the Drummond's Checklist.

Results: The searches identified 354 records. We are currently screening the identified abstracts. We will describe the cost-effectiveness findings narratively. If feasible, we will report the cost-effectiveness results based on type of cancer, age group (pediatric and adult), T-cell origin (autologous versus allogeneic), and construct type (including but not limited to antigen type and costimulatory domain).

Conclusion: The results of this study will summarize existing evidence on the economic value of CAR-T therapy and highlight their methodological quality and identify any knowledge gaps. Our study findings will also inform future economic evaluations of CAR-T therapy for cancers.

PD-L1 enhances the efficacy of the oncolytic virus VSVD51

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Background: Oncolytic viruses (OVs) are a novel immunotherapy showing great promise in the treatment of cancer. Due to the impaired anti-viral response in cancer cells, OVs such as VSVD51 preferentially replicate in cancer cells. PD-L1 is a surface protein, often expressed on tumor cells, that binds to the inhibitory checkpoint receptor PD-1 to inhibit anti-cancer immunity. In addition to its role as a PD-1 ligand, emerging evidence suggests that PD-L1 modulates cellular responses of tumour cells in a cell-intrinsic fashion, including suppression of type I interferon signaling.

Objective: Given that the type I interferon pathway is responsible for inducing the cellular anti-viral response, we hypothesized that PD-L1 will enhance the infection of the OV VSVD51 in cancer cells.

Methods: To this end, a PD-L1 knockout line was generated from the PD-L1-expressing cancer cell lines TRAMP-C2 and CT26 by CRISPR/Cas9 (TRAMP-C2 Cd274$^{-/-}$ and CT26 Cd274$^{-/-}$, respectively). These cell lines were infected with VSVD51, and analyzed by various methods.

Results: TRAMP-C2 Cd274$^{-/-}$ cells are less susceptible to VSVD51 infection and oncolysis compared to WT TRAMP-C2, and restoration of PD-L1 expression enhances oncolysis. TRAMP-C2 Cd274$^{-/-}$ cells exhibit severe defects in viral replication and virion production. Mechanistically, TRAMP-C2 Cd274$^{-/-}$ secrete greater amounts of IFN-b compared to WT TRAMP-C2 post-infection (with subsequent enhanced expression of IFN-stimulated anti-viral genes), and have altered JAK/STAT signaling in response to IFN-b stimulation. These PD-L1-mediated changes in the interferon pathway are also observed with the use of the virus simulator polyI:C. Importantly, all differences in infection between WT TRAMP-C2 and TRAMP-C2 Cd274$^{-/-}$ are abolished when the activity of IFNAR is blocked. Additionally, CD80 surface expression may be required for the function of PD-L1, while PD-1 surface expression is not required for the observed phenotype. Preliminary evidence suggests that inhibition of STAT3 activity post-infection abolishes differences in infection, implicating this transcription factor as a key downstream effector of PD-L1. Lastly, PD-L1 expression in CT26 also enhances VSVD51 infection, suggesting that this is not a cell-type or cell-line specific function of PD-L1.

Conclusions: PD-L1 enhances VSVD51 infection, and this is associated with impaired type I IFN signaling. Ultimately, we aim to characterize PD-1-independent functions of PD-L1.

Utilizing effector proteins to enhance oncolytic viral efficacy

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Background: Oncolytic Viruses (OV) are promising anti-cancer biotherapeutics but face challenges in resistant tumors. We have identified small molecules termed Viral Sensitizers (VSes) that potentiate OV infection in such resistant tumors. We have also shown that the combination of VSes working by different mechanisms can result in synergistic enhancement of viral growth. Different classes of VSes target various cellular pathways and structures, in part inhibiting antiviral signaling pathways in unique ways that leads to increase OV spread and bystander killing in cancer cells.

Objective: Despite improving OV efficacy, some classes of Vses suffer from does limiting systemic toxicities. We deem that OVs encoding effector proteins that function similarly to VSes, termed VSEPs, will be more potent against resistant cancer cells, and will lead to tumor-focused viral-sensitization and anticancer effects, resulting in less systemic toxicity. Indeed several bacterial effector proteins have been identified that functions similarly to VSes, such as VSEP1.

Results: Comparing growth kinetics of VSV?51-VSEP1 to VSV?51 expressing firefly luciferase (VSV?51-Fluc), we observed faster growth and higher overall titers in multiple cancer cell lines that are generally more resistant to OVs. We also observed an increase in plaque size, and therefore viral spread, in cancer cells infected with VSV?51-VSEP1 compared to VSV?51-Fluc. *In vivo*, VSVΔ51-VSEP1 treatment delays tumor growth compared to VSVΔ51-Fluc in Balb/C mice implanted with CT26wt tumours.

Conclusion: Our results suggest VSEPs, and strategic combinations of these, could potentiate OV infections *in vitro* and control tumor growth *in vivo.*

Experts prioritize osteoarthritis interventions from Cochrane Systematic Reviews for translation into "Evidence4Equity" summaries.

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Background: Osteoarthritis carries substantial health and socioeconomic burden, which is particularly marked in marginalised groups. It is imperative that practitioners have ready access to summaries of evidence-based interventions for osteoarthritis that incorporate equity considerations. Cochrane summaries can provide this.

Objective: The present study surveyed experts to inform the selection of interventions to generate Cochrane Evidence4Equity (E4E) summaries.

Methods: We identified 29 effective interventions to prioritise. Key findings from these interventions were summarised and this information was provided to 6 experts in the field of osteoarthritis. Expert participants were asked to rate interventions based on feasibility, health system effects, universality, impact on inequities, and priority for translation into equity-based E4E summaries.

Results: Expert participants rated exercise and land-based exercise interventions across a number of dimensions within interventions treating osteoarthritis with land-based exercise rating highest for priority for translation into E4E summaries.

Conclusion: The survey generated information that can be used to direct and support knowledge translation efforts.

Fabrication and Characterization of Biocellulose Based, Composite Delivery System For Spinal Cord Regeneration

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Background: Spinal cord injury (SCI) is a devastating condition for which there is no cure. Endogenous neural stem/progenitor cells in the spinal cord have been proposed to be promising targets for promoting regeneration after injury. The delivery of growth factors to the injury site may induce endogenous regeneration through stimulating endogenous neural stem/progenitor cells. One potential delivery method is the biomaterial-based systems. Biocellulose (BC) has been reported to be biocompatible; however, BC has not been tested for the treatment of SCI.

Objective: We hypothesize that growth factor loaded composite BC tubes can act as a sustained protein delivery system to be used for SCI applications.

Methods: The composite delivery system is composed of 2 parts: BC tube and PLGA microspheres loaded with bovine serum albumin (BSA) as a model drug. PLGA microspheres were produced by solvent evaporation method in presence and absence of an osmotic agent. This was followed by morphological characterization using scanning electron microscopy (SEM), encapsulation efficiency and release profile assessment. BC tubes were produced through modification to cellulose membrane synthesized by Gluconacetobacter Hansenii bacteria. Microspheres were loaded on BC tubes to form composite membranes and the incorporation was confirmed by SEM as well as microsphere retention assessment. BC tubes were mechanically characterized and the release profile using BSA as a model drug was assessed.

Results: Optimization of the fabrication process was performed. SEM analysis revealed successful production of microspheres with variable size and surface morphology that was dependent on the sodium chloride content. Encapsulation efficiency was significantly dependent on the volume of internal aqueous phase used, reaching 10.4%±1.4 versus 69.9%±2.1 for low and high internal phase volume, respectively. Structural analysis revealed the successful incorporation of microspheres into BC tubes with retention percentage of 97%±12%. Assessment of swelling ratio revealed the high liquid absorption capacity of BC tubes, reaching 967% (±50%) of its original weight within 72 hours. In addition, mechanical testing for composite BC tube revealed a compressive modulus of 130±10 kPa as compared to 8±1 kPa for the rat spinal cord. Finally, release profile assessment revealed a sustained BSA release behavior over an extended period of time.

Conclusion: Microsphere loaded BC tubes are a promising sustained release system to deliver GFs in case of SCI. Next steps will be in vitro bioactivity assessment of growth factor loaded composite membranes on primary neural stem/progenitor cells.

CHROMATIN CROSS-TALK IN TLX1 MEDIATED T-ALL

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Background: T-cell Acute Lymphoblastic Leukemias (T-ALLs) are aggressive hematological tumors caused by malignant transformation of T cell progenitors. Amongst T-ALL patients, 5-10% pediatric and nearly 30% adult were characterized by aberrant expression of a homeobox family transcription factor TLX-1. Normal physiological function of TLX-1 is restricted to spleen development during embryogenesis. However, ectopic over-expression of TLX-1 is caused by translocation of the gene to the very strong enhancer elements of the T-cell receptor loci. Until recent years, very little was known about the specific mechanisms that mediate T-cell transformation downstream of TLX1. Interestingly, TLX1 induced leukemias have unique genetic alterations including NUP214-ABL1 oncogene rearrangement and mutation of tumor suppressor genes like WT1 and PHF6. Chromatin profiling using Chip-seq data (from our lab and others) including TLX1, architectural protein CTCF and repressive histone modification, H3K27me3 revealed interesting observation. Several TLX1 binding sites overlapped with CTCF binding sites. More importantly many of these overlapping sites marked the boundary of the repressed chromatin. This suggests a regulatory role of TLX1 in chromatin organization of the leukemia.

Objective: To identify TLX1-mediated loops in TLX1 expressing T-ALL cell line (i.e. ALL-SIL cell) and further study new loop formation upon TLX1 over-expression in a human cell line.

Methods: To address this, the study involves use of Next Generation Chromatin Conformation Capture assay (Capture-C) in different T-ALL cell lines and in a tagged-TLX1 overexpressed cell line.

Results: Preliminary chromatin conformation capture (3C) assay has revealed specific interaction between TLX1 binding sites in TLX1 expressing leukemic cell line, ALL-SIL. Capture-C analysis further reveals a strong interaction between TLX1 binding sites in ALL-SIL cells compared to the other non-TLX1 expressing T-ALL cell line.

Conclusion: Strong interaction between the TLX1 binding sites reveal regulatory role of TLX1 in shaping the chromatin structure in T-ALL cells, hence influencing the downstream gene expression. Further validation will help us decipher TLX1 role in the higher order chromatin organization of T-ALL cells and might reveal new loops with functional implication in TLX1 dependent T-ALL.

A double dose of DNA damage: Overcoming PARPi resistance using targeted oncolytic viruses

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Hereditary breast and ovarian cancers (HBOC) represent 5-10% of breast and 10-15% of ovarian cancer cases. These cancers tend to be difficult to treat and progress in an aggressive manner. Poly(ADP-ribose) polymerase inhibitors (PARPi), a family of drugs that inhibit DNA repair, are a promising therapy for cancers harbouring mutations in their DNA repair machinery, such as HBOC. Unfortunately, nearly all patients ultimately become resistant to PARPi, leaving limited options for definitive treatment. Oncolytic or "cancer-killing" viruses are an innovative immunotherapeutic platform capable of selectively targeting cancer cells, leaving normal cells unharmed. Our group has demonstrated that oncolytic rhabdoviruses may be used to deliver therapeutic payloads by encoding targeting sequences to act on genes via RNA interference. I aim to engineer cancer specific viruses that will target genes essential for DNA repair, sensitizing them to PARPi. shRNA sequences targeting the DNA repair pathway have been cloned into the genome of an oncolytic rhabdovirus and downregulation of targeted genes has been validated by both qPCR and Western blot analysis. Preliminary validation experiments testing the combination of siRNA knockdown of target sequences with PARPi have revealed a significant decrease in survival compared to PARPi alone via clonogenic assays of both human and mouse breast tumour cell lines; current experiments aim to demonstrate a similar effect combining viral infection with PARPi using *in vivo* models of ovarian and breast cancer. By engineering oncolytic viruses capable of delivering PARPi sensitizing sequences, this combination approach may enhance and expand the utility of oral PARPi therapeutics.

Inhibition of Human Adenovirus Replication by Curcumin

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Background: Human adenovirus (HAdV) is a relatively common “cold” virus that can rapidly spread through confined populations such as schools, day care centres and retirement homes. Though typically self-limiting, HAdV infection can cause severe morbidity and mortality in certain populations, including pediatric, geriatric, and immunocompromised patients. No effective anti-HAdV therapeutics are currently available to limit infection or spread of the virus.
Our research group has developed a high-throughput screening protocol to identify compounds that inhibit HAdV replication. Such compounds may act as effective anti-HAdV therapeutics, in addition to providing novel insight into basic virus biology. We have determined that curcumin (a major component of the spice turmeric) inhibits HAdV replication. Previous studies showed that curcumin treatment impacts several cellular proteins and pathways within the cell, including upregulation and activation of the cell cycle regulatory protein p53. Given that HAdV naturally inhibits p53 function during infection, it suggests a mechanism by which curcumin may act to inhibit the HAdV replicative cycle.

Objectives: Determine the mechanism by which curcumin inhibits HAdV infection.

Methodology: A549 cells were treated with varying concentration of curcumin (0-100 μM) and infected with wildtype HAdV. Crude protein samples were isolated at varying time post-infection (0-24 hr, the time required for one complete HAdV replicative cycle), and probed for expression of viral proteins indicative of early and late virus replication, to determine the stage of the virus lifecycle impacted by curcumin. Cell viability during treatment was analyzed using a metabolic activity assay. Finally, recovery of functional virus from treated cells was analyzed by plaque assay –infected cells were harvested into the medium, freeze/thawed to release virus, and the virus titer determined on A549 cells.

Results: Treatment of cells with curcumin caused a dose-dependent decrease in HAdV early and late protein expression, which also resulted in reduced recovery of virus. However, although treatment of cells with up to 50 μM of curcumin had no effect on cell viability, treatment of cells with 100 μM led to a significant reduction in cell viability, suggesting curcumin may be toxic to the cells at high doses.

Conclusion: Our results suggest that curcumin is an effective anti-HAdV therapeutic, but may have a narrow therapeutic window.

Evaluation of Cancer-testis Antigens in Osteosarcoma and Dedifferentiated Liposarcoma as Targets for Immunotherapy

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Background: T-cell based immunotherapies are a promising alternative to traditional cancer treatments due to their ability to target only malignant cells, leaving benign cells unharmed. The development of successful immunotherapy requires the identification and characterization of targetable immunogenic tumor antigens. Cancer testis antigens (CTA) are a group of highly immunogenic tumor-associated proteins that have emerged as potential targets for CD8+ T-cell recognition. Unlike other auto-antigens, CTAs exhibit restricted expression in normal tissue, limiting potential therapeutic side effects. Other parameters that are crucial for CD8+ T-cell recognition of tumor cells, such as their ability to infiltrate the tumor and the expression of HLA-peptide complexes on the surface of cancer cells, also play important roles in the outcome of immunotherapy.

Objective: The goal of this study is to screen for CTA expression, HLA expression, and tumor T-cell infiltration in human dedifferentiated liposarcoma (DDLPS) and osteosarcoma (OS) by IHC , in order to identify targetable immunogenic antigens for T-cell based immunotherapy.

Methods: Human tissue micro-arrays composed of 50 cores of OS and 18 cores of DDLPS were obtained, along with matched control tissues from the same patients. IHC for the cancer testis antigens NY-ESO-1, MAGE-A3, and SSX/SSX2, as well as for CD3 and CD8 T-cells was performed.

Results: Immunohistochemical analysis of CTA expression showed considerable inter- and intra-tumoral heterogeneity. DDLPS showed relatively low expression of all CTAs tested, with only 11% of samples exhibiting MAGE-A3 and 1 sample each (5.5%) showing expression of SSX2 and NY-ESO-1 in low percentages of tumor cells. By contrast, in osteosarcoma, 100% of samples expressed MAGE-A3 and 89% expressed SSX, both with >80% of positive cases showing moderate to high expression. NY-ESO-1 was expressed in 78% of OS samples, predominantly at low levels. Brisk infiltration of CD8+ T cells was observed in over 70% of both sarcoma types tested. Furthermore, all sarcoma samples tested were positive for HLA expression.

Conclusions: To date, these results show promising expression of CTAs MAGE-A3 and SSX in human OS, which may be used as targets in the future development of an immunotherapy for sarcoma. Contrarily, DDLPS shows relatively low expression. The data generated throughout this project will provide insight into the immune profile of DDLPS – information that is critical for immunotherapy design.

A Novel Lung Tropic AAV Vector Dramatically Improves Survival and Surfactant Homeostasis in a Mouse Model of Inherited Surfactant B Deficiency

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Background: Surfactant protein B (SP-B) deficiency is an autosomal recessive disease that impairs surfactant homeostasis and phenotypically manifests as lethal respiratory distress. A compelling argument exists for the use of gene therapy to treat this disease, as *de novo* protein synthesis of SP-B in alveolar type 2 (AT2) epithelial cells is required for proper surfactant production. However, the challenges in delivering transgenes to AT2 cells have been formidable due to obstacles such as the immune response, the requirement of cell-surface receptors for entry, and the barrier properties of respiratory mucus and alveolar fluid.

Objective: We examine whether gene therapy using an adeno-associated virus (AAV)-vector rescues respiratory distress and improves survival in a mouse model of SP-B deficiency.

Methods: We rationally designed an adeno-associated virus (AAV)6 capsid to deliver SP-B cDNA into a transgenic mouse model that conditionally expresses SP-B under the control of doxycycline. In the absence of doxycycline, these mice suffer progressive respiratory failure and death occurs within 2 days.

Results: Intratracheal administration of this vector delivering mouse or human SP-B cDNA into the SP-B deficient mouse model restored surfactant homeostasis, prevented lung injury, maintained lamellar body structure, and improved lung physiology. Untreated SP-B deficient mice developed fatal respiratory distress within 2 days. Gene therapy resulted in an unprecedented improvement in median survival to more than 6 months, with the longest living mouse surviving >7 months. This vector also efficiently transduces human lung tissue.

Conclusion: AAV-SPB demonstrates unambiguous potential for clinical translation against SP-B deficiency.

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Insights into remyelination failure in progressive multiple sclerosis – miR-145-5p regulates oligodendrocyte differentiation and its loss promotes remyelination in a model of chronic demyelination

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Background/Objectives: Progressive multiple sclerosis (pMS) is a debilitating disease in which demyelinated lesions form in the central nervous system (CNS). Normally, demyelination leads to recruitment of oligodendrocyte progenitor cells (OPCs), which differentiate into oligodendrocytes (OLs) to regenerate lost myelin. However in pMS, OPCs fail to differentiate despite their active recruitment. One characteristic of pMS lesions is abnormally high expression of microRNA miR-145-5p. In OPCs, miR-145-5p is also expressed at high levels but is strongly downregulated as they begin to differentiate. This downregulation may be required as OPCs transition to OLs, and thus high levels of miR-145-5p play into the OL differentiation block observed in pMS. We aimed to determine how altering normal expression of miR-145-5p affects OL maturation, and whether its expression could be manipulated to affect remyelination in an animal model of chronic demyelination.

Methods/Results:*In vitro*, differentiating OLs overexpressing miR-145-5p showed severe defects in branching and myelin protein expression, and extensive gene expression changes in major pathways including myelination, Wnt signaling and CNS development. Conversely, OLs in which miR-145 was knocked out showed enhanced differentiation. Following a course of chronic exposure to the demyelinating agent cuprizone, *in vivo* analyses revealed that miR-145 knockout mice exhibited striking levels of remyelination while wild-type littermates remained chronically demyelinated. Further, behavioural assessments showed that the remyelination in miR-145 knockout mice led to functional recovery of both motor deficits and anxiety-driven behaviours caused by chronic cuprizone exposure.

Conclusions: Taken together, these data suggest that downregulation of miR-145-5p is required for OL differentiation, and that loss of that downregulation – as is observed in pMS - is detrimental to OL differentiation. Excitingly, loss of miR-145 expression in our mouse model of chronic demyelination allows extensive remyelination and full functional recovery following chronic demyelination.Thus, the overabundance of miR-145-5p in the pMS lesion microenvironment may be a factor in the OL differentiation block observed there, and miR-145-5p may serve as a relevant therapeutic target to help overcome this aspect of remyelination failure.

Metformin promotes CNS remyelination and improves social interaction following focal demyelination through CBP Ser436 phosphorylation

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Background: Individuals with demyelinating diseases often experience difficulties during social interactions that are not well studied in preclinical models. A simple and reliable experimental animal model particularly addressing the relationship between white matter demyelination and social impairment is still lacking.

Objective: To developed a murine juvenile focal lysolecithin-induced demyelination model exhibiting a robust social interaction deficit, manifesting social impairments, that could be ameliorated with treatment with metformin. And also to promote oligodendrocyte regeneration from both NPCs and OPCs and enhances remyelination by stimulating CBP Ser436 phosphorylation using metformin

Methods: In this study, we describe a novel juvenile focal corpus callosum demyelination murine model exhibiting a social interaction deficit. Using this preclinical murine demyelination model, we discover that the application of metformin, an FDA-approved drug, in this model promotes oligodendrocyte regeneration and remyelination and improves social interaction.

Results: The beneficial effect of metformin acts through stimulating Ser436 phosphorylation in CBP, a histone acetyltransferase. In addition, we found that metformin acts through two distinct molecular pathways to enhance oligodendrocyte precursor (OPC) proliferation and differentiation, respectively. Specifically, metformin enhances OPC proliferation through the autophagy pathway, while metformin promotes OPC differentiation into mature oligodendrocytes through CBP Ser436 phosphorylation.

Conclusions: In summary, we identify that metformin is a promising remyelinating agent to improve juvenile demyelination-associated social interaction deficits by promoting oligodendrocyte regeneration and remyelination.

Characterization of histone modifications associated with MyoD-bound enhancers in regenerating muscle

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Myogenesis defines the cellular processes involved in muscle formation and repair of damaged muscle tissue. Regeneration is promoted by super-enhancers, a large group of neighbouring transcriptional enhancer regions. Coordinated activation of super-enhancers is required for the regulation of lineage-specific genes that ultimately determine cell identity. These regions are delineated by signature histone marks, such as acetylated histone H3 lysine 27 (H3K27ac) and methylated H3K4 (H3K4me1), and an abundance of transcription factors and cofactors. The fate of satellite cell differentiation into muscle tissue is likely reliant on histone marks found at active, muscle-specific enhancer regions. However, the identity of all the histone marks required for an active enhancer is not comprehensive as the methods for discovery have thus far been limited to histone-specific antibodies. In muscle, MyoD acts to establish active enhancers of genes involved in myogenesis. Here we examined whether proximity labelling (PL) using MyoD would allow for the identification of histone modifications associated with muscle enhancers in an unbiased manner. Using a miniTurbo-MyoD fusion protein for proximity ligation, target histones were biotin labelled, purified, and subjected to mass spectrometry. Ongoing work is expected to reveal the full set of epigenetic marks that define muscle-specific active enhancers. It has been shown that genes correlated to disease are enriched in super-enhancer regions, indicating that these regions could act as biomarkers for disease diagnosis. This investigation will uncover key components of muscle differentiation which is important for the development of myopathic therapeutics.

Role of Integrin ß1 in Disseminated Tumor Cell Dormancy in Prostate Cancer Bone Metastases

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Background: Our previous work identified that integrin β1 (ITGβ1) is an important regulator of prostate tumor cell invasion. We thus speculated it played an important role in metastasis of prostate tumors *in vivo*. Using an intracardiac bone metastasis model, we found that ITGβ1-depleted PC3 cells had delayed growth of bone metastases *in vivo* however tumors eventually arose in all animals.

Objective: As it is known that disseminated prostate tumors in the bone undergo a period of bone microenvironment induced dormancy, we speculated that ITGβ1 may facilitate the ability of disseminated tumor cells to overcome bone microenvironment-induced dormancy in part via cellular cross talk with bone mesenchymal stromal cells (MSC).

Methods: Our *in vitro* experiments utilized siRNA to reduce ITGβ1 expression in PC3 cells. To investigate the role of cellular cross-talk, cell-conditioned supernatant transfer assays were used to assess the ability of ITGβ1-depleted versus ITGβ1-expressing PC3 cells to modify protein expression in MSC. The subsequent ability of the conditioned supernatants from treated MSC to modulate cell growth and viability of parental PC3 cells was assessed using AlamarBlue cell viability assays. Expression of various factors associated with dormancy or quiescent phenotypes were also assessed by western blot and qRT-PCR.

Results: We found that ITGβ1-depleted cells induced proliferation of MSC and additionally had increased expression of SPARC, a matricellular protein which has been previously shown to influence growth factor secretion (such as BMP7) by MSC. As it was previously shown that SPARC can induce dormant quiescent cell phenotypes in tumor cells, we tested the effects of conditioned media from MSC that had been stimulated with conditioned media from ITGβ1-depleted or expressing PC3 cells. This conditioned supernatant from MSC cells treated with either conditioned media from ITGβ1-depleted or ITGβ1-expressing PC3 had altered ability to modulate growth and viability of parental ITGβ1-expressing PC3 cells.

Conclusion: These findings highlight an important role for ITGβ1 in mediating inter-cellular cross-talk to facilitate growth of tumor cells in metastatic locations.

Investigating Immunomodulatory Effect of Mesenchymal Stem Cell Treatment on Innate Immune Cells

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Background: Sepsis is a life-threatening pathobiology driven by an uncontrolled innate immune response to infection, leading to multiple organ failure and death. While some studies have demonstrated that MSCs modulate monocyte/macrophage functions and inhibit T cell activation, the exact immunomodulatory effects of MSCs on host innate immune cells in sepsis is still unknown.

Objective: Here, we sought to determine whether exposure to an inflammatory milieu would enhance or decrease the immunomodulatory properties of MSCs. Our hypothesis is that a hyper-inflammatory state in sepsis does not impair MSC's ability to modulate immune cell functions.

Methods and Results: Human bone marrow MSCs, derived from healthy volunteer donors, were used between passages 3 to 6 and were all characterized to meet ISCT criteria of MSC. To mimic overstimulated inflammatory state (known as cytokine storm) that can be experienced by septic patients in vitro, MSC were incubated with cytomix, which consists of three key sepsis relevant inflammatory cytokines. Transcriptome analysis revealed that cytomix-stimulated MSC (vs. unstimulated) showed marked changes of immune-related genes, such as ptges and ptgs2. Furthermore, stimulated MSC showed an upregulation of cytokines (i.e., IL-6, IL-8, vs. unstimulated), accompanied by greater capacity of MSC to improve monocytic phagocytosis in co-culture assay. Finally, using an animal model of sepsis (cecal ligation and puncture, CLP model), monocytes isolated from the peritoneum of septic animals treated with MSCs (at 6 hrs after CLP) showed significant improvement in their phagocytic activity, accompanied by increased bacteria clearance by the host and reduced levels of systemic inflammatory cytokines.

Conclusions: Our results demonstrated that cytokine storm-like microenvironment may educate MSCs to modulate immune cell functions in vitro and in septic animals. Further experiment will be performed to confirm the ability of MSC to promote interactions between immune cells and potentially promote long-term recovery after sepsis.

Outcomes and Costs of ICU Patients Experiencing Adverse Events

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Background: Adverse events (AE) occur in the intensive care unit (ICU) setting more frequently than in general hospital units and are associated with increased length of stay, higher costs, and unfavourable patient outcomes.

Objectives: This study was conducted to identify pre-existing conditions, interventions, mortality, and costs associated with the occurrence of adverse events within two tertiary-care ICUs in a single hospital system in Ontario, Canada.

Methods: A retrospective cohort study of 17,173 patients 18 years of age and older who were admitted to an ICU at The Ottawa Hospital between January 1, 2011 and December 31, 2016. Patients were categorized according to whether or not they experienced an adverse event and chi-squared tests were performed to test for differences between the two groups. The primary outcome was in-hospital mortality.

Results: Older age (64.9 vs 63.1, $p<0.001$) and higher Elixhauser comorbidity scores (5.92 vs 4.83, $p<0.001$) were associated with the occurrence of adverse events. In-hospital mortality was higher among AE patients (26.3% vs 18.4%, $p<0.001$). ICU interventions occurred more among patients who had an adverse event (eg. dialysis, 14.9% vs 8.7%, $p<0.001$); invasive mechanical ventilation, 64.1% vs 46.8%, $p<0.001$; central line, 69.6% vs 42.9%, $p<0.001$). Hospital and ICU length of stay were longer for patients with adverse events (32.1 vs 9.1, $p<0.001$ and 11.1 vs 5.2, $p<0.001$, respectively). A smaller proportion of patients with adverse events went home without support (18.7% vs 44.3%, $p<0.001$) and a greater proportion received continuing care (20.2% vs 8.2%, $p<0.001$). Total hospital and ICU costs were higher among patients with adverse events ($72,609 vs $20,475, $p<0.001$ and $46,706 vs $16,180, $p<0.001$, respectively).

Conclusions: Patients with adverse events had greater mortality, longer length of stays, and higher costs compared to patients who did not have adverse events. Increased age and the presence of comorbidities were associated with adverse events. A subsequent study may investigate particular causes of adverse events among patients with comorbid conditions, and policy changes or innovations in care may be required to reduce the occurrence of adverse events among these patients.

Improvement of Survival in Septic Mice by Treatment with Mesenchymal Stem Cells Overexpressing Acyloxyacyl Hydrolase

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Background: Sepsis is one of the main causes of mortality in intensive care units and can be caused by an unbalanced immune response upon an infection. As a key component of the outer membrane of Gram-negative bacteria, Lipopolysaccharide (LPS) is a pivotal pathogenic trigger of bacterial sepsis, which accounts for 67.1% of patients around the world. Our group has previously shown that the treatment with mesenchymal stem cells (MSCs) is beneficial in animal models of sepsis, including significant improvement in survival. Acyloxyacyl hydrolase (AOAH), an enzyme that can inactivate LPS, are usually found in mammalian antigen presenting cells. We hypothesized that transfection of MSCs with acyloxyacyl hydrolase (AOAH) can further enhance MSCs' therapeutic action in septic mice infected with *E. coli*.

Methods & Results: Human AOAH gene was cloned into two different expression DNA plasmids, and AOAH-plasmid #1 exhibited higher expression level compared with AOAH-plasmid #2, and was used in all the following experiments. After transfection, the expression of both intracellular and extracellular AOAH was assessed by Western Blot and ELISA, respectively. After 24 h of expression, MSCs with or without transfection of AOAH-plasmid were delivered through the right jugular vein of mice at 1 hour post intraperitoneal bacteria administration. Results showed that overexpressed AOAH could be found in both intracellular and extracellular fractions of cell lysate/supernatant. Gram-negative bacteria induced septic mice showed significant improvement in survival with the treatment of MSC-AOAH, compared with vehicle-treated animals. MSC-AOAH treated animals showed a slightly better survival rate compared with MSCs treated animals.

Conclusions: Overexpression of AOAH protein has been confirmed in MSCs transfected in cell lysates and culture supernatant. Treatment of MSC-AOAH showed survival benefit in gram-negative bacterial septic mice. These results support the potential benefits of the AOAH gene enhancement of MSC therapy to treat sepsis caused by Gram-negative bacteria.

Abnormal temporal dynamics of resting state shapes stimulus-related activity in depression – An EEG study on rest-stimulus interaction

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Background: Major depressive disorder (MDD) is a complex psychiatric disorder characterized by changes in both resting state and stimulus-related activity. Whether resting state changes are carried over to stimulus-related activity, however, is unclear.

Objective: We conducted a combined rest (3 mins) and task (auditory oddball paradigm) EEG study in acute depressed MDD patients (*n*=28), comparing them with healthy participants (*n*=25).

Methods: Our focus was on the temporal dynamics of both resting state and stimulus-related activity, for which reason we measured frequency band peak frequency (PF), coefficient of variation (CV), Lempel-Ziv complexity (LZC), and trial-to-trial variability (TTV).

Results: Our main findings are: (i) abnormal temporal dynamics in resting state, specifically in the alpha and theta bands as measured by their peak frequency (PF), coefficient of variation (CV) and power; (ii) decreased reactivity to auditory target deviant stimuli as measured by decreased changes in stimulus-related variance and complexity – TTV, LZC, and power and frequency sliding (FS, PS); (iii) correlation of task related measures (TTV, LZC, PS, FS) with resting state measures.

Conclusions: Together, our findings show that resting state dynamics alone are abnormal in MDD and, even more important, strongly shapes the dynamics of subsequent stimulus-related activity. We thus conclude that MDD can be characterized by an abnormal temporal dynamic of its rest-stimulus interaction. This in turn makes it difficult for depressed patients to react to relevant stimuli such as the deviant tone in our paradigm.

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Interventions to prevent anastomotic leak after esophageal cancer resection: A systematic review and meta-analysis

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Background: Esophagectomy is critical to gold standard curative intent treatment of esophageal cancer in combination with radiation and chemotherapy. Up to 50% of patients with esophageal cancer experience esophagogastric anastomotic leak after surgery, which is associated with a prolonged length of stay, stricture formation, and increased morbidity and mortality [1]. Many standardized interventions have been investigated to attempt to reduce leaks, including reinforcement of the anastomosis using a pedicle omental flap (omentoplasty).

Objective: To provide a comprehensive summary of all randomized control trials (RCTs) reporting the efficacy and safety of standardized interventions designed to prevent esophagogastric anastomotic leak.

Methods: We searched MEDLINE and Embase from 1946 to 2019. RCTs from investigating any intervention to minimize anastomotic leakage in patients with esophagectomy were included. Two reviewers independently reviewed 441 abstracts and 73 full manuscripts, 11 RCT articles were eligible for meta-analysis. A random effects method was used to generate a pooled risk ratio (RR) for anastomotic leak across standardized interventions. The quality of evidence for anastomotic leak was graded as high, moderate, low, or very low [2].

Results: Eleven RCT articles that investigated interventions to prevent anastomotic leak after esophagectomy among patients treated for esophageal cancer were included in meta-analysis. Patients treated with an omentoplasty intervention showed a significant 78% reduction of risk for anastomotic leak [RR: 0.22; 95% CI: 0.10, 0.50; I^2 0] compared to conventional esophagectomy (3 studies, 650 patients). Patients treated with early (1 to 2 days) or no nasogastric tube decompression showed a significant 88% reduction of risk for esophagogastric anastomotic leak [RR: 0.12; 95% CI: 0.02, 0.65; I^2 28.4] compared to prolonged decompression lasting 2 to 7 days (2 studies, 307 patients). Patients treated with a stapled anastomosis had a 10% reduction of risk for esophagogastric anastomotic leak [RR: 0.90; 95% CI: 0.51, 1.5; I^2 28.4] compared to hand-sewn anastomosis (6 studies, 1630 patients). Omentoplasty had high quality of evidence while early nasogastric tube decompression and stapled anastomosis interventions had low and moderate quality of evidence, respectively.

Conclusion: There is a high level of evidence that omentoplasty significantly reduces the risk of esophagogastric anastomotic leak. There is low to moderate level of evidence that early nasogastric tube decompression and stapled anastomosis interventions reduce the risk of leak. There is a need for further RCT evidence to clarify the role of standardized interventions to prevent anastomotic leak.

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Chest Tube (CT) Management after Lung Resection Surgery using Machine Learning Classification

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Background: After lung surgery, air and liquid may be drained from the pleural space to maintain subatmospheric pressure. Pleural effusion (fluids accumulation), pneumothorax (air in chest cavity), and prolonged air leaks can lead to poor lung function and may cause the lung to collapse. Inserting a chest tube (CT) into the pleural space and connecting it to a sealed drainage system with a one-way valve for fluid evacuation is a common procedure. CT management is highly specialized and requires careful decision-making. While the duration of drainage significantly impacts patient experience, outcomes, length of stay, and postoperative costs, CT cannot be safely removed until air leak and fluid output have met specific safety criteria.

Objective: Optimizing CT management remains a significant challenge to minimize risks associated with premature removal (e.g. pneumothorax, fluid re-accumulation, chest tube re-insertion) and to avoid prolonged air leaks that extend more than 5 days after surgery. Early, and accurate, identification of patients who require CT maintenance is crucial. Conversely, patients who meet CT removal criteria should not be delayed to achieve higher efficiency. Using machine learning methods, we develop a clinically manageable approach to CT management without compromising safety and accuracy.

Methods: after surgery, patients struggle to attain a state of recovery which prevails after a period of time. This produces discrepancies and inconsistencies reflected in patient monitoring data. We identify this time window -- we call it the predictive time window, when most patients achieve this state of stability. We use monitoring data collected during this predictive time window to construct a machine-learning model that recommends CT maintenance or removal. To identify this predictive time window, we partition the data by equal-width time intervals, and using a sliding-window algorithm, we build machine learning classifiers from early data to predict CT maintenance or removal in subsequent time frames.

Results: we analyzed data from 115990 pleural space measurements and 1264 CXRs. Data collection in 36-48 hours postoperatively is the optimal period for building CT management recommendations. The AUC of safe CT removal within the following 96 hours was 95% (95% CI, 90-95%). The unsafe CT removal was 8% (95% CI, 4-10%) false positives resulting in 92% (85% CI, 90-96%) specificity. The efficiency of CT removal (sensitivity) was 71% (95% CI, 66-79%).

Conclusions: machine learning can safely and efficiently predict CT removal with error consistently under 10%. Prospective development and testing are further needed.

Mechanisms of Periostin acquired expression by epithelial cells in an ErbB2 driven breast cancer animal model.

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Background: ErbB2 is an oncogene overexpressed in about 30% of all breast cancer tumors. About 50% of these tumors acquire epithelial Periostin (Postn) expression while the fibroblast population of these tumors always expresses it. The expression of Postn in the epithelial compartment has been correlated with a more aggressive phenotype. Postn itself has been shown to be involved in various processes of tumor development like angiogenesis, invasion, cell survival and metastasis. It seems like the effect are worse when the expression is present in the epithelial cells since those are the cells that needs to escape the primary tumors to establish at a distant site for metastasis to happen. The regulation of Postn is poorly understood.

Objectives: Therefore, we assessed the role of Postn in ErbB2+ tumors using a global knockout model. We hypothesize that Postn is driving ErbB2-mediated tumor growth and that depleting it completely from the tumor microenvironment will significantly delay tumorigenesis and metastasis rate. Furthermore, we believe that we will identify specific factors that are regulating the expression of Postn in epithelial and fibroblastic cells distinctively.

Methods and Results: Using the NeuNDL model (ErbB2 positive animal model) along with the Postn global knockout model, we are tracking tumor onset, tumor progression and overall survival. Collection of endpoint tumors along with lungs from those animals are used for staining and analysis of the number and size of lung metastases. We also have already identified a complex supplement (Bovine Pituitary Extract; BPE) that is able to repress Postn in epithelial cells, using RNA-sequencing technology we were able to identify potential key regulators of Postn in BPE treated cells. Furthermore, dissecting this supplement into individual components using different chromatography approaches combined with mass spectrometry was also helpful in identifying potential key players in the regulation of Postn. Multiple pathways (TGFβ, AKT, MAPK) are being investigated as potential regulator of Postn.Using Q-PCR and western blotting analysis we can assess the expression of Postn in those epithelial cells *in vitro*.

Conclusion: This project will give insight into the role and effect of Postn in breast cancer as well as elucidate the regulation mechanisms of Postn specifically in breast cancer. Considering the differences in the regulation between epithelial and fibroblast cells. We believe these findings will elucidate a new way of treating this disease by targeting epithelial cells in order to prevent them from expressing Postn.

Expression and activity of peptidylarginine deiminase 4 in hypertension and diabetes

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Background: Hypertension and diabetes are important risk factors of vascular disease and are the leading causes of chronic kidney disease (CKD). Endothelial dysfunction frequently precedes development of CKD in hypertension and diabetes. However, little is known on the mechanisms behind endothelial dysfunction and its role in the progression of CKD. Peptidylarginine deiminases (PADs) are a family of enzymes that convert arginine to citrulline in protein, generating neoantigens that are foreign to the immune system. As such, PADs have been involved in a number of inflammatory autoimmune diseases such as lupus and multiple sclerosis. More specifically, PAD4 has been associated to the development of endothelial dysfunction in such diseases. PAD4 is known to citrullinate histones leading to the formation of neutrophil extracellular traps (NETs). Dysregulated NET formation, as seen in lupus and diabetes, has been linked to vascular damage and delay in tissue healing. Whether PAD4 has a direct effect on the vasculature is not known. This project aims to investigate the role of PAD4 and NETs on endothelial function in models of hypertension and diabetes.

Method/Results: An endothelium-specific deletion of PAD4 in mice was generated through a Cre-LoxP system. Endothelial-dependent vasorelaxation was assessed in the $PAD4^{endo-/-}$ mice using wire myography. Blood pressure was also measured at 8 and 16 weeks. We observed no significant difference in endothelial-dependent vasorelaxation or blood pressure in the $PAD4^{endo-/-}$mice, under non-diabetic normotensive conditions. Levels of NETs were assessed in the kidney of hypertensive, diabetic, and hypertensive-diabetic mice by immunohistochemistry and Western blot analysis of citrullinated histone H3, neutrophil elastase, and PAD4. We observed a significant increase in PAD4 activity and NET formation in the kidney of hypertensive-diabetic mice, associated with interstitial fibrosis and reduced renal function. Total protein citrullination was also significantly increased.

Conclusion: Our current results suggest that endothelial PAD4 has no effect on vascular function under baseline conditions. However, PAD4 activity and NET formation are increased in hypertensive diabetic mice, suggesting that PAD4 could be a potential therapeutic target for endothelial dysfunction and CKD in early hypertension and diabetes.

Preclinical Multicenter Acute lung injury Trials In Canada (PreMATIC): A pilot study assessing feasibility

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Background: 'Bench-to-bedside' failure may be attributable to pitfalls of typical single-center preclinical laboratory studies (e.g. lack of study design rigor, or lack of generalizability to other laboratories).

Objective: Use methodologically rigorous multicenter preclinical studies to address some of these issues.

Methods: We performed a pilot study using a mouse model of acute lung injury (ALI). We chose to conduct a non-therapeutic study where the intervention was disease induction [lipopolysaccharide (LPS, ALI disease) intratracheally vs phosphate buffer saline (PBS, control) intratracheally]. 8-week old C57/Bl6 mice (Charles River) were used at four labs ('centers'). Mice were anesthetized with ketamine/xylazine and either intubated with 50mg of LPS in 50uL PBS or 50uL PBS alone. Randomization and blinding to the intervention occurred as LPS and PBS were in identical vials identified only by mouse number. Mice were recovered, monitored for 24h and then sacrificed. Feasibility (primary outcome) was assessed by tracking protocol violations. Plasma and bronchoalveolar lavage fluid (BALF) were collected to assess secondary outcomes including inflammation (cytokines) and barrier disruption (protein concentration in BALF).

Results: We successfully harmonized protocols for disease induction between centers, gained multi-institutional animal care approval and synchronized the conduct of experiments. Minimal protocol violations were noted. Despite large between-center variation, LPS treated mice demonstrated significant changes in cytokines and barrier function.

Conclusion: The PreMATIC pilot is the first multicenter preclinical study in Canada. By using this approach, we will next conduct the world's first multicenter preclinical trial of a stem cell therapy. This may provide a more robust evidence-base to consider clinical translation.

Feasibility of a coaching App in the management of patients with post-concussion symptoms.

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Background: Patients with persisting mTBI/concussion symptoms can experience significant somatic, social, economic and psychological impacts. Following specialist consultations, patients often exhibit challenges with treatment recommendations, including medication and lifestyle modifications. An internet-based health coaching tool may allow for more timely and consistent patient contact, which could provide the support needed to help patients optimize their health outcomes.

Objectives: To assess the feasibility of a scheduled interactive personal health log with a coaching component, in the management of patients with post-concussion syndrome.

Methods: Seven patients were recruited prospectively to use the App and track 2-4 individual treatment goals derived from their initial consultation, for 5-6 weeks. Patients completed weekly progress questionnaires and the health coach and physician provided scheduled weekly health coaching, through the App. Data collected included patient App activity/compliance, patient satisfaction and feedback, physician and coach satisfaction, and pre and post symptom comparison using the Rivermead post-concussion symptom questionnaire.

Results: All questions received scores of “Strongly Agree”, “Agree” or “Neither Agree nor Disagree” from all participants, with most responses being positive. All participants found the App easy to use, and five participants (71.4%) felt that the coaching and App helped them improve their symptoms. All participants believe that this is an effective tool that could be used in patient care. Six out of seven patients (85.7%) had improved scores on their Rivermead Post-Concussion Questionnaire after the 6 weeks. Six out of seven patients showed a compliance of 90% or higher with using the app.

Conclusion: Our results show promising evidence that an online coaching App is a feasible tool to improve the adherence and symptom management of patients with post-concussion symptoms. Further studies should focus on determining the effectiveness of this intervention on a larger scale.

Potential use of 17BIPHE2 as a vaginal contraceptive/microbicide

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Background: We have demonstrated that antimicrobial peptide LL-37 and its truncated form, GF-17, have spermicidal activity on both human and mouse sperm (Kiattiburut et al., Human Reprod 2018). LL-37 and GF-17 at the spermicidal concentration (10.8 □M) also exert microbicidal activity against *Neisseria gonorrhoeae*, a sexually transmitted infection (STI) pathogen. Therefore, LL-37 and GF-17 can potentially be developed into vaginal contraceptives/anti-STI agents. However, both peptides were susceptible to degradation by proteases in human cervicovaginal lavage (CVL). Finding LL-37/GF-17 mimetics that are resistant to proteases is one avenue to circumvent this challenge. 17BIPHE2 is a mimetic of GF-17 with 5 unnatural residues, thus likely to be resistant to protease degradation.
Objective: Determine whether 17BIPHE2 is more resistant to CVF proteases but still possesses spermicidal activity and microbicidal effects against *N. gonorrhoeae*, like LL-37 and GF-17.
Methods: Degradation of 17BIPHE2 (20 □M) preincubated with CVL samples was determined by immunoblotting. Mouse and human sperm treated with various concentrations of 17BIPHE2 were assessed for their motility and membrane permeabilization. The ability of 17BIPHE2-treated mouse sperm to fertilize eggs in vitro and in vivo was assessed as described previously (Srakaew et al., Human Reprod 2014; Kiattiburut et al., ibid). Naturally cycling female mice were evaluated for a pregnancy outcome after transcervical injection of sperm with 17BIPHE2 (Srakaew et al., ibid). Antimicrobial effects (MIC/MBC) of 17BIPHE2 on *N. gonorrhoeae* were determined following standard methods.
Results: 17BIPHE2 was much resistant to CVF proteases than LL-37 and GF-17; <20% degradation was observed after 48 h co-incubation of 17BIPHE2 with CVL in 2 of 3 samples. Treatment of mouse sperm with 17BIPHE2 induced sperm immotility and membrane permeabilization in a concentration-dependent manner. At 10.8 and 21.6 □M 17BIPHE2, 100% of mouse and human sperm, respectively, became immotile, with 80% showing membrane permeabilization. Mouse sperm treated with 10.8 □M 17BIPHE2 completely lost the ability to fertilize eggs both in vitro (n = 46) and in vivo (n = 40). None of female mice (n = 10) transcervically injected with sperm + 10.8 □M 17BIPHE2 became pregnant. MIC and MBC of 17BIPHE2 against *N. gonorrhoeae* were both at 1.8 □M.
Conclusion: Our results suggest that 17BIPHE2 is a promising candidate as a multipurpose prevention technology (MPT) agent with both contraceptive and anti-STI activities.
We thank CIHR for funding.

Endothelial Colony-Forming Cell-derived Extracellular Vesicles Attenuate Experimental Bronchopulmonary Dysplasia

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Background: Bronchopulmonary dysplasia (BPD) complicates the course of preterm infants requiring positive pressure ventilation and oxygen supplementation. BPD interferes with vascular and alveolar lung development, which is critical to establish a functional gas-exchanging unit. To date, no effective therapy for BPD is available, leading to life-long morbidities. Evidence suggests that angiogenesis drives lung development. Endothelial colony-forming cells (ECFCs) represent a subset of vascular progenitors capable of self-renewal and de novo vessel formation. We hypothesize that extracellular vesicles (EV) secreted by ECFC carry pro-angiogenic properties.

Objective: To restore disrupted lung vascular and alveolar growth in experimental BPD by ECFC-EV therapy.

Methods: EVs were isolated by sequential centrifugation combined with tangential flow filtration from media conditioned by human ECFC cultures for 48h. EV integrity and function was assessed by Western Blot, Zetaview, electron microscopy and in vitro uptake and angiogenesis assays. To create experimental BPD, rat pups were housed in hyperoxic conditions (85% O_2) from birth until postnatal day (PN) 14. On PN4 and PN11 rats received ECFC-EVs intravenously with a cell equivalent of $250 \cdot 10^6$ cells/kg. On PN21, pulmonary hypertension was analyzed by ultrasound and right ventricular hypertrophy (RVH). Pulmonary function was analyzed by Flexivent and pulmonary structure was quantified histologically by mean linear intercept (MLI).

Results: ECFC-EVs express typical EV-markers (CD9, CD81, TSG101) and measure between 40 and 120nm. In vitro functional assays demonstrated that ECFC-EVs (i) are able to fuse with human umbilical vein endothelial cells (HUVEC) and (ii) induce HUVECs to form endothelial networks on matrigel in a dose-dependent manner. Neonatal hyperoxic injury induces pulmonary hypertension and arrested alveolar development, reminiscent of BPD. ECFC-EV therapy improved pulmonary artery acceleration time and RVH. In line with these results, we expect an improved pulmonary function and structure.

Conclusion: ECFC-EVs are a promising novel treatment for BPD. Future studies will confirm dose-dependent effects and safety. Next-generation sequencing and proteomic analysis will provide mechanistic insight.

Effects on the embryonic cerebral cortex development after low-dose methylmercury exposure

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Background: Methylmercury (MeHg) is an environmental neurotoxicant affecting health of millions of people globally. Humans are exposed to MeHg through the consumption of seafoods and it bioaccumulates in human body. MeHg is toxic to the central nervous system and the developing brain is extremely vulnerable to MeHg neurotoxicity. A study reported that developmental exposure to MeHg in mice results in memory disturbances and depression-like behavior in their adulthood. Although MeHg is known to be neurotoxic, especially to fetal brain development, the underlying mechanisms responsible for MeHg-induced neurological deficits remains unclear. Our published data showed that extra-low doses of MeHg affect embryonic neural precursor cell (NPC) development in vitro. 0.25 nM MeHg increased neuronal differentiation and decreased proliferation of the embryonic NPCs.

Objectives: Based on our in vitro data, we would like to investigate whether extra low-dose MeHg exposure to pregnant dam in vivo can have impact on embryonic brain development.

Methods and Results: Pregnant mice were treated with extra low concentrations of MeHg via drinking water starting from the first day of their pregnancy until E15. Half of litter brains collected from E15 embryos were used to measure mercury concentration in vivo, and the other half were used to perform immunohistochemistry to assess NPC proliferation, and their development into newborn neurons in the cerebral cortex. We expect to observe that extra low concentrations of MeHg will promote neuronal differentiation of embryonic NPCs at the expense of NPC populations.

Conclusion: The study will provide potential mechanism for late onset of neurodegenerative disease following early mercury exposure during fetal development, at least partly due to the depletion of NPC pools that are triggered by premature fetal neuronal differentiation.

Management of post-operative atrial fibrillation (POAF) after thoracic surgery

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Background: New onset post-operative atrial fibrillation (POAF) is the most frequent adverse event following major thoracic surgery and contributes to increased risk of stroke, morbidity and mortality. Current guidelines recommend pharmacotherapy to be continued for 1-6 weeks after discharge, but these recommendations are based on minimal evidence. The lack of clarity in recommendations and lack of studies likely contribute to variation in patients who are receiving pharmacotherapy after discharge. The impact of choice and duration of pharmacotherapy on POAF recurrence remain to be determined.

Objectives: This single-center study aims to understand current practices in management of POAF after discharge from hospital and recurrence of new onset POAF in patients within the immediate 30-day discharge period following non-cardiac thoracic surgery.

Methods: This retrospective, single-center cohort study included patients who underwent major non-cardiac thoracic surgery and subsequently developed POAF at a single institution between 2008-2017. Demographic, clinical and surgical variables/outcomes were collected to evaluate the management of POAF and incidence of POAF recurrence in the 30-day post-operative period. Comparison of means was done using two-tailed T-test.

Results: A total of 164 out of 2054 patients developed POAF following major thoracic surgery and 154 patients were managed with pharmacotherapy alone. 68% of patients were continued on rate control therapy upon discharge for a mean of 56.1 days with plans for reassessment by primary care provider. 15% of patients were discharged on anticoagulation therapy, of which 74% had a CHA_2DS_2-VASc >/= 2. Among those who had therapy discontinued on discharge (n=164), none were found to have recurrence of POAF in contrast to 3 patients who continued on therapy ($p>0.05$).

Conclusions: POAF management after hospital discharge is variable in practice with multiple treatment regimens used. Despite recommendations of continuing discharge therapy for 1-6 weeks, 32% are not discharged home on any management therapy and this does not appear to have any impact on AF recurrence. Further research, in the form of prospective studies, is needed to understand appropriate discharge management of POAF.

The Role of ARL-6 in Ventral Nerve Cord Assembly in C. elegans

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Background: Neural tube defects (NTDs) are a common cause of perinatal and infant mortality occurring in roughly 1 in every 2000 births in Canada. NTDs arise when the developing neural plate fails to form a closed neural tube. The Colavita lab has previously shown that assembly of the ventral nerve cord (VNC) in *C. elegans* uses molecular and cellular mechanisms similar to neural tube formation in vertebrates. Embryonic ventral neuroblasts (DA, DB, and DD) undergo a rosette mediated convergent extension (CE) to form the VNC. Arf-like GTPase 6 (Arl-6) is a member of the Arf family of small GTPases that are classically known to act as molecular switches. A recent genetic screen has identified mutations in the *C. elegans* orthologue of Arl6 (*arl-6)* that result in VNC assembly defects. We seek to investigate the role of *arl-6* in ventral cord assembly. In particular, we will determine if *arl-6* plays a role in convergent extension of the VNC and determine where *arl-6* is expressed during VNC assembly

Methods: Time-lapse microscopy of loss and gain of function *arl-6* mutants to characterize CE and rosette formation in mutants. Generation of GFP transcriptional fusions of *arl-6* to identify *arl-6* expression.

Results: We have identified DD neuron spacing defects in the gain of function mutations in the *arl-6* promotor region (*zy44, zy43*) indicative of CE defects. The loss of function *arl-6* mutant (*zy61*) displays normal DD neuron spacing. We have identified *arl-6* expression in a subset of neurons that intercalate to form the VNC. In transgenic animals carrying *zy43* and *zy44* promoter mutations, this VNC expression is absent.

Conclusions: The results of this investigation suggests that *arl-6* is expressed in a subset of ventral nerve cord neurons during convergent extension. This VNC expression appears to be lost in *zy43* and *zy44* mutants.

Modelling and Evaluation of an Energy Return Ankle-foot Orthosis with Quick Release Strut

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Background:

Ankle-foot orthoses cross the foot, ankle, and shank to assist people with ankle weakness. Posterior dynamic element ankle-foot orthoses (PDE AFO) use a stiff carbon fibre strut to store and release energy for different needs. Since current PDE AFO uses bolts to connect the strut to the orthosis, swapping struts for specific activities could improve mobility but would be time consuming and impractical in daily living. Therefore, a quick release ankle foot orthosis (QR-AFO) was designed to allow fast strut swapping for different activities but also maintain robustness and durability.

Objective:

Develop a quick release ankle-foot orthosis and evaluate performance and user satisfaction of the new design.

Methods:

A scoring model was created and applied to a variety of proposed mechanical designs before selecting a viable approach for modelling in SolidWorks. Safety factor design and Von Mises stress theory were applied to determine the quick release mechanism's critical dimensions. Finite Element Analysis (FEA) in SolidWorks provided preliminary safety and failure load outcomes. A functional test model was produced by a 3D printer to check the function and effort to use the quick release mechanism on an orthosis. A physical model was produced in aluminum and tested on an Instron machine to verify performance under expected loads.

Results:

The new QR-AFO consisted of an orthosis shank, orthosis foot plate, quick release mechanism, and strut. The quick release fastener and weight bearing pin were designed to be affixed to the strut. An anchor was molded inside the orthosis with a receptacle for the quick release fastener. Simulated stress on the weight bearing pin, anchor and surrounding carbon fiber structure, under maximum load, did not exceed the yielding stress after multiplying the safety factors. The simulation result was verified in the mechanical since no visible failure and measurable plastic deformation occurs under 3600N axial load. The quick release mechanism on the 3D printed QR-AFO worked smoothly with no occurrence of interferences.

Conclusions:

The QR-AFO was sufficient to support a person's weight during daily activities. The novel quick release mechanism should save effort and allow people to swap struts with different stiffness to addressing requirements for different activities. The successful results in mechanical simulation, axial loading test, and functional test model show confidence in the further tests on humans wearing the actual device.

Willingness of volunteers from Canadian Blood Service's Stem Cell Registry to donate blood, marrow, and other tissues for regenerative therapy

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Background:
As research surrounding cell-based regenerative medicine advances towards human trials, greater demand for clinical-grade cell products sourced from healthy donors will arise. A means to obtain these cells is not readily available in Canada. The extent to which volunteers in Canadian Blood Services Stem Cell Registry would be willing to donate cells to support regenerative therapy is not known and warrants exploration.

Objective:
Our objective was to conduct a survey to assess factors that would influence donor willingness to donate various tissues (blood, skin, fat, and bone marrow) for regenerative therapy.

To our knowledge this is the first and largest national survey of stem cell registrants addressing the donation of biospecimens to support regenerative therapy.

Methods:
We conducted a web-based survey to assess factors that would influence donor willingness to donate various tissues (blood, skin, fat, and bone marrow) for regenerative therapy. The survey was provided to 15 000 randomly selected donors who registered between 2013 and 2018. Data from the 1118 respondents was analyzed.

Results:
Despite a mixed degree of familiarity with regenerative medicine, potential donors were very supportive of donating for direct patient care and for research, and increasing their familiarity by reading a brief paragraph of information on regenerative medicine increased willingness to donate. Canadian Blood Services' stem cell registrants greatly preferred supporting non-profit groups in research and development in comparison to entities that represent profit-seeking industry involvement. The most important factors influencing donor willingness to donate were having an impact on patients, safety of donation, advancing knowledge in regenerative medicine, a manageable time commitment, and tolerable pain that could be managed. Donors were most willing to donate blood and had mixed responses to donating other tissue types.

Characterization of gastrointestinal pathologies in the dystonia musculorum mouse model for hereditary sensory and autonomic neuropathy type VI

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Background: Hereditary sensory and autonomic neuropathy type VI (HSAN-VI) is a newly identified neuronal disorder caused by mutations in the human dystonin gene (*DST*). Patients may present with joint contractures, problems with eating and breathing, motor deficits, autonomic irregularities, as well as gastrointestinal symptoms such as chronic diarrhoea, and abdominal pain. Similarly, a mouse disease known as *dystonia musculorum* (*Dst*^{dt}) also arises due to mutations in the dystonin gene. In our studies, we have also come to recognize gastrointestinal (GI) pathologies present within this mouse model.
As HSAN-VI and *Dst*^{dt} are primarily sensory neuron disorders, we hypothesize that the underlying cause for these gastrointestinal defects are due to impairments in the enteric nervous system (ENS).

Objective: Here we aim to assess gut morphology and function, as well as evaluate the integrity of the enteric nervous system.

Results: Our results indicate that by P15 the GI tract of *Dst*^{dt} mice is intact, and there are no signs of neurodegeneration or neuronal loss in the ENS. Functional analysis reveals small but significant reductions in GI motility, though absorption is unchanged.

Conclusions: Thus far we have observed no major changes to the ENS of *Dst*^{dt} mice. Although we have determined that dystonin isoforms are expressed in the ENS, their role my not be as critical to neuronal survival as in dorsal root sensory neurons. It may also be that autonomic dysfunction could be responsible for observed gastrointestinal defects. Investigation into higher nervous system centres inputting onto the gut will be analysed in future work.

Thyrotropin Stimulation of Thymic Stromal Lymphopoietin Production by Human Adipocytes

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Background: Thymic stromal lymphopoietin (TSLP) is an inflammatory cytokine recently recognized to be expressed in human adipocytes stimulated by thyrotropin (TSH).

Objective: This study aimed to identify TSH-dependent signaling routes that up-regulate TSLP and to examine if TSLP production is adipose depot-dependent.

Methods: Abdominal subcutaneous adipose tissue was obtained from 9 female patients undergoing elective surgery (approved by the Ottawa Health Science Network Research Ethics Board, #1995023-01H). Human abdominal differentiated adipocytes were stimulated with TSH. Activation of signal transduction pathways was measured by immunoblotting, and TSLP levels in cell culture medium were assessed by ELISA. TSLP responses from abdominal subcutaneous and omental adipocytes were compared.

Results: TSH-stimulated TSLP secretion from human abdominal subcutaneous differentiated adipocytes was enhanced by IBMX (raises cAMP levels) and was blocked by UO126 (inhibitor of MEK1/2- ERK1/2 pathway). No inhibition was observed with SB203580 nor SP600125. Interferon-γ, but not IL-4, inhibited TSLP responses to TSH. Intra-abdominal omental adipocytes also released TSLP in response to TSH.

Conclusions: TSLP is produced by human differentiated adipocytes derived from subcutaneous or omental depots in response to TSH. Our data suggest a role for PKA and the MEK1/2-ERK1/2 pathway in mediating the TSH effect on TSLP. TSLP may be of relevance to the extra-thyroidal action of TSH associated with adipose dysfunction and the cardiovascular disease risk observed with the elevated TSH levels of subclinical hypothyroidism.

Modulating Human Embryonic Stem Cell-Derived Skeletal Muscle Satellite-like Cell Division with Wnt7a and EGF

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Background: Satellite cells are skeletal muscle stem cells that are characterized by Pax7 expression. Satellite stem cells, which have not expressed myogenic factor Myf5, express the Wnt-receptor Frizzled-7 and upon stimulation with its ligand Wnt7a, they selectively undergo a symmetric division and expand the stem cell pool by giving rise to two daughter satellite stem cells. Conversely, epidermal growth factor (EGF) stimulation of satellite stem cells activates the EGF receptor and induces an asymmetric division, generating one daughter stem cell and one Myf5-positive progenitor cell committed to the myogenic lineage and contributing to muscle repair.

Objective: The aim of this study is to confirm these satellite cell division mechanisms in a human model using human embryonic stem cells (hESC) differentiated into the myogenic lineage to generate satellite-like cells.

Methods: hESC line 9 (H9) cell colonies were dissociated and plated as single cells prior to differentiation into satellite-like cells. From day 12 of differentiation, cells were treated with either Wnt7a or EGF to modulate satellite-like cell division mechanisms. After treatment, Pax7 protein expression was analyzed in the treated and untreated cells by immunofluorescence (IF) staining and Western blotting. Array scanner analysis of IF staining for Pax7 and myogenic factors MyoD and Myogenin was used to quantify myogenic cell numbers and assess population dynamics in response to treatment with Wnt7a and EGF. RT-qPCR was used to investigate global gene expression of myogenic markers within differentiating cell populations and to identify genes that were differentially expressed between treatment conditions.

Results: The presence of satellite-like cells among hESC-derived differentiated cells was confirmed by their expression of Pax7. Moreover, some cells expressed MyoD and Myogenin, which are transcription factors expressed in committed myogenic precursor cells. The proportions of cells expressing different myogenic factors were affected by treatment with both Wnt7a and EGF. Western blot analysis of Pax7 protein expression revealed that cells treated with Wnt7a expressed more Pax7 when compared to untreated cells or cells treated with EGF.

Conclusion: It is possible to model myogenic differentiation *in vitro* using hESC-derived progenitors. A trend towards higher Pax7 expression after treatment with Wnt7a was observed, suggesting an expansion of the satellite-like cell pool among the differentiating cells.

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Cyclophosphamide for the Treatment of Skin Fibrosis in Systemic Sclerosis: A Systematic Review

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Background: Systemic sclerosis (SSc) is a chronic disease characterized by multi-organ involvement. Excess fibrosis is thought to result from a complex interplay between vasculopathy and inflammation. Skin fibrosis is a cardinal feature of SSc. Evidence for the pharmacologic management of skin involvement is limited and there is a need for further clinical guidance. Cyclophosphamide is an alkylating agent with potent immunomodulating effects.

Objective: To assess the efficacy of Cyclophosphamide in the treatment of skin fibrosis in patients with SSc.

Methods: Embase, MEDLINE and Cochrane Central Register of Controlled Trials were searched for all randomized control trials (RCTs), quasi-randomized studies, case-control studies, controlled before-after studies, prospective and retrospective cohort studies, case series and cross-sectional studies. Case reports were excluded. The Health Canada registry, clinicaltrials.gov, the ISRCTN registry, and the World Health Organization (WHO) international clinical trials registry were searched for grey literature. Studies pertaining to patients with a diagnosis of SSc were included. The main intervention of interest was Cyclophosphamide with no limitation of regimens or route of administration. Comparators were other standard disease modifying agents or placebo. The outcome of interest was extent of skin fibrosis defined by the modified Rodnan skin score (mRss). Two review authors completed independent and duplicate abstract, full text screening, data extraction and quality appraisal. Meta-analysis was performed for the primary outcome (mRSS) through random-effects models.

Results: Thirty-one studies conducted between 1994 and 2018 were included: 11 RCTs, 13 case series, 4 retrospective cohort studies and 3 prospective cohort studies. The majority of the 11 RCTs showed some concerns in regards to risk of bias. After 12 months of treatment with Cyclophosphamide, mean mRSS decreased 6.30 points (95% CI, 4.95 to 7.64). The effect remained significant when pooling both 6- and 12-month outcomes – mRSS decreased 4.11 points (95% CI, 0.97, 7.25). Heterogeneity of comparators, use of steroids, and outcome endpoints did not allow for subgroup analyses to be completed as planned.

Conclusion: Statistically and clinically significant improvements in mRSS with administration of Cyclophosphamide were demonstrated in patients with SSc. The lack of consistent standard of care for the treatment of skin fibrosis in SSc was highlighted by the variability in the type and duration of comparator treatments used in clinical trials. There is a need for more consistent recommendations regarding treatment regimens, and longer clinical follow-up to better understand the role of immunosuppression as a treatment for this disease manifestation.

Extracellular vesicles and the quest for molecular biomarkers in ALS.

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Background: Amyotrophic lateral sclerosis (ALS) is a relentlessly progressive and fatal neuromuscular disease with no effective treatment or cure. In the early stages of ALS, it can be difficult to provide a diagnosis as patients do not meet diagnostic criteria until after they become symptomatic, a sign of neuron degeneration. Early diagnosis is therefore crucial to provide access to current or experimental therapies before significant motor neuron loss has occurred. Moreover, other than clinical motor function and respiratory evaluation, there are no simple assays to follow ALS disease progression or response to treatment. A quantitative, biological test would be an ideal complimentary assay to these functional tests.

Objective: We are identifying biomarkers for ALS through analysis of proteins contained in extracellular vesicles (EV) isolated from cell lines and blood samples from patients. EV are small membrane-bound particles released from all cells in the body and contain a heterogenous mix of proteins reflective of the physiological state of the cell.

Methods: Our initial studies focus on ALS disease caused by mutations in the vasolin containing protein (VCP) gene, which is responsible for 1-2% of familial forms of ALS. Using an optimized differential ultracentrifugation protocol, we will analyze the protein content of these particles by mass spectrometry to identify proteins differentially present in the EV of ALS cell lines relative to normal controls. Immunofluorescence microscopy and cell fractionation are being used to characterize presence of the pathological properties of ALS14 in these cell lines, including nuclearization of VCP and mislocalization of TDP-43.

Results: We isolated EV from VCP-mutant carrier fibroblast cell lines. Preliminary mass spectrometry findings have identified a total of 166 proteins, 52 of which are differentially present in EV from VCP mutant fibroblasts. Current experiments are focused on validating the proteomic findings via immunoblot analysis. Future experiments will be testing the wider applicability of our findings in plasma samples obtained from patients with VCP-related ALS.

Conclusions: Successful completion of our work will identify blood-based proteins which may act as effective diagnostic, prognostic, or pharmacodynamic biomarkers in patients with ALS.

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The association of blood transfusions with adverse graft outcomes in kidney transplant patients: a retrospective cohort study.

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Background: Anemia is a frequent problem in kidney transplant recipients and these individuals often require blood transfusions. Little is known about the potential immunological risks from blood transfusions in kidney transplant patients in terms of risks for rejection and graft loss.

Objective: The purpose of this study is to examine the association between receipt of a red blood cell transfusion (RBCT) in individuals with a kidney transplant and adverse graft outcomes.

Methods: This is a retrospective cohort study of all adult kidney transplant recipients at The Ottawa Hospital from January 1, 2002 to December 31, 2018. Study participants, exposures and outcomes were determined through available databases (Nephrology Transplant database and Ottawa Hospital Data Warehouse) as well as manual chart review. The exposure of interest was receipt of a RBCT after kidney transplant. The outcomes of interest were a biopsy-proven graft rejection episode, graft-loss and death-censored graft loss. A time-to-event analysis was done using date of kidney transplant as the index date and follow-up until occurrence of the outcome of interest or a censoring event (death, graft loss, loss to follow-up or end of study period 31 December 2018). Hazard ratios (HR) adjusting for baseline characteristics were calculated with cox proportional hazard regression using RBCT as a time-varying exposure.

Results: There were 1,258 study participants. In all, 468 individuals (37.2%) received a total of 2,373 RBCT throughout the study period. The median number of RBCT received per individual was 3 (IQR 2 to 6) and the median time to first RBCT was 5.7 days (IQR 2.1 to 72.2). There were 197 episodes of rejection, 318 graft losses and 114 death-censored graft losses. The 1-, 5- and 10-year event-free survival probabilities were 88.1%, 83.6% and 80.3% for rejection, 94.7%, 82.8% and 65.4% for graft loss, and 97.3%, 92.7% and 83.9% for death-censored graft loss, respectively. The adjusted risks (HR, 95% confidence interval) for graft loss and death-censored graft loss for every 1 RBCT received were significantly increased (1.14, 1.13 to 1.16 and 1.12, 1.08 to 1.15, respectively). For rejection, the risk was not statistically significant (1.05, 0.99 to 1.10).

Conclusion: Receipt of RBCT in kidney transplant recipients is associated with overall worse graft outcomes. Despite the potential for residual confounding whereby individuals receiving RBCT are inherently at higher risk for adverse graft outcomes, clinicians caring for kidney transplant receipts should carefully consider these potential risks before ordering RBCT for their patients.

Analysis of the Enhanced Recovery After Thoracic Surgery (ERATS) pathway

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Background: Enhanced Recovery After Surgery (ERAS) programs aim to improve patient recovery through a multidisciplinary shift in perioperative care as supported by evidence from ERAS pathways in multiple surgical specialties.

Objectives: The purpose of this perioperative implementation study is to evaluate the impact of an Enhanced Recovery After Thoracic Surgery (ERATS) program at The Ottawa Hospital (TOH) for patients undergoing elective thoracic surgeries. The main objective of the program is to demonstrate its usefulness in improving patient care, quality of life and patient satisfaction. This initiative aims to improve patient outcomes, decrease adverse events (AEs), and improve patient satisfaction by maintaining a patient-centered focus in care and standardizing best care processes.

Methods: ERAS elements which were targeted from literature searches were unanimously selected bya multidisciplinary ERATS team at TOH. Patients were engaged in the recovery process through patient education sessions which provided clear expectations for shorter length of stay (LOS), patient diary for daily goals, and the benefits of early mobilization and enteral feeding. Patients were asked tocomplete a six-minute walk test at 4 weeks post-surgery to evaluate recovery and patient satisfaction was evaluated upon discharge through standardized QOL questionnaires post-operatively at 4 weeks, 3 months and 6 months. This interrupted time series analysis aids in determining the impact of an ERAS program on AEs and patient satisfaction with care.

Results: Eligible patients included 350 from the pre-implementation phase and 330 patients from the post-implementation phase. A significant decrease in LOS was noted for patients undergoing open esophagectomy (24.2 ± 12.8 to 13.7 ± 8.0; $p = 0.04$), open lobectomy (8.2 ± 8.6 to 5.0 ± 1.6; $p = 0.04$) and sublobar resection (3.4 ± 3.3 to 2.6 ± 2.5; $p = 0.04$). Through implementation of the ERATS pathway, there was a reduction in the average time for patients to ambulate and mobilize independently following a surgical procedure. ERATS has successfully decreased the time to first intake of tolerated diet and fluids PO for elective GI procedures.

Conclusions: Implementation of ERATS at TOH has demonstrated the ability to reduce LOS which could promote faster recovery of elective surgery patients. Analysis of the impact of ERATS on patient experience, function, and, overall hospital costs are necessary to determine the benefit of this program. The ERATS program aims to act as a template for other thoracic surgery programs in other tertiary hospitals.

Characterization of novel bifluorescent transgenic mice for Parkinson's disease research

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Background: Parkinson's disease (PD) is an incurable, neurodegenerative disease with an extensive prodromal phase. The Braak hypothesis suggests a pathological spread of alpha-synuclein aggregates from the enteric nervous system (ENS) to the midbrain in a prion-like fashion occurring over a five- to twenty-year prodromal period. Transmission of a-syn aggregates through the vagus nerve is supported by both human and mouse vagotomy data, however the initiation site of a-syn aggregation and the mechanism of cell-to-cell propagation have yet to be elucidated. Our novel BiSyn (i.e., bimolecular fluorescence complementation of a-syn aggregates) system was developed to address these gaps.

Objective: We aim to demonstrate the use of our novel BiSyn system as a tool for addressing gaps in PD research, specifically the underlying initiation and propagation mechanisms. This will be achieved through the characterization of our transgenic mice and via preliminary in vivo validation studies.

Methods: Our BiSyn system exploits bimolecular fluorescence complementation, which generates a quantifiable fluorescent signal upon alpha-synuclein aggregation. In characterizing our novel mouse model, we used X-gal staining to determine the efficiency of transgene expression.

Results: Expression of the BiSyn transgene was found to be highly mosaic and variable between individuals from the same litter, despite homozygosity. Silencing occurred as early as the oocyte stage. SAHA, a histone deacetylase complex inhibitor, and 5-Azacytidine, a DNA methyltransferase inhibitor, did not restore expression in mouse embryonic fibroblasts derived from our BiSyn mice. We are now utilizing an adeno-associated virus (AAV) packaged with our BiSyn construct to deliver our transgene to various sites throughout the ENS. We will evaluate exosomal transfer as one mechanism of prion-like transmission. We have shown that our AAV/BiSyn system infects cells and have detected fluorescence upon rotenone exposure. We are currently awaiting in vivo validation results for AAV/BiSyn-injected mouse substantia nigra.

Conclusions: Our AAV/BiSyn system has potential as a strategic tool for investigating the PD prodromal phase, which could lead to novel therapeutic interventions that delay or preclude brain infiltration of a-syn aggregates and subsequent nigral dopaminergic degeneration-mediated Parkinsonism motor deficits.

A Survey of Nurses Knowledge, Attitudes and Beliefs about a Hospital-Wide Pragmatic Trial Evaluating Fluid Administration

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Background: FLUID is a hospital-wide pragmatic cluster cross-over randomized trial evaluating whether Ringer's lactate reduces death and re-admission to hospital compared to Normal Saline. The FLUID pilot trial included 4 centres and demonstrated feasibility of our design and execution.
Objectives: Due to FLUID's pragmatic and novel design (hospital-wide intervention, waiver of consent, requirement for nurses to implement an automatic fluid substitution), we surveyed clinical nurses at FLUID pilot centres to understand the effectiveness of FLUID communication strategies, as well as knowledge, attitudes and beliefs about FLUID's pragmatic trial design.
Methods: A web-based survey was developed targeting nurses who provided direct patient care at FLUID pilot centres by using hospital email distribution lists. The survey was developed by the FLUID scientific team and piloted for clinical sensibility and readability with six nurses. The 5-minute survey included the following domains: nurse demographics, FLUID communication strategies and knowledge and attitudes and beliefs about FLUID's pragmatic design. Completion was voluntary. Communication effectiveness and knowledge, attitudes and belief questions about FLUID were answered using a 5-point Likert scale. Responses were described with counts and proportions and with mean, medians and interquartile ranges as appropriate.
Results: A total of 500 nurses responded. 90% (451/500) of the respondents were Registered Nurses and 52% (258/500) were in practice for more than 10 years. The two most common reported nursing specialties were surgical 26% (128/500) and intensive care 18% (93/500). 73% (367/500) reported they were aware of the FLUID trial prior to study launch. The top four reported communication strategies were posters, word of mouth, email and nurse educators (very effective/effective: 82% (411/500), 78% (389/500), 73% (364/500), and 72% (362/500) respectively). The majority of nurses reported being comfortable performing the automatic substitution order (agreed/strongly agreed 88% (440/500)). Although 72% (362/500) agreed or strongly agreed that waiver of consent was appropriate, 8% (41/500) disagreed or strongly disagreed. Over the course of the two study periods, 98% (489/500) of nurses reported either never receiving resistance (72%) or receiving resistance from = 5 different physicians (26%) when performing the automatic substitution. Furthermore, 72.0% (360/500) of nurses reported never/rarely encountering Do Not Substitute Orders from the treating physicians.
Conclusions: Registered Nurses at participating FLUID pilot centres reported a high level of FLUID awareness and knowledge as well as overall comfort performing study related tasks such as automatic fluid substitution. Communication to enhance pre-study implementation awareness can be improved for the larger trial.

Network-level sphingolipid alterations differentiate Spinal Muscular Atrophy with Progressive Myoclonic Epilepsy from Farber's Disease

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Background: The genetic determinants of Spinal Muscular Atrophy with Progressive Myoclonic Epilepsy (SMA-PME) and Farber's Lipogranulomatosis are mutations in the *ASAH1* gene, encoding for the lysosomal acid ceramidase enzyme. SMA-PME is characterized by the onset of muscle weakness and generalized muscle atrophy due to the degeneration of spinal motor neurons accompanied by myoclonic seizures, while Farber's disease is characterized primarily by painful and progressively deformed joints, hoarseness of the voice and subcutaneous nodules. In both diseases, pathogenic mutations impair acid ceramidase activity, leading to an accumulation of ceramides in the lysosomes of patient cells.

Objective: While an underlying ceramide accumulation is common to both diseases, to date no group has investigated whether alterations in the sphingolipidomes differentiate between the two disease phenotypes. Therefore, in this study, we examine the sphingolipidomes of patient-derived fibroblasts, to determine how these disease-causing mutations affect the sphingolipidome of the cells, and to see if the diseases can be distinguished by their lipid profiles.

Methods: To investigate how these mutations in the *ASAH1* gene affect network-level sphingolipid metabolism, we profiled seven SMA-PME patients, three Farber's disease patients, as well as age-matched controls. Using a novel quantitative-discovery targeted nanoLC-ESI-MS/MS approach and EPI-IDA structural identification, we quantified the repertoire of ceramides (d18:1), sphingosine, glucosylceramides, glucosylsphingosine, galactosylceramides, galactosphingosine, sphingomyelins, lactosylceramides, and globotriaosylceramides in fibroblasts.

Results: We found that the metabolic profile was sufficient to distinguish between Farber's Disease and SMA-PME, indicating that these two diseases can be distinguished metabolically by their sphingolipid profile.

Conclusion: To our knowledge, this is the first time a lipidomic difference between these two diseases has been reported, and these findings could lead to further mechanistic insights, and drive us towards therapeutic strategies for these two rare diseases.

A Low Computational Cost Deep Learning Approach for Binary Pneumonia Classification

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Background: Current state of the art approaches for pneumonia diagnosis have utilized various machine learning algorithms such as basic logistic-regression, support-vector machines, and various implementations of neural networks. A high accuracy model while also using minimal computing resources is needed for future artificial intelligence (AI) implementations.

Objective: The objective of this project was to design a low computational cost AI model for pneumonia diagnosis.

Methods: A modified Visual Geometry Group 16 (VGG-16) convolutional neural network architecture was created in Python using Keras and NumPy libraries. The model was trained using data obtained from CheXNet and Kaggle, with a total of approximately 10,000 images. Images were labelled as “PNEUMONIA” or “NORMAL” and normalized with a zero-mean unit-variance approach. The model was trained on an AMD RX-580 4Gb graphics card while maintaining under 2Gb of total GPU memory usage. Model variations were compared using accuracy, specificity, and sensitivity metrics. Desired model accuracy, specificity, and sensitivity were selected based on physician results from a pneumonia diagnosis study where physicians scored: 89% accuracy, 67% specificity, and 58% sensitivity. A binary pneumonia classification problem was selected to match the goal of the other pneumonia diagnosis study.

Results: The final model consisted of 8 layers for this binary classification problem. The final training accuracy was 96%. After testing on a separate subset of data, accuracy was found to be 90.7%. The testing specificity and sensitivity were 94.4% and 90.0% respectively. During testing the model produced 47 false negative and 77 false positive results. The network was lightweight such that the trained model with weights could be executed on an i5-6200U CPU running at 2.30GHz and with an inference speed of less than 1 second. The final training time required for this network was under 2 hours.

Conclusions: A convolutional neural network was designed for binary pneumonia classification. The network achieved 90.7% accuracy, 94.4% specificity, and 90.0% sensitivity on the test data. The final model was computationally efficient and able to execute on a low-grade processor. This approach provides support for future work on video-based analysis using embedded artificial intelligence boards for processing.

Sex-Dependent Requirement of 16p11.2 Locus for Metabolic Response to Ischemic Stroke

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Background: Metabolic disorders, including obesity, hypertension and diabetes have been associated with higher incidence of cerebrovascular disease, including ischemic stroke (IS), leading cause of long-term disability and death worldwide. Pre-existing vascular conditions are present within 75% of stroke patients and are important predictors of health outcomes. The Lacoste lab recently discovered that a copy number variation (i.e. deletion) at the 16p11.2 locus was associated with vascular phenotypes, including increased resting-state brain perfusion, dysfunction of endothelium-dependent vasodilation, and neurovascular uncoupling. The 16p11.2 deletion is also known to influences both risk of obesity and insulin concentration. However, involvement of the 16p11.2 locus in stroke outcomes/recovery has never been investigated. Since metabolism is directly related to vascular health, we hypothesize that pre-existing vascular conditions associated with 16p11.2 deletion will alter the outcomes of cerebrovascular injury (i.e. IS).

Objective: To test our hypothesis, mice harboring the 16p11.2 deletion were used to monitor metabolic outcomes following ischemic stroke (IS).

Methods: Baseline metabolic measures were used as a reference point to detect alterations in cardiovascular fitness post-stroke. Mice were subjected to IS in either the somatosensory or motor cerebral cortex, and outcomes measures were repeated at 2 weeks and 4 weeks post-IS.

Results: Preliminary results obtained following somatosensory cortex IS suggest that 16p11.2 deletion is protective in females, but not males.

Conclusions: Further investigation is required to support preliminary findings, and work is being performed to generate data from motor cortex IS. Future research will incorporate mice harboring the reciprocal 16p11.2 duplication.

The relationship between opioid use and obstructive sleep apnea: A systematic review

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INTRODUCTION: The relationship between opioids and obstructive sleep apnea (OSA) is an understudied topic. Given limited and controversial data available, the purpose of this review was (1) to investigate the impact of opioid use on the presence and severity of OSA, (2) to explore adverse health consequences of opioid use in patients with OSA, and (3) to compare the effects of treatment for OSA among opioid and non-opioid users.

METHODS: All studies in the English language from 1946 to 2018 found through MEDLINE, EMBASE, CINAHL, PsycINFO, Cochrane Databases and bibliographies of identified articles and reviews were eligible. Quality of the included studies was assessed based on published guidelines.
Randomized controlled trials and observational studies with adult participants (≥ 18 years) were considered after fulfilling our inclusion and exclusion criteria.

RESULTS: From 2,114 articles retrieved, 68 were selected for the full-text review and 15 (6 clinical trials and 9 observational studies) were included in this review.
For the first objective, 8/14 (57%) studies found no significant relationship between opioid use or dose and presence and/or severity of OSA as measured by apnea-hypopnea index (AHI) or degree of nocturnal desaturation, 5/14 (36%) studies found that opioid use exacerbated the severity of OSA. Only 1/14 study (7%) found that opioid use may ameliorate OSA.
None of the studies addressed objective 2.
For the third objective, 3/4 (75%) studies found that CPAP reduced AHI by 30/h to 17/h in opioid users. 1/4 reported a decrease in oxygen desaturation index by 11/h and increased the mean oxygen saturation by 3%. 1/4 (25%) study found a higher percentage of CPAP treatment failure in patients on opioids (33%) vs non-opioids (12%).
1/4 study (25%) found that Bilevel therapy with a backup rate reduced AHI by 42/h in opioid users. Another study (25%) found that Auto servo-ventilation (ASV) reduced AHI by 31/h in opioid users. Both Bilevel therapy and ASV were found to successfully resolve AHI (≤5) in 94% with no difference between opioid and non-opioid users.

CONCLUSION: There was no clear evidence on the negative impact of opioid use on the presence and severity of OSA; although the results were limited by paucity of good-quality studies. Data were lacking on the primary adverse consequences of opioid use in OSA patients. There was some evidence that CPAP, Bilevel therapy and ASV may be beneficial for patients with OSA on opioids.

Building Capacity for Relapsed/Refractory ALL patients needing CAR-T cells

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Introduction: The prognosis of relapsed/refractory (r/r) acute lymphoblastic leukemia (ALL) remains poor in adults. Chimeric antigen receptor (CAR) T-cell therapy is a promising therapeutic in r/r ALL. In preparation for the first Canadian investigator-led clinical trial of CAR-T cell therapy (CLIC-01), we sought to evaluate current outcomes for patients who would be deemed eligible for this trial.

Methods: We conducted a retrospective chart review of all patients diagnosed with ALL at 2 Canadian academic hospitals between 2010-2015 (Ottawa and Vancouver). All patient, therapy and outcome-based factors were extracted. Included patients were then cross-checked against the eligibility criteria of the upcoming CLIC-01 trial.

Results: 173 patients were diagnosed with ALL over a 5-year period in Ottawa and Vancouver. 124 patients (72%) achieved complete remission with induction chemotherapy and 74 patients (43%) relapsed after a median of 11.8 months (range, 1–85.6). Sixty-two patients (36%) would have been eligible for the upcoming CLIC-01 trial. Median overall survival of the entire cohort was 26.8 months (range, 0.4-88.9), compared to 17.0 months (range 0.4–71.6) for trial eligible patients.

Conclusion: CAR-T cell therapy has shown promising results based on early phase data in patients with r/r ALL. Due to the complexity of manufacturing and administering CAR-T cells, it is important for retrospective reviews to help prepare for potential challenges of CAR-T cell trials. This study has demonstrated that approximately one third of ALL patients will be eligible for this clinical trial and has helped in preparation for a more feasible and practical trial design.

Inpatient Transthoracic Echocardiograms Following Acute Ischemic Stroke: Low Utility, High Costs

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Background: Determining ischemic stroke etiology is essential to secondary prevention. The extent of cardiac testing required in hospital is currently a topic of controversy. It is unclear whether it is clinically necessary and cost-effective to obtain a transthoracic echocardiogram (TTE) during inpatient admission for ischemic stroke.

Objective: To determine the diagnostic yield of TTE after acute ischemic stroke and costs associated with performing this investigation during admission.

Methods: We assessed patients admitted with acute ischemic stroke at a The Ottawa Hospital from 2015 to 2017; patients who obtained transthoracic echocardiogram (TTE) were included for analysis. The primary outcomes were clinically significant findings on TTE warranting urgent intervention, and subsequent changes in management. We assessed costs associated with delaying discharge to obtain TTE as an inpatient using a data repository containing administrative and financial information.

Results: We included 516 patients in our analysis. TTE findings were potentially clinically significant in 30 (5.8%) patients, and these findings changed management in 17 (3.3%) patients. Cost data were available for 507 patients. Discharge from hospital was delayed to expedite TTE in 24 patients while TTE occurred after discharge in 77 patients; the mean difference in cost of admission was $809.32 per patient. We estimated that if all patients had their discharge delayed to expedite TTE, it would cost $25 524 per change in management.

Conclusions: TTE after acute ischemic stroke has low yield of findings that infrequently change management. Given costs associated with prolonging inpatient admission, discharge should not be routinely delayed to expedite this investigation.

Molecular control of genome organization

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Background: The importance of the nuclear lamina is demonstrated by over 400 disease-causing mutations that have been found in the lamin A gene, which encodes a key cytoskeletal component of the lamina scaffold. Lamin A mutations cause a number of “laminopathies” with diverse symptoms and target tissues. Lamin A also has a role in physiological aging as the C-terminal mutant of lamin A, progerin, is associated with the most common form of premature aging syndrome, Hutchinson-Gilford progeria syndrome (HGPS). Progeria-expressing cells demonstrate dysmorphic nuclei, loss of heterochromatin (HC) marks, compromised DNA repair and altered gene expression. How all these processes contribute to progeria has yet to be fully disentangled.

Objectives: The question we wish to address is to what degree does disrupted nuclear organization contribute to premature aging? What is the purpose of tethering HC at the lamina? Although the tethering of HC to the nuclear lamina is a well-described phenomenon, the molecular mechanisms mediating this process remain poorly understood. We propose to use adult mouse rod photoreceptors, which are devoid of any tethering proteins, as a model system to identify and characterize the proteins that tether HC at the lamina. Our goals are 1) to perform a structure-function analysis of lamin A, in order to identify the critical region responsible for HC tethering, 2) to elucidate the genomic and transcriptomic consequences of HC tethering. We will integrate this information to develop a model explaining mechanistically how lamin A organizes the nucleus.

Methods: To determine how potential tethering proteins affect genome organization, I ectopically express them in mouse rod photoreceptors using DNA transfection by in vivo electroporation. To explore how lamin A affects genome accessibility, we performed ATAC-seq experiment on Control, and lamin A transfected mouse rod photoreceptors. To explore how lamin A affects gene expression, we will perform single-cell RNA-seq on the same sample groups**.**

Results: We show that lamin A is sufficient for HC tethering and that C-terminus only is sufficient for tethering of facultative HC (fHC). These results complement our observation that progerin does not tether HC like lamin A, which means that the key DNA binding region is in the C-terminal part of lamin A.

Conclusions: Lamin A C-terminal domain is crucial in binding HC, probably in cooperation with other regions of the protein. We conclude that loss of HC tethering might be an important feature of some laminopathies.

Is My Kidney Tumour a Cancerous Tumour? A Prediction Nomogram Using Clinical Information

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Background: With the increased use of cross-sectional images, incidental kidney tumours are frequently discovered. Most patients with kidney tumours choose to have them surgically removed. However, nearly 20% of surgically removed kidney tumours are found to be benign. Furthermore, some cancerous tumours are not aggressive and may be safely managed with close observation, rather than surgery. A predictive model to assess the risk of cancer for a kidney tumour would help patients and physicians with management decisions.

Objective: To determine the clinical and radiographic predictive factors of kidney tumour malignancy and to develop a nomogram to distinguish between benign and malignant renal masses.

Methods: Patients diagnosed with solitary renal masses with tumour pathology information were identified from the Canadian Kidney Cancer information system. Demographic, clinical and imaging data of included patients were compared to the pathologic diagnosis. Tumours were categorized into malignant or benign. Tumours were also classified as aggressive (high-grade malignant) or indolent (low-grade malignant and benign). Logistic regression models were constructed to identify predictors of each category. Nomograms were created using the statistically significant (p-value<0.05) risk factors and were internally validated using bootstrap methods.

Results: Of 3,991 patients diagnosed with a solitary kidney tumour since January 2011, 3,708 (93%) patients had malignant tumours, and 1,515 (43%) had aggressive tumours. On multivariate analysis, factors independently associated with cancer and high-grade cancer were age (Odds Ratio [OR] 0.99, 95% confidence interval [CI] 0.98-1.00; OR 1.01, 95%CI 1.00-1.02, respectively) and tumour size (OR 1.25, 95%CI 1.00-1.02; OR 1.28, 95%CI 1.25-1.31, respectively), while male sex was also predictive of aggressive cancer (OR 1.48, 95%CI 1.26-1.73). The final nomograms were able to discriminate between malignant/benign tumours (c-statistic=0.71, 95%CI 0.68-0.74), and aggressive/indolent tumours (c-statistic=0.77, 95%CI 0.75-0.78).

Conclusion: Patient and tumour characteristics are independently associated with cancer risk and aggressive-cancer risk. Our point-of-care nomograms have good accuracy in distinguishing renal mass pathology. This prediction tool can be used by physicians and patients with kidney tumours to help determine an optimal management plan.

Oncolytic Measles Virus armed with apoptosis-promoting transgene for enhanced anti-tumor potency

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Background

Many types of non-resectable cancer remain incurable despite recent advances in radio-, chemo-, and immunotherapy. Oncolytic viruses (OV) which replicate selectively in tumor cells are an emerging modality of cancer treatment. The oncolytic Measles virus (MeV) has quickly risen as a leading OV candidate that demonstrated a good safety profile in addition to indications of efficacy in recent phase I/II clinical trials. However, it has been observed that the OV alone is not always sufficient in fully eliminating the tumor burden and that a synergy with additional factors is needed for a curative treatment.

Objective

By encoding apoptosis-promoting transgenes within the MeV genome, cell death can be achieved independently from the virus-induced cytopathic effect leading to a greater anti-cancer potency. By encoding pro-apoptotic factors in a Measles oncolytic virus, we will synergize the tumor homing ability and excellent safety profile of the measles OV with the potency of the apoptosis-promoting transgenes.

Methods

The virus was produced and is being characterized for its oncolytic potency via cytotoxicity assays on numerous cancer cell lines. Its ability to induce apoptosis is being confirmed with real-time caspase-3 activation assays as well as microscopy analysis.

Results

The novel construct has demonstrated its ability to induce a significant apoptosis response in cancer cell lines. Furthermore, the armed MeV is an improvement as an oncolytic in certain cancer cells versus the parental strain.

Conclusion

The synergy of the well characterized Measles OV with encoded pro-apoptotic transgenes has proven effective *in vitro* demonstrated by increased killing ability versus the MeV alone. Further studies will aim to elucidate mechanisms of action as well as identifying additional cancer candidate types that are susceptible to the new construct.

Translation of highly promising basic science research into clinical applications: a systematic review

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Background:
Results from animal experiments often form the rationale for first-in-human trials. However, previous work has suggested that less than 5% of therapeutics studied in preclinical models become licensed for clinical use with an average lag time between preclinical publication to first clinical trial of approximately 10 years. Contemporary rates of "bench-to-bedside" translation are unknown.

Objective:
Identify the contemperoary rate of successful "bench-to-bedside" translation.

Methods:
All studies published in *Science* and *Nature* from 1995-2015 were screened independently in duplicate by pairs of reviewers to identify preclinical studies highlighting promising interventions. Studies which used an *in vivo* animal model and demonstrated therapeutic benefit of an intervention were included. Then, a predefined search strategy was applied to determine how many of these promising therapies have been translated clinically.

Results:
15,941 articles were screened, 470 of which met our eligibility criteria. Of the 470 interventions studied preclinically, 24.5 % progressed to a clinical trial with an average time of 4.4 years (95% CI; 3.6-5.3). Of the studies that went to clinical trial, 59% had a published trial, 77% of which had a positive result. 1.8% of interventions studied preclinically earned regulatory approval, with an average time to approval of 8 years (95% CI; 4.3-11.7).

Conclusions:
The findings from our study demonstrate that the rate of translation from bed to bedside remains exceedingly low (<2%). Interestingly, the speed of translation may be increasing. Future studies will investigate factors that promote or impede "bench-to-bedside" translation.

What ethical challenges do pragmatic trials pose for the oversight of human subjects' research? An interview study with key stakeholders

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Background: Pragmatic trials are intended to inform clinical or health policy decisions. However, despite pragmatic trials more closely resembling real-world clinical practice they may also raise new ethical concerns.

Methods: To identify ethical issues emerging from pragmatic trials, we conducted semi-structured interviews with key stakeholders (methodologists, clinical investigators, ethicists, regulators, lay members of study teams). Interviewees were identified through published articles, funding decisions, centers known to be engaged in the design or conduct of pragmatic trials, and our investigator network. Interview topics included: (i) experiences with pragmatic trials, including experiences of ethical issues; (ii) perceptions of ethical issues relevant to pragmatic trials; and (iii) perspectives on oversight and regulation of pragmatic trials. The examination of interview transcripts followed a thematic analysis approach in which transcripts were coded and labeled inductively, with no prior coding scheme. Interviews were coded by two researchers. Interim and final analyses were presented to the broader team for comments and further discussion before producing a final analytic framework.

Results: We conducted 45 interviews between April-September 2018, with interviewees largely from the USA (16), Canada (10), and UK (10). Interviewees included lay members of study teams (16), clinical investigators (10), methodologists (7) and ethicists (7). We identified six issues that were deemed particularly relevant to the oversight of pragmatic trials: (1) Risk identification and determination of what constitutes minimal risk; (2) The distinction (and relevance) between research, quality improvement, and practice; (3) The potential for broader populations to be affected by the trial and how to determine their ethical status with respect to the trial; (4) The broader range of trial stakeholders and determining their roles and responsibilities; (5) Issues around determining and reporting interventions or comparators as "usual care", and; (6) Determining when alterations to traditional informed consent approaches are appropriate.

Discussion: We identified six key areas for ethics guidance. How risk is conceptualized and managed in the oversight of pragmatic trials was central and related to risks from the intervention, but also how risks inherent in a broader and potentially ill-defined population are managed. There is a need for greater discussion of risk, and minimal risk, and how this is managed in pragmatic RCTs. Further, the reporting of usual care interventions poses important questions for initial review, but also with respect to the scope of research regulation beyond the trial conduct.

The Caenorhabditis elegans inositol pentakisphosphate kinase 1 (IPPK-1) homolog is involved in Ventral Nerve Cord (VNC) assembly

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Background: Neural tube defects (NTDs) are a devastating and common condition within the Canadian population, affecting approximately 1/1000 newborns. A proportion of severe NTDs are caused by a developmental process called convergent extension (CE). Our lab has shown that ventral nerve cord (VNC) assembly in *C. elegans* is a CE-mediated process. The stereotypic arrangement of neurons in the VNC require proper CE. CE is controlled through multiple signaling pathways including the planar cell polarity (PCP) pathway or SAX-3/ROBO-containing pathway. To uncover novel pathways, our lab has conducted a forward genetic screen for VNC assembly defects. This identified mutations in the worm orthologue of Inositol-pentakisphosphate 2-kinase (*ippk-1*). Here we describe our initial characterization of IPPK-1 in VNC assembly.

Methods: In-vivo time-lapse florescent microscopy was used to examine the timing and position of neuroblasts involved with the formation of the VNC. This characterization was then used to quantify genetic interactions with other genes that we have found cause VNC motor neuron assembly defects. To examine the expression pattern of IPPK-1 in C. elegans, we tagged the endogenous ippk-1 locus (IPPK-1::GFP) with GFP using CRISPR/CAS9. In the future, we hope to examine if IPPK-1 functions cell-autonomously using a ZIF degron strategy that can target and degrade IPPK-1::GFP in a tissue specific manner.

Results: Loss of IPPK-1 function during embryogenesis resulted in CE defects, incorrect neuroblast migration and disorganization of VNC motor neurons at the time of hatching. This role is synergistic with that of VANG-1 (A core PCP pathway component) and SAX-3/ROBO; as loss of either protein enhances the phenotype caused by loss of IPPK-1.

Conclusions: Our current results describe a novel pathway, which functions in parallel to the *sax-3*/ROBO and *vang-1*/PCP pathways, to regulate VNC formation through convergent extension.

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Reducing Pfkt1 as a therapeutic approach to decreasing alpha-Synuclein levels

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Background: Parkinson's Disease (PD) is a debilitating neurodegenerative disease characterized by the abnormal accumulation of alpha-Synuclein (a-Syn) in Lewy bodies and neurites. In patients and model organisms, overexpression of a-Syn appears to drive toxicity, and its reduction is neuroprotective. Therefore, reducing a-Syn levels may be a promising therapeutic approach to treating PD. Moreover, achieving this via classical pharmacology is a minimally invasive approach, of particular importance in treating a chronic illness of the ageing population. Thus far, no treatments mitigating neurodegeneration have passed clinical trials, indicating the need for new therapeutic approaches.

Objective: We previously identified druggable modulators of a-Syn levels via a large-scale screen of over 7,500 genes. The screen identified PFTAIRE protein kinase 1 (PFTK1), a kinase of largely unknown function, as a robust regulator of a-Syn levels. Given that kinases are generally good drug targets, pharmacological inhibition of PFTK1 may hold therapeutic promise. We are studying the long-term effects of the potential therapy in mice, and determining if the intervention is sufficient to decrease a-Syn levels and therefore neurodegeneration in a model of PD.

Methods: In order to study the functional relationship between a-Syn and PFTAIRE1, we created a double mutant mouse line by crossing *Pfkt1*$^{+/-}$ mice to a-Syn overexpressing (*mThy1-SNCA*$^{Tg/y}$) mice. Currently, we are testing this genetic interaction by behavioural, histological and biochemical analyses to determine if the double mutant (*Pftk1*$^{+/-}$; *mThy1-SNCA*$^{Tg/y}$) mice mitigate Parkinson-like symptoms via reduction of transgenic a-Syn levels.

Results: Preliminary data suggest that the double mutant mice may show improved locomotion, initiation of movement, procedural ability and gut motility, through pole, nesting, and fecal count tests. Preliminary biochemical analysis of a-Syn overexpressing mouse brain suggests that knocking-down PFTK1 may decrease a-Syn levels by as much as 20% and toxic pSer129 a-Syn levels by 14%.

Conclusion: Thus far, our results support the potential of PFTK1 reduction in decreasing a-Syn levels and the effects of its overexpression in a mouse model of PD. Further testing will shed light on the potential of PFTK1 as a clinically relevant target for reducing a-Syn toxicity and ultimately treating PD.

A proteomic approach to understanding protein mislocalization in neurodegenerative disease

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Background: A commonality between neurodegenerative diseases such as Parkinson's disease (PD) and amyotrophic lateral sclerosis (ALS) is the presence of protein aggregates, comprised of a-synuclein (aSyn) and TDP-43 (for PD and ALS, respectively), primarily occurring at the end-stages of the disease. There is a question of whether these aggregates are drivers of disease or a consequence of other aberrant mechanisms. In PD, aSyn mislocalizes from its normal presynaptic locale to the nucleus. In contrast, in ALS, TDP-43 is sequestered from the nucleus to the cytosol, thereby forming aggregates. Given that the subcellular location of a protein is highly dynamic and regulated by post-translational modifications (PTM) and protein-protein interactions (PPIs), we posit that identifying novel PTMs and interactors of these proteins could reveal insight into networks involved in driving neurodegeneration.

Objective: We aim to identify nuclear post-translational modification signatures on aSyn in PD-relevant brain sections and to elucidate the interactors of the ALS-linked TDP-43 Q331K variant for further validation and downstream characterization of their role in neurodegeneration.

Methods: Our lab has generated a knockin mouse line targeting endogenous aSyn to the nucleus using a c-terminal nuclear localization signal (NLS) and flag tag (for biochemical manipulations). Through a series of behavioural tests, we have found that these mice (aSynNLS-Flag/NLS-Flag) exhibit age-dependant motor defects, reflecting those seen in PD. Using this model in conjunction with a mouse expressing WT aSyn (aSynFlag/Flag), we will isolate aSyn from the nuclear and cytosolic fractions of brain regions that are known to be more susceptible (i.e. midbrain and cortex) and less affected (i.e. cerebellum) in PD. In parallel, we have generated cell lines expressing GFP-tagged WT TDP-43 and an ALS-relevant Q331K mutation. TDP-43 Q331K is an aggressive mutation, resulting in ALS in all cases. Using these in vivo and in vitro tools, we will perform immunoprecipitation coupled to mass spectrometry to elucidate TDP-43 interactomes and aSyn PTMs. We will then validate and characterize promising candidate through cellular and biochemical approaches.

Results: We expect to identify novel PTMs of aSyn, correlating with its nuclear toxicity and to elucidate WT and Q331K TDP-43 protein networks with shared and distinct interactors.

Conclusion: Due to the aging population, incurable neurodegenerative diseases including PD and ALS place an increasing burden on the health care system. Using unbiased approaches, we will identify novel interactors and modifications involved in neurodegeneration, giving insight into pathways that can be targeted for their therapeutic potential.

Assessing the impact of platelet storage extension from 5 to 7 days: A pre-post study at a large multisite academic center

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Background: Platelets (PLTs) have a short storage duration, which can result in shortages when blood donation is sparse, and in high outdate rates at hospital blood banks during times of low PLT demand. In August 2017, Canadian Blood Services extended the maximum PLT storage duration from 5 to 7 days, however, this practice change has not yet been rigorously evaluated in Canada.

Objective: This study assessed the impact of PLT storage extension on PLT utilization, clinical effectiveness, and safety at the Ottawa Hospital.

Methods: We conducted a retrospective cohort using the Ottawa Hospital Data Warehouse. All hospital encounters with PLT transfusion at the Ottawa Hospital from February 2012 to February 2019 were included. Outcomes were compared between the 5-day PLT period (February 1, 2012 to August 16, 2017) and the 7-day PLT period (August 22, 2017 to February 28, 2019) using descriptive statistics and interrupted time series analysis. The transition period from 5 to 7-day PLTs (August 17-21, 2017) was excluded from analysis due to the possibility of receiving both types of PLTs. Measures of PLT utilization were (a) number of PLT doses per encounter, and (b) blood bank monthly PLT outdate rates. PLT effectiveness outcomes were (a) PLT increment after a single dose, (b) time from first to next PLT transfusion episode within an encounter, and (c) number of red blood cells (RBCs) transfused per encounter. PLT safety was measured by reported transfusion reactions.

Results: The 5-day PLT period included 9,463 hospital encounters and 23,749 transfused PLT doses, while the 7-day period included 2,933 encounters and 7,660 PLT doses. Mean PLT age at transfusion increased from 3.9 days during the 5-day period to 5.4 days during the 7-day period (mean difference = 1.5, 95% CI: 1.5, 1.6). The average monthly PLT outdate rate decreased from 11.9% during the 5-day period to 7.2% during the 7-day period (relative reduction = -39.5%, $p<0.05$). Overall, there was no significant difference in platelet utilization per encounter, time from first to next PLT transfusion, PLT increment, and PLT transfusion reaction rate between the study periods. The average RBC utilization per encounter decreased by 1.3 units/encounter from the 5 to 7-day PLT periods (95% CI: -1.9, -0.7).

Conclusion: Extending PLT storage duration from 5 to 7 days greatly reduced PLT outdate rates at our center without significantly affecting PLT effectiveness and safety.

High throughput small molecule screening with 3D lung-mimetic hydrogels in the "benign metastasizing" disease Lymphangioleiomyomatosis

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Background: Lymphangioleiomyomatosis (LAM) is a rare lung disease characterized by immature smooth muscle-like cells invading the lung parenchyma and axial lymphatics. The disease is typically described as "benign metastasizing" since the LAM cells originate from an unknown primary, immediately metastasize to the lungs, and digest the pulmonary tissue in a slowly destructive process. LAM is a monogentic disorder characterized by loss of TSC2, leading to hyperactivation of the mTORC1 signalling cascade. An mTOR inhibitor, rapamycin, is the only treatment option for LAM patients, yet the treatment solely exerts cytostatic effects with negligible LAM cell-specific cytotoxicity. Consequently, there exists a critical need for more effective therapy options, particularly those that eliminate the LAM cells without potentiating their invasiveness. Recently our lab developed a novel drug screening platform which combines a 3D lung tissue-mimetic culture system and high-content imaging. This assay has allowed us to screen a variety of small molecules, identifying compounds with LAM-specific therapeutic efficacy by both selective cytotoxicity and anti-invasive metrics.

Objective: To identify a LAM cell-specific therapeutic that demonstrates selective cytotoxicity and anti-invasiveness.

Results: We first screened an 800-drug library of Health Canada-approved cancer therapeutics, identifying both anti-invasive and cytotoxic effects via our 3D assay. Small molecules were tested in the presence and absence of rapamycin to assess compound synergy. We subsequently narrowed down our candidate list by highly ranking drugs that demonstrated selective cytotoxic and anti-invasive effects; preferred drugs were those that performed well in both categories. In addition, our drug library contained multiple structurally diverse compounds that inhibited similar targets, so we employed enrichment analysis to identify targets which consistently demonstrated superior efficacy irrespective of the small molecule treatment. After reducing our candidate list to 98 small molecules, we repeated the screen at a variety of concentrations to establish dose-response curves. While this screen is nearing completion, we have identified several molecules which demonstrate anti-invasion and therapeutic efficacy at a range of concentrations.

Conclusions: Using a novel drug screening approach, we identified effective candidate therapeutics. Future work will involve target validation, elucidation of the mechanism of action, and *in vivo* testing. As these small molecules are Health Canada-approved, we anticipate rapid clinical translation of our preferred candidate.

Development of a Human Accompanying Wheelchair using Ultrasonic Tethering

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Background: In social situations where a person accompanies a powered wheelchair user, conversations between the wheelchair user and the accompanying person could result in distracted navigation, potentially leading to accidents. Researchers have been pressed to develop human-following smart wheelchairs with hands-free navigation when moving behind a caregiver/guide or moving beside the person and participating in social interactions, thus reducing distractions while navigation the wheelchair. Current human-following technologies implemented in smart wheelchairs are only capable of following behind a person. This does not allow wheelchair users to appropriately converse with the accompanying person. Furthermore, current human-following technologies use complex devices that require extensive wheelchair modifications and cannot be easily transferred between different powered wheelchairs.

Objective: This research developed and evaluated a human-following smart wheelchair prototype using ultrasonic sensors (ultrasonic tethering). The objectives were to develop an ultrasonic tethering prototype to enable side-by-side following in social situations and, test and validate the ultrasonic tethering prototype for smooth hands-free powered wheelchair navigation.

Methods: Ultrasonic tethering can be considered for human-following and wheelchair navigation. This technique provides contactless tethered steering between a powered wheelchair and an accompanying person (AP) using robust ultrasonic sensors to detect and calculate AP location for side-by-side following. Ultrasonic sensors are cost effective, have a small form factor, and are reliable for short distances.

Results: The prototype was mounted on a Permobil F3 Corpus using a SmartChair Remote as an interface between the ultrasonic tethering system and the power wheelchair. The system successfully navigated the wheelchair based on the AP trajectory, maintaining an average user-selected conversation distance of 0.36 ± 0.1 m and controlled wheelchair direction with 8% error.

Conclusion: Results demonstrated that the system can navigate a wheelchair beside an accompanying person and maintain a comfortable conversation distance, which is advantageous for users who require hands-free wheelchair control during social activities.

A Prioritization Framework for Test-Ordering Feedback Interventions

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Introduction: Lab testing is argued to be a precursor for 70% of all medical decisions.[1] Considering that 20-50% of tests may be inappropriately ordered,[2] reduction of inappropriate use is a clear target for improving efficiency and care. Audit and Feedback (A&F), the collection of performance data and presentation of the results to care providers, can be an effective intervention for changing practices like test ordering, on average showing improvement of 4% in desired behaviours, with 25% of the studies showing better than a 16% improvement.[3] While many decision makers have large datasets on test-ordering practices that could serve as the basis for an A&F intervention, there is no clear guidance on how to prioritize which tests should be targeted for intervention first.

Objectives: Our objective is to develop a prioritization framework that will assist feedback developers in determining which tests should be the targets of feedback intervention.

Methods: This work will involve two approaches, 1) a scoping review of the factors that guided previous test ordering prioritization decisions, and 2) interviews of experts who will inform and modify the draft framework developed based on the review.

Discussion: Our framework will ensure that feedback providers are able to systematically choose which tests are the best candidates (e.g. most cost effective, highest volume) to target for intervention. The framework will provide decision makers with the factors and considerations relevant to selecting between target tests. In the long term, the impact should be an increased efficiency of resource utilization and improved clinical decision making.

Sex differences in cerebral blood flow following ischemic stroke

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Background: Ischemic stroke (IS) leads to a reduction in cerebral blood flow (CBF) in the affected brain region. Reduced CBF is detrimental to neural health, as the brain's capacity for energy storage is limited. Prior to menopause, women have a lower risk of IS. This is attributed to a protective effect of estrogens on vascular health. Signalling through estrogen receptors stimulates endothelial nitric oxide synthase (eNOS). eNOS activation increases nitric oxide production, causing relaxation of vascular smooth muscle cells, promoting vasodilation and neuroprotection against ischemia. Despite these known differences, the role of sex hormones on CBF following IS is not well understood. Our previous findings have found that female mice show higher baseline CBF values and altered hemodynamic responses compared to males. More recent work from our lab (unpublished) found an immediate drop in CBF by ~50% in female mice following a photothrombosis (PT) model of IS, while CBF in males did not drop until 48 hours post-stroke.

Objective: To elucidate potential mechanisms contributing to the acute CBF response between male and female mice following PT stroke.

Methods: To mimic the action of estrogens on eNOS, we used a genetic mouse line ($ROCK2^{+/-}$) characterized by increased eNOS expression in brain endothelial cells (ECs). Mice were gonadectomized to remove endogenous hormone production, or alternatively received sham surgeries ('intact'). Using laser-doppler flowmetry (LDF), CBF was measured under ketamine-xylazine anesthesia before and after inducing a PT stroke in the somatosensory cortex. MRI images were taken at 48 hours post stroke.

Results: Congruent with our previous findings, intact wildtype (WT) males displayed no drop in CBF immediately following PT stroke, however, at the 48-hour post-stroke timepoint CBF values were comparable to intact WT females. Both intact $ROCK2^{+/-}$ males and intact $ROCK2^{+/-}$ females had an immediate drop in CBF following PT stroke. WT female mice that were ovariectomized prior to PT stroke showed similar CBF values as intact WT males, suggesting these sex differences in CBF may be due to a particular action of estrogen on ECs. Lastly, more recent work without the use of anesthesia to induce PT and measure acute CBF responses suggests there is a sex-and-anesthesia dependent CBF response to PT stroke.

Conclusions: These data suggest that increased eNOS expression promotes a hormone-dependent drop in CBF observed immediately following PT. This study provides novel insight into how hormones influence CBF regulation after IS.

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 selection of high quality tumour-related specimens and data obtained directly by dedicated tumour bank staff, who follow a stringent set of procedures and ethical guidelines. The biospecimens and clinical data are an important resource for scientists engaged in translational research who are developing better diagnostic tools and new drug therapies. Researchers depend on the OTB to provide research biospecimens of high quality, diversity, and integrity.

The OTB coordinates the collection, storage, analysis, annotation, and distribution of tumours and peripheral blood samples. Working alongside local pathologists, medical oncologists, surgeons and other hospital personnel, specially trained OTB staff obtains patient consent, collect tissue and assemble comprehensive clinical information for each donor and their corresponding samples. To date the OTB has collected approximately 175,000 aliquots from over 20,000 consented patient donors.

TOH-OTB site has been acknowledged in many high impact scientific journals over the past years including Integrated Genomic Characterization of Carcinomas. The most recent publication was in the August 2019 issue of Biopreservation and Biobanking revealing that RNA and DNA integrity can remain stable in frozen tissue after long-term storage at cryogenic temperatures.

TOH-Ontario Tumour Bank team, Dr. Harman Sekhon (PI); Dr. Angel Arnaout and Dr. Sebastien Gilbert (Co-PI's); TOH-OTB staff, Nikita Rayne and Dheeksha Reddy.

Characterization of Neurofilament Light Chain as a Potential Blood Biomarker for Spinal Muscular Atrophy

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Background: Spinal Muscular Atrophy (SMA) is a childhood disease characterized by motor neuron loss and skeletal muscle atrophy, ultimately leading to early death. With the increasing development of new therapeutics, the field is in need of reliable biomarkers to monitor disease severity and treatment response. Elevations of neuronal proteins in blood and cerebrospinal fluid have been associated with brain injury and neurological disorders like ALS and MS. These proteins have therefore been suggested as potential blood biomarkers for SMA.

Objective: To determine whether plasma levels of neurofilament light chain (NfL), tau, and UCHL-1 serve as biomarkers in mild and severe forms of SMA, and to evaluate their potential as pharmacodynamic and disease progression biomarkers in pre-clinical models of the disease.

Methods: Using the Ultra-sensitive Simoa immunoassay, protein levels were first measured over time to determine whether they were representative of disease progression. Plasma samples were collected at 4, 7, 11, 15, and 19 days of age in our severe SMA mouse model (*Smn*$^{2B/-}$, 25 day lifespan), and 3, 6, 9, 13, 15, and 18 months of age in our mild mouse model (*Smn*$^{2B/-}$;*SMN2*$^{+/-}$, 2 year lifespan). Plasma was also collected from *Smn*$^{2B/-}$ mice treated with *SMN* gene-replacement therapy to determine whether treatment lowers NfL levels, and thus whether the biomarker effectively represents treatment response.

Results: In the severe *Smn*$^{2B/-}$ model, all proteins were elevated in symptomatic mice. NfL levels mirrored the progression of the disease, with elevations beginning concurrently with SMA symptoms and more striking elevations correlating with the presence of motor neuron pathology. Interestingly, protein levels were not elevated by 15 months in the mild model. Though muscle and weight symptoms are present at 6 months in this model, motor neuron pathology is not observed until 18 months, which could explain why no elevations were observed.

Conclusions: NfL shows promise as a biomarker of disease progression in severe but not mild SMA. The lack of elevations observed in our mild model might however be explained by motor neuron degeneration being delayed until 18 months of age. Further analysis at later time points will evaluate this relationship. Further assays will also include plasma samples from *Smn*$^{2B/-}$ mice treated with *SMN* gene-replacement therapy to evaluate NfL as a pharmacodynamic biomarker. As SMA is a multi-organ disease, treated mice will be characterized for changes in muscle, motor neuron, and neuromuscular junction pathology to assess which SMA symptoms NfL levels represent.

Extracellular vesicles as a biotherapeutic for spinal muscular atrophy.

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Background: Spinal muscular atrophy (SMA) is a genetic neuromuscular disorder caused by a deficiency in survival motor neuron (SMN) protein due to mutation or deletion of the SMN1 gene. This leads to inadequate levels of SMN protein, resulting in degeneration of the alpha motor neurons, causing atrophy of the skeletal muscle, and death in severe forms of the disease. Extracellular vesicles (EV) are nano-sized, membrane-bound particles released from all cell types, and can be found in all body fluids. EV are naturally involved in cell-to-cell communication, which may make them an idea platform for development as a novel therapeutic for SMA.

Objective: Explore the utility and efficacy of extracellular vesicles as a novel therapeutic for spinal muscular atrophy in tissue culture and animal models of the disease.

Methods: We have generated several reagents necessary for our studies, including a stable cell line that overexpress the SMN protein, and an adenovirus-based gene therapy vector encoding the SMN gene. We will use these reagents to examine loading of the SMN protein into EV, and their ability to deliver the protein to recipient cells using immunoblot and immunofluorescence.

Results: We have shown that the SMN protein is naturally released from cells in EV. Cells overexpressing the SMN protein either stably or through adenovirus vector-mediated transduction release EV containing elevated levels of the protein. Our current work is focused on examining whether the delivered protein localizes correctly within recipient cells and interacts with normal cellular protein partners.

Conclusions: Successful completion of our work may contribute to the development of a novel therapeutic for SMA.

Generation of Skeletal Muscle Satellite-like Cells from Healthy and Dystrophic Induced Pluripotent Stem Cells

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Background: Satellite cells are skeletal muscle adult stem cells capable of self-renewal and muscle repair in response to activation cues. Duchenne muscular dystrophy (DMD) is a muscle wasting disorder caused by mutations in the *DMD* gene, which encodes the dystrophin protein. Loss of dystrophin leads to sarcolemmal fragility in skeletal and cardiac muscle, as well as dysfunctions in satellite cell homeostasis in skeletal muscle. Satellite cells have a limited expansion capacity once removed from their niche within muscle tissue. This hinders research into human satellite cell dynamics, especially in muscle wasting conditions due to limited sample availability. Recent breakthroughs in induced pluripotent stem cell (iPSC) myogenic differentiation have provided methods for investigating patient-specific mechanisms of muscle diseases.

Objective: To differentiate healthy and DMD patient donor iPSCs into satellite-like cells for investigating human satellite cell biology.

Methods: DMD patient and healthy donor cells were episomally reprogrammed to generate iPSC lines. A standard myogenic differentiation protocol was used to generate satellite-like cells, assessed at several stages of differentiation with immunofluorescence staining, flow cytometry and qRT-PCR. Primary satellite cells were isolated from donor muscle tissue with fluorescence-activated cell sorting. These donor cells and muscle progenitors from iPSCs were transplanted into cardiotoxin-injured tibialis anterior (TA) muscles of immunocompromised mice. Human cell contribution to engraftment and localisation to the muscle satellite stem cell niche were assessed by immunohistochemistry on TA cryosections. Mouse hind limb irradiation was performed to reduce the inherent satellite cell pool and provide a favourable environment for human progenitor cells to engraft and contribute towards regeneration and the satellite cell pool.

Results: Transplanted primary satellite cells and iPSC-derived progenitors both contributed towards muscle regeneration post-injury. Irradiation of mouse TA muscles prior to injury and human cell transplantation was performed to enhance engraftment. Primary cells were able to generate humanised muscle in mice, with a resident human satellite cell population, characterised by Pax7 transcription factor expression. Both dystrophic and healthy donor progenitors derived from iPSCs were also able to contribute towards muscle fiber regeneration in recipient mice and gave rise to human $Pax7^+$ cells *in situ*.

Conclusions: This project improves use of xenotransplant models for investigating human satellite cell biology. Human dystrophic satellite-like cells created from readily expandable iPSCs can be used to verify molecular mechanisms discovered in model organisms, as well as testing therapeutic candidates for DMD.

The delivery of IL-15 and IL-21 in an infected cell vaccine platform reshapes the immune response for the treatment of ovarian cancer

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Background: Ovarian cancer is the most lethal gynecologic cancer with overall survival of <45%. Intratumoral CD8+ T lymphocytes are activated to generate cytotoxic T lymphocytes (CTLs) responses in part because of immunogenicity of the growing tumor.

Objective: Because cancer cells can be rendered more immunogenic by direct infection with oncolytic viruses (**OVs**), we exploited an OV platform to deliver the antitumoral cytokines IL-15 and IL-21 into the tumor microenvironment of ID8 model of ovarian cancer.

Methods and Results: To test the therapeutic potential, ID8 cells were injected orthotopically and, four weeks later, mice were injected IP with 5 consecutive daily doses of 1e8 pfu of the OV variants or PBS as control. Mice treated with OV-IL21 survived a median of 81 days compared to the other groups (~70 days), a difference that was not significant. To increase cytokine release and production by virus-infected cells, we tested how an infected cell vaccine (ICV) platform would shape the immune response. Gamma-irradiated ID8 cells were infected with different OV variants and injected IP. Within 20 hours, the ICV-OV-IL15, -OV-IL21 and -OV-IL15+21 activated and recruited different immune cells compared to the ICV-OV-Ctrl. ICV-OV-IL15+21 significantly increased the frequency of CD8+ T lymphocytes in the draining lymph nodes and peritoneal cavity, suggesting a synergistic effect of IL-15+21 on antigen-independent proliferation of CD8+ T cells. These strategies hold considerable promise for improving intratumoral T cell responses, CTL memory generation and tumor-associated antigen presentation.

Exploring the role of alpha-synuclein in innate defenses

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Background: Typical Parkinson disease (PD) is thought to be caused by a combination of both genetic susceptibility and unknown environmental trigger(s). The SNCA (α-synuclein) protein is central to PD pathogenesis, and both gene multiplication events and point mutations in SNCA are linked to familial PD. The exact function of this protein, and how it contributes to disease pathogenesis remain unknown. We, and others, have recently found that SNCA has an antimicrobial function: it protects mice from both bacterial and neurotropic viral infections. The mechanisms associated with this function, and ultimately, any possible relevance to PD remains to be determined.

Objective: To provide further support for a possible function of the SNCA protein by elucidating the role of the SNCA gene in both systemic health of the host and brain health during infections. We hypothesized that SNCA plays a systemic role in innate defenses and that SNCA dosage will modulate the outcome of virulent brain infections following the host's exposure to a pathogen.

Methodology: Delivery of reovirus isotype-3 Dearing (T3D) to the nose pad of mouse pups causes systemic illness, which leads to encephalitis starting at day 9 post inoculation; mortality is increased in Snca-null animals. In the current study, intracranial inoculations of 5x10^2 virus into the left forebrain were used to bypass systemic immune responses to differentiate the relative contribution of Snca-mediated protection in the brain vs. the periphery. Two outcomes were monitored: survival and viral titres in select organs.

Results: The effect of gene-dosage on survival was studied by comparison between Snca WT, heterozygous (WT/-), and Snca knock-out mice, and it was found that α-synuclein did not confer any significant advantages in this experimental context. These results paralleled our cellular overexpression models carried out in neural as well as non-neural culture systems. Thus, our preliminary results show that the anti-viral properties of α-synuclein, which were recorded by us and others in a systemic inoculation paradigm with dsRNA viruses, were not replicated, in the context of a direct brain infection.

Conclusions: Unexpectedly, our preliminary results showed that the antimicrobial property of Snca, which was observed systemically with 3 distinct dsRNA viruses, did not extend to when neural cells are directly exposed to reovirus-T3D. Our study suggests a complex, anti-viral role for Snca in host defenses that may be mediated, at least in part, outside the central nervous system. Future studies will address if this occurs in peripheral neurons or non-neural cells of hematopoetic lineages.

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Impact of treatment on Client Quality of Life in a First Episode Psychosis Program

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Background: Quality of life has become one of the most important components assessing outcomes in first episode psychosis populations as it measures broad aspects of life including satisfaction, functioning, socialization, physical health, & psychological well-being.

Objective: The aim of this study was to assess the quality of life of clients with first episode psychosis following treatment in a community-based mental health outpatient program specialized in assessment & treatment of early psychosis. The program is based on a recovery model and offers comprehensive treatment for up to 3 years to individuals aged 16 to 35 years residing in North-Eastern Ontario, Canada.

Methods: The study used a longitudinal approach to evaluate the impact of the treatment on clients' quality of life one and two years following treatment. All patients with a schizophrenia spectrum diagnosis were included for the period between September 2008 and 2018. Quality of life was assessed through a standardized self-reported Wisconsin Quality of Life (WQoL) questionnaire which measures eight domains including General Satisfaction, Activities and Occupation, Psychological Well-being, Symptoms, Physical Health, Social Relationship and Support, Money, and Activities of Daily Living. The WQoL was completed at three time points: baseline (T0) + 3 months of enrolment, one year (T1), and two years (T2) following start of treatment.

Results: In total 394 WQoL questionnaires were completed (T0=150, T1=150, T2= 94). Most patients reported being single (91%), white (61%), and male (66%), with an average age of 25 years (SD=8.1). The majority also reported living with their parents (64%), and having completed high school (75%).
Paired or repeated measures analysis indicated a highly significant improvement on all quality of life domains from baseline to one year and two years post treatment ($p<0.001$-0.001); however, only Physical Health domain had a significant mean change from one year to two years following treatment ($t(62)=1.98$, $p=0.05$). Client Weighted Quality of Life Index also improved significantly from baseline to one year ($t(93)=6.8$, $p<0.001$) and two years post treatment ($t(46)=6.2$, $p<0.001$). Clients also reported a higher Quality of Life Index from one year to two years following the treatment ($t(62)=2.52$, $p=0.01$).

Conclusion: Our findings demonstrate that the quality of life of patients with first episode psychosis improved significantly in various domains following one and two years of comprehensive medical and psychological treatment in a specialized early intervention program.

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Determining the anatomical cells-of-origin of alpha-synuclein using a novel mouse model

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Background: Alpha-synuclein (a-syn) is an aggregation-prone protein typically associated with synucleinopathies such as Parkinson's Disease, dementia with Lewy bodies and multiple system atrophy. Many studies focus on the resulting a-syn aggregates in disease; however, fewer studies aim to understand the native roles. While studying the end stages of disease is important, it is equally as vital to study the origin of a-syn to improve our understanding of the progression of its dysfunction. As a-syn is found primarily in the cytoplasm and synapses of neurons, identifying cell-types and brain regions enriched with a-syn remains a challenge. Determining the cells-of-origin of a-syn will provide novel insight to help focus synucleinopathy research.

Objective: Determine the cells-of-origin of a-syn in the $Snca^{NLS\text{-}Flag}$ mouse brain, with the future direction to identify the cells-of-origin of a-syn throughout all major mouse organs.

Methods: Our lab has generated a novel knock-in mouse model endogenously expressing a-syn with a nuclear localization signal (NLS) and flag-tag. This NLS tag localizes endogenous a-syn to the nucleus, enabling distinct nuclear staining to identify the cells-of-origin using immunohistochemical analysis of sectioned mouse tissues including the brain, heart, kidneys, and intestines.

Results: We show that this novel mouse model is effective for identifying cells-of-origin through distinctive staining patterns observed in the $Snca^{NLS\text{-}Flag}$ mice.

Conclusions: Using this model, we will be able to generate heat maps based on the density of a-syn positive cells in organ tissues, enabling additional insight to the onset and progression of synucleinopathies. This heat map generation will be beneficial in elucidating additional information regarding synucleinopathies. This study will also provide a novel method for studying the anatomical origins of other highly synaptic or cytoplasmic proteins.

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The chromatin re-modeller Chd4 regulates neural progenitor multipotency

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Background: In the developing CNS, a huge diversity of neuronal and glial sub-types are generated during development. To produce this diversity, neural progenitors must undergo transitions in their multipotency, for example, when they switch from producing neurons to glia. However, the mechanisms that regulate these transitions remain unclear. This study intends to characterize those mechanisms and determine factors that regulate neural stem cell potential, using the mouse retina as our model system. The 7 neuronal and glial sub-types of the retina are exclusively generated from a single population of retinal progenitor cells (RPCs). RPCs exhibit distinct phases of multipotency, resulting in temporally dependent generation of early and late born neuronal and glial cell fates. Previously, the lab identified and characterized the temporal transcription factors (TTFs) Ikzf1 and Casz1, which regulate the temporal competence transition of neural progenitors in the retina. Through proteomic studies, Mi2/NuRD chromatin remodelling complex was identified as a shared cofactor to the TTFs, suggesting that a common epigenetic pathway regulates RPC potential.

Objective: Our hypothesis is that the Mi2/NuRD complex restricts RPC potential via the epigenome and our objective is to determine whether and how the Mi2/NuRD complex regulates the production of retinal cell types from RPCs.

Method: Utilizing a conditional genetics approach, we focused on genetically knocking out the key Mi2/NuRD subunit Chd4 (Mi-2β). Chd4 is known to impart the crucial nucleosome remodelling enzymatic activity exhibited by the complex. We obtained a floxed *Chd4* allele, that was cross-bred with a progenitor-specific *Chx10-Cre* allele to excise an essential region of the *Chd4* gene at the beginning of retinal neurogenesis. Subsequently, we performed histology to observe any changes caused by the conditional knockout of Chd4.

Results: Preliminary histology studies showed drastic phenotypic changes in the mutant retinas, where the outer nuclear layer (ONL), mainly consisting of late born neuronal rod photoreceptors, had considerably thinned out. Moreover, ectopic presence of other cell types were also observed in the ONL and a general over-representation of the early born neuronal sub-types was seen at the expense of late born rod photoreceptors.

Conclusion: The preliminary results suggest that that the Mi2/NuRD complex might play a crucial role in retinal development by regulating the temporal competence transitions of neural progenitor cells. Future “omics” studies will help identify key players of a major pathway that regulates RPC potential.

The accuracy and feasibility of clinically applied frailty instruments before surgery: a systematic review and meta-analysis

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Background: A barrier to preoperative frailty assessment is the large number of frailty instruments described. Previous systematic reviews have pooled all frailty instruments into a single effect; none evaluate accuracy at the individual instrument level or considerations necessary for clinical use, which must combine accuracy with feasibility.
Objective: To systematically review clinically applied frailty instruments in the preoperative setting to: 1) estimate their predictive accuracy; and 2) assess their feasibility.
Methods: Registration was completed, and a peer reviewed search strategy was applied to Medline, EMBASE, CINAHL, and Cochrane databases from inception to November 18, 2018; included articles and related reviews were also searched. The review adhered to Cochrane guidelines. Included studies described: 1) surgical patient participants $\geq$18 years-of-age; 2) explicit frailty instrument application; 3) relevant outcomes and association of frailty with outcomes. Excluded studies: 1) included mixed populations with <50% surgical patients; 2) applied the frailty instrument solely to electronic data; 3) based frailty status on comprehensive geriatric assessment only; 4) based frailty status on single laboratory or imaging results. All stages of review were conducted independently, in duplicate. Consensus was required for exclusion. Piloted data extraction forms were used to collect key measures. Risk of bias was assessed using the QUIPS tool. Results were pooled according to the specific type of frailty instrument used, using random effects models; otherwise narrative synthesis was employed. The primary outcome was in-hospital or 30-day mortality. Secondary outcomes included complications, discharge disposition, delirium, length of stay, and function or disability. Feasibility outcomes were also collected.
Results: We screened 985 titles and abstracts for eligibility; 70 were included; 45 contributed to meta-analysis. Frailty was defined using 35 different instruments. The Clinical Frailty Scale was most strongly associated with mortality (OR 4.89, 95%CI 1.83-13.05) and non-favourable discharge (OR 6.31, 95%CI 4.00-9.94); the Edmonton Frailty Scale with complications (OR 2.93, 95%CI 1.52-5.65); and the Frailty Phenotype with delirium (OR 3.79, 95%CI 1.75-8.22). Frailty defined using any instrument was typically associated with worse functional outcomes and increased length of stay. The Clinical Frailty Scale had the strongest measures of feasibility. Non-effect size measures of accuracy were rarely reported. Measures of feasibility were limited and heterogenous.
Conclusion: Clinicians should consider accuracy and feasibility when choosing a frailty instrument. Strong evidence in both domains support the Clinical Frailty Scale, while the Fried Phenotype may require a trade-off of accuracy with lower feasibility.

Relationship between Human Spinal Cord Neural Stem/Progenitor Cell Proliferation and Donor Demographics

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Background: Neural Stem/Progenitor cells (NSPCs) have a well-established use in therapy to treat injury, with their ability to replace numerous types of cells lost due to injury, especially in animals. The literature has readily demonstrated the encouraging capabilities that stem cells possess in their role for regenerative medicine, specifically for injury in animals to the spinal cord. Modern regenerative medicine has shown the presence of neural stem cells in the human spinal cord, and as a result, is actively looking to determine if these stem cells may possess a similar and reliable role in the treatment of spinal cord injuries in humans. There is evidence of neural spinal stem cells ability to proliferate and regenerate neurons, which subsequently leads to functional improvement. Unfortunately, given the difficulty in obtaining human tissues comparatively little is known about human NSPC behaviour compared to that of animals. Thus, we will be using our unique ability to obtain healthy human donor tissue to assess the biological (spinal location) and demographic markers (sex and age) associated with NSPC proliferation rates.

Objective: This study aims to investigate certain relationships between NSPC proliferation rate and organ donor demographics, as well as their relationship with other basic biological factors such as spinal cord location.

Methods: Correlational analyses were carried out, with potential post-hoc regression for those factors found to be significantly associated with neural spinal stem cell proliferation.

Results: Preliminary analyses suggest no significant correlation between donor demographics and doubling rates.

Conclusion: This study provides insight into the insignificant clinical indication of an association between general donor demographics and NSPC doubling rates. This indicates that donor age and sex are not a strong indicators of neural spinal stem proliferation rates.

From retrospective review to prospective trial: prediction and prevention of post-operative atrial fibrillation after thoracic surgery

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Background: Post-operative atrial fibrillation(POAF) occurs commonly after thoracic surgery. Although several medications are effective at preventing POAF (e.g. amiodarone, considered to be most effective) and prophylaxis is recommended for individuals at high risk of POAF, concern regarding medication side effects and lack of a validated predictive model has lead to widespread absence of prophylaxis. Passman and colleagues previously developed a prediction model for POAF after thoracic surgery using three simple factors (sex, age, and pre-operative resting heart rate), not yet externally validated.

Objectives: This research program has two objectives,: (1) we assessed the external validity of a model to risk stratify patients of developing POAF after major thoracic surgery.; (2) among individuals identified as “high risk” of POAF, we will assess the feasibility and safety of a randomized controlled trial of administering prophylactic amiodarone to prevent POAF.

Methods: A retrospective cohort analysis was conducted of all patients who underwent major thoracic surgery at TOH (2008-2017). The prospectively documented incidence of POAF was used to assess the external validity of the POAF prediction model. Second, a multi-centre randomized controlled trial (RCT) is designed to assess if amiodarone is effective at reducing POAF among individuals who are “high risk” of POAF after thoracic surgery. A single-center blinded, feasibility RCT protocol to assess feasibility and safety of prophylactic amiodarone in patients with elevated risk of POAF is under ethics review and pending funding application.

Results: Of 2054 patients undergoing thoracic surgery, 164(7.9%) developed POAF. The model demonstrated positive correlation between risk scores and POAF incidence (2.4% of individuals scored as 1 developed POAF, 4.1% if 2, 3.4% if 3, 8.5% if 4, 10.2% if 5, 16.2% if 6). We translated the POAF model to a binary designation with “high risk” including those undergoing esophagectomy, lobectomy, or pneumonectomy and a score >3.

Pulmonary Arterial Hypertension and the Balance Between Endothelial Cell Damage and Lung Microvasculature Repair/Resolution

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Rationale:
Endothelial cell (EC) apoptosis is a critical trigger for pulmonary arterial hypertension (PAH), resulting either in the direct loss of fragile distal lung arterioles by a degenerative mechanism, and/or in aberrant repair resulting in occlusive arterial remodeling. To better elucidate the mechanisms of microvascular loss and repair in the lung, we generated a murine model for the ablation of lung ECs by targeted expression of the human diphtheria toxin receptor(DTR) using an EC specific promoter.

Methods&Results:
C57BL/6 mice expressing CRE under the control of Ve-Cadh were crossed with animals harbouring the human DTR and exposed to DT via intra-tracheal delivery. Right ventricular systolic pressure (RVSP) progressively increased after 24 hours, reaching a peak at 72 hours post DT treatment (n=19, 23.9±0.35 vs 33.7±1 mmHg; saline vs. DT, respectively; $p<0.0001$), progressively returning to baseline levels by day 7. At one-week post a single DT treatment, there was a delayed increase in right ventricular hypertrophy (RVH) (n=15, 0.24±0.004 vs. 0.27±0.006; saline vs. DT, respectively; $p=0.001$). The changes in RVSP correlated with an initial increase in active caspase3 and TUNEL staining, as well as a >50% loss of lung ECs as shown by flow cytometric analysis (DTR, CD144, CD31 and CD34) at 72 hours post treatment; followed by a recovery in EC counts by day 7. Micro-CT analysis showed a 35% decrease in total lung microvascular volume, mainly in vessels <150um in diameter at 3 days followed by the full recovery in microvasculature by day 7.

Conclusion:
Administration of DT resulted in a substantial loss in total numbers of lung microvascular ECs associated with rapid onset of PH, followed by full recovery by 1 week. This model will be used to define the molecular and cellular mechanisms underpinning of this remarkable regenerative process, potentially leading to novel therapeutic targets to enhance microvascular repair.

Scurvy in a Canadian hospital. No longer just an old-world plight. A case series.

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Background: Vitamin C, or ascorbic acid, is a water-soluble vitamin involved in multiple hydroxylation reactions, plays a major role as a cofactor in collagen biosynthesis, and promotes iron absorption and mobilization. A deficiency in vitamin C and its attributed symptoms is known as scurvy. Vitamin C deficiency is relatively uncommon in developed countries. Recent review of cases presenting to services at The Ottawa Hospital hematology service, suggested several possible conditions and treatments may be associated with an increased incidence of scurvy, including a prolonged use of proton pump inhibitors (PPIs).

Objective: To review all patients in whom vitamin C levels have been measured at The Ottawa Hospital from 2014-2019, to determine if similarities can be identified which suggest causes of vitamin C deficiency.

Methods: We conducted a retrospective chart review of vitamin C deficiency patients (<25 µmol/L) between June 2014 and January 2019. The list of patients was obtained from the Division of Biochemistry's database, and patient information was gathered using the hospital's electronic medical record system.

Results: A total of 93 patients were found to have vitamin C deficiency, with 40 patients (43%) having values deemed undetectable (<5 µmol/L). Our patient population had a male to female ratio of 0.72, and a mean age of 57. 55 (59%) patients had some form of gastrointestinal (GI) disorder, ranging from GERD to small bowel obstruction. The second most common comorbidity was cardiovascular in nature, seen in 40 (43%) patients. 43 (46%) patients were taking a PPI at the time of testing, and 21 (53%) of patients with an undetectable vitamin C level were prescribed a PPI.

Conclusion: While medical professionals are usually taught that scurvy is a disease of only historical significance, it seems that it has not totally been eradicated, even in high income countries. While it is not possible to comment on prevalence in our retrospective study including in- and out-patients of the hospital, it is possible to surmise that scurvy remains a cause of disease in 2019. We found a high prevalence of GI disorders and PPI use in our cohort. Future prospective studies will need to compare rates of these conditions in patients with vitamin C deficiency to normal controls and also assess for additional medical and social factors which may be contributing to the condition.

Do long-term care residents receive continuous family physician care? A population-based retrospective cohort study

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Background: Relational continuity of care – a key aspect of family practice – has been shown to improve patient-provider satisfaction, while reducing hospital utilization and lowering healthcare costs; these findings are especially applicable to vulnerable populations with complex needs, such as long-term care residents. However, few studies have investigated the proportion of residents that retain their family physician when they transition into long-term care.
Objective: To describe the provision of relational continuity of care delivered by family physicians to long-term care residents, and to explore the resident, facility and provider characteristics associated with the receipt of continuous care.
Methods: Using linked administrative health databases, we conducted a population-based retrospective cohort study of Ontario residents aged 60 years or older who were admitted to a long-term care facility between March 2014 - March 2017. The index date was defined as the date of long-term care entry. Continuity was defined as at least 1 visit by a primary care physician within both 0-90 days and 90-180 days of the index date.
Results: We identified 50,089 long-term care residents. The mean age was 83.8, 65.9% were female and 14.4% resided in rural communities. Only 12.2% of our cohort retained their family physician post-entry. Factors associated with reduced odds of receiving continuous care include: presence of physical and cognitive impairments (respectively, odds ratio(OR)=0.59, 95% confidence interval(CI): 0.42-0.83 and OR=0.39, 95% CI: 0.33-0.47), greater distance from family physician's clinic (residing ≥30 kilometers; OR=0.41, 95% CI: 0.35-0.48), having a pre-entry physician who was female (OR=0.90 95%CI: 0.83-0.98), or a foreign medical school graduate (OR:0.89, 95%CI: 0.81-0.97). Factors associated with increased odds of continuous care receipt include: residing in a rural long-term care facility (OR=2.23, 95%CI: 1.78-2.79), receiving treatment by a rural practitioner (OR=1.70, 95%CI: 1.52-1.90), or a practitioner who has billed long-term care codes in the past year (OR: 2.64, 95%CI: 2.45-2.85).
Conclusion: Less than 1 in 8 Ontario long-term care residents maintain relational continuity with their family doctors. Factors influencing continuous care provision include resident health, facility geography and physician practice patterns. This study informs health professionals and policymakers about the state of relational continuity of care in Ontario long-term care facilities.

High-content imaging approach to identify regulators of TDP-43 localization in the context of ALS.

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Background: Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease of the motor neurons. Over 20 genes have been identified to cause familial ALS, although the majority of cases occur sporadically due to *de novo* mutations or unknown mechanisms. Despite this, a pathological hallmark of *nearly all cases* of ALS is the nuclear-to-cytoplasmic mislocalization and aggregation of TDP-43. Mislocalization of TDP-43 was recently identified as a precursor to aggregation to recapitulate pathology, however the mechanisms driving cytoplasmic accumulation remains unclear. Identifying the forces underlying TDP-43 mislocalization will not only provide insight into the modes of neurotoxicity, but also identify potential avenues of therapeutic interception.

Objectives: Identify modifiers of TDP-43 localization using high throughput approaches to understand ALS.

Methods: Using a CRISPR/Cas9 knock-in approach, we generated cell lines that label endogenous TDP-43 with Green Fluorescent Protein (GFP). We ensured that the TDP-43-GFP fusion does not impact native TDP-43 function by assessing its localization, levels, and downstream targets. Building on this new cell line, we made and characterized a clone bearing the ALS-causing mutation Q331K. We performed a high content imaging screen using an siRNA library against the human kinome to identify kinases that regulate *wild type* TDP-43 localization. To validate using an orthogonal approach, we used CRISPR/Cas9 to target hits from the siRNA screen and analyzed the effects on TDP-43 localization.

Results: We show that the ALS-linked mutation, Q331K, confers basal mislocalization and loss-of-function of TDP-43 further supporting mislocalization as a precursor in pathology. We identified kinases that effect the localization of TDP-43 and may contribute to ALS pathogenesis. Furthermore, we demonstrate that this high throughput approach is effective for identifying proteins involved in regulating TDP-43 localization.

Conclusions: Our results provide insight into potential mechanisms causing TDP-43 pathology in ALS. Further studies will dissect the mechanisms in the context of ALS to identify avenues of therapeutic interception and better understand the causes of ALS.

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In Vivo Evidence Showed Comparable Immunomodulatory Potency Between Freshly Thawed and Freshly Cultured Mesenchymal Stem Cell Products

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Introduction: Mesenchymal stem cells (MSCs) have been shown to exert important immunomodulatory effects in both acute and chronic diseases. However, in acute inflammatory conditions, like septic shock, immunomodulatory cell therapy must be administered within hours of diagnosis. While "off-the-shelf" cryopreserved allogeneic cell products are best suited for sepsis therapy, the immunomodulatory potency of freshly thawed cells injected immediately has not been well documented. In this study, we compared the abilities of "freshly thawed" versus "freshly cultured" MSCs in modulating immune responses, specifically in an animal model of sepsis.

Methods & Results: MSCs from bone marrow donors were either: 1) thawed and cultured for 2 days before being re-suspended as the "freshly cultured" (FC) cell product; or 2) thawed directly as the "freshly thawed" (FT) cell product. FC and FT donor-matched MSCs exhibited similar surface marker expression profiles by flow cytometry and viability (Trypan blue assay 92% ± 2% vs. 93% ± 2%; Annexin V/Propidium iodide analysis 95% ± 0.6% vs. 92% ± 2%). *In vivo* potency of the MSCs was assessed by their ability to improve phagocytic activity of monocytes in whole blood and peritoneal lavage cells in an animal model of sepsis. To induce sepsis in mice, cecal ligation and puncture (CLP) was performed, followed by the infusion of MSCs at 6h later. After twenty-four hours post-sham or CLP operation, peritoneal lavage fluid and plasma samples were collected. CLP mice had a reduction in phagocytic capacity of CD11b+ cells compared to sham-operated mice both in whole blood ($p<0.05$) and peritoneal lavage. Administration of either FC or FT MSCs showed a trend of rescuing phagocytosis of CD11b+ cells in whole blood, as well as significantly improved the phagocytic ability of peritoneal lavage cells in a comparable level (with 2-fold increase in phagocyting cells). Plasma lactate levels were measured and showed a significantly elevation in the CLP animals compared to the sham-operated mice ($p<0.01$). Both FC and FT MSC treatment markedly reduced plasma lactate level ($p<0.05$).

Conclusions: *In vivo* immunomodulatory potency comparing a freshly thawed MSC product to freshly cultured cells showed comparable results, providing further evidence for the utility of a cryopreserved MSC product for acute inflammatory diseases.

Implementation of a First Responder Operational Stress Injury Clinic Using Theoretical Domains Framework and Consolidated Framework for Implementation Research

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Background: First Responder vocations (i.e., firefighters, volunteer firefighters, paramedics, police officers) involve repeated, often chronic, exposure to trauma and critical stress. Exposures, insufficient social supports, and reduced healthcare-seeking behaviours contribute to increased risk for First Responders to develop mental health problems relative to the general population. First Responders are at particularly elevated risk for posttraumatic stress disorder (PTSD), depression, anxiety, suicidal ideation, and alcohol abuse and dependence. Among First Responders, paramedics report the highest rates of PTSD, significantly higher than those for firefighters and police officers.
The acceptability of health interventions to healthcare providers and end-users is increasingly acknowledged as a vital component of implementation and sustainability. The general population typically accesses mental health care at First Responder workplaces (e.g., emergency departments), which can exacerbate significant stigma-related barriers. To effectively implement services specific to paramedics in an acceptable, accessible, and feasible fashion, stakeholders have been integrated into the research design to reduce barriers to the utilization of mental health services.

Objective: This study has been designed to explore paramedic treatment preferences for mental health care access in a First Responders Operational Stress Injury Clinic. The study examines paramedic preferences to address their mental health, internal and external barriers to care, dissemination of information about the clinic, availability of prevention services, and barriers impeding implementation and sustainability.

Methods: Thematic analysis of a minimum sample of 12 semi-structured qualitative interviews with paramedics will be used to identify access to care preferences in Ottawa. Organizational and individual determinants likely influence access to care preferences; accordingly, the Theoretical Domains Framework (TDF) will guide interviews for frontline staff, and the Consolidated Framework for Implementation Research (CFIR) will guide interviews with mid- and senior-level management. The frameworks will be used to understand multiple stakeholder perspectives. Interviews will continue until evidence of data saturation. The coding of treatment and access to care preference utterances will be guided by two manuals, one for each of the TDF and CFIR domains. Demographic data will be summarized using descriptive statistics.

Results: Anticipated emergent themes include the complexities and practicalities of stigma, anonymity, confidentiality, and trust.

Conclusions: This study is the first of its kind in Canada and will be instrumental in developing evidence-based approaches to mental health care for paramedics. The study will build on recent research assessing attitudes of Public Safety Personnel towards professional and non-professional mental health support, and associations between training programs and mental health support.

Amputee Foot Strike Identification Accuracy from 6 Minute Walk Test Raw Data

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Background:

Many people with a lower extremity amputation have problems with stability when walking. These difficulties can result in activity avoidance, stumbles or falls, and lower quality of life. For amputees, proper Foot Strike (FS) labeling can be problematic due to irregular gait patterns and asymmetries. This project explores foot strike identification within a new amputee dataset using a custom Matlab program.

Objective:

To evaluate FS detection accuracy from The Ottawa Hospital Rehabilitation Center (TOHRC) Walk Test app data during a Six Minute Walk Test (6MWT).

Methods:

A sample of 117 individuals with lower limb amputations (64.2±13.1 years old) was recruited from the University Rehabilitation Institute in Ljubljana, Slovenia. All participants completed the 6MWT along a 20m walkway (i.e., multiple turns).
An Android 6MWT smartphone application captured kinematic data (acceleration, angular velocity, orientation), with the smartphone on the posterior pelvis.
A custom Matlab program used a rule-based algorithm to identify FS from the vertical linear acceleration signal. The positions of FS were visually verified with acceleration graphs and corrected, as necessary. The time indices for calculated and corrected FS were compared.

Results:

Initial analysis on 10 participants showed, on average, 21±15% of the FS needed to be corrected from the calculated time index. The correction differed by an average of 11.11±5.25 frames from the calculated value. 17±13% of the errors were false positives and 4±3% were false negatives. 79±15% of the identified FS were true positive and 95±1% were true negative. Average accuracy across all participants was 97±1%.

Conclusion:

These preliminary results demonstrated the need for more robust FS detection methods when extracting movement information from smartphone sensor data obtained from lower limb amputees due to their irregular gait patterns and asymmetries. In future work, the remaining participants will be analysed to confirm these preliminary results. Finally, new machine learning methods will be developed to improve for gait event identification.

Analysis of Autophagy Induction Through Detection of ATG16L1 Phosphorylation

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Background: Autophagy is a degradative program that maintains cellular homeostasis and is responsible for clearing potentially toxic elements from the cell. Defects in autophagy have been described in pathophysiology including neurodegeneration, cancer, and inflammatory bowel disease. However, analysis of autophagy rates can be challenging, particularly in rare cell populations or in vivo, due to inherent limitations of current tools available for measuring autophagy pathway induction.

Objective: Characterize and validate antibody-based assays measuring ATG16L1 phosphorylation as novel methods to monitor autophagy induction, which would be free of common caveats found in current tools to measure autophagy induction.

Methods: Tissue culture and in vivo models, western blotting, immunofluorescence microscopy, immunohistochemistry, electron microscopy

Results: We developed and characterized a monoclonal antibody that can detect phospho-ATG16L1 endogenously in mammalian cells by western blot, immunofluorescence, and immunohistochemistry. We demonstrate that phosphorylation of ATG16L1 provides a readout for the level of active E3-like enzyme responsible for lipidating LC3B, which can be used to determine autophagy rates. Importantly, phospho-ATG16L1 is only present on newly-forming autophagosomes. Therefore, its levels are not affected by prolonged stress or late-stage autophagy blocks, which can confound autophagy analysis. Moreover, we show ATG16L1 phosphorylation by the autophagy kinase ULK1 is a conserved signalling pathway that is activated by numerous autophagy-inducing stressors.

Conclusions: In summary, our results demonstrate phospho-ATG16L1 can be used as a marker to monitor autophagy induction across a wide range of samples applied through multiple commonly used biological assays, with unique advantages over currently employed methods in the field. Phospho-ATG16L1 presents itself as an exciting new tool for researchers to study autophagy induction.

Are mesenchymal stem cell-derived extracellular vesicles a promising cell-free therapy? A comprehensive systematic review of preclinical methodology and outcomes

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Background: Mesenchymal stem cells (MSCs) promote healing and reduce inflammation for many different conditions. These protective effects are believed to be due to release of “extracellular vesicles” (EVs): small particles that carry biologically active factors to modulate cell processes. Hence, EVs represent a possibility to harness the benefits of MSCs as a ‘cell-free’ product. Prior to translating this therapy to first-in-human trials, there is a critical need to synthesize existing preclinical evidence of EVs.
Objective: This systematic review aims to provide the most comprehensive evidence map of methods, safety, and efficacy for MSC-EV research to date.
Methods: MEDLINE and Embase were systematically searched for preclinical, in vivo interventional studies using MSC-EVs as a therapy. Two reviewers extracted data in duplicate for: (1) methodology, (2) experimental design, (3) interventional traits, and (4) efficacy/adverse events.
Results: After screening 754 articles, 208 studies met our eligibility criteria. MSC-EVs were used to treat a wide variety of diseases, including renal (15%), brain (13%) and cardiac (11%) conditions. Most studies delivered EVs intravenously (48%), while others used direct tissue injections (20%). Overall, benefits were described in 95% of studies across all organ systems and adverse effects were reported in only three studies; two showing tumour growth. However, several key methodological concerns were evident. Based on size criteria for EV subtypes (exosomes/small EVs ~30-150nm, microvesicles ~150-1000nm) only 60% of studies used appropriate nomenclature. Ultracentrifugation (70%) and isolation kits (21%) were the most common isolation methods despite their marked differences in yield and purity. EVs were inconsistently dosed by by protein amount (67%), particle number (16%), animal weight (5%) or MSC cell count (5%); this limited our ability for inter-study comparisons. Techniques to determine size, protein markers, and morphology was highly heterogeneous, reflecting a lack of consensus on best approaches for characterization. Finally, more than 50% of studies did not incorporate randomization, thus representing a high risk for bias. Only a quarter of studies performed biodistribution experiments to ascertain whether EVs reached their target tissue of interest.
Conclusions: This systematic review of preclinical studies using MSC-EVs reveals extensive heterogeneity in methodology and interventional characteristics. Nonetheless, almost all studies showed significant benefits in a wide range of conditions; however, this is confounded by high risk of bias. The knowledge gaps we have identified highlight important opportunities for improving preclinical design and the need for more standardized approaches in this growing field of EV therapeutics.

Initiation of independent cell volume regulation in the mouse oocyte at ovulation

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Background:
Oocytes and early embryos are highly sensitive to changes in cell volume. It is now understood that cell volume dysregulation was a major cause of developmental arrest that occurred in traditional embryo culture. Early (1- to 2-cell) mouse embryos use a novel mechanism to control cell volume, in which glycine is accumulated intracellularly via the GLYT1 transporter (SLC6A9 protein). In vivo, GLYT1 activation normally occurs in parallel with release of an oocyte from meiotic arrest. It is also activated shortly after oocytes are removed from antral follicles, implying active suppression within follicles. The specific factor(s) responsible for GLYT1 activation in oocytes are completely unknown. In intact follicles, the endogenous ligand natriuretic peptide precursor C (NPPC) acts on cumulus cells to suppress meiotic resumption of the oocyte. We therefore evaluated this peptide as a potential GLYT1 suppressor.

Objective:
The objective of this project is evaluate the direct and potential indirect roles of natriuretic peptide precursor C (NPPC) as a potential GLYT1 suppressor.

Methods:
Cumulus-oocyte complexes (COCs) were collected from the ovaries of female CD1 mice primed with Pregnant Mare Serum Gonadotropin (PMSG). COCs were cultured for 4 hours after removal from ovarian follicles, and GLYT1 activity was measured with a [3H]glycine activity assay to measure glycine uptake of oocytes.

Results:
We observed that the addition of NPPC to COC culture medium partially reduced GLYT1 activity. Unexpectedly, GLYT1 activity in oocytes of COCs cultured in NPPC increased as culture medium volume increased, and activity decreased as the number of oocytes group-cultured together in a constant volume increased. Furthermore, this suppressive effect was reversed when NPPC was removed, and the small volume culture of COCs did not impact the ability of oocytes to resume meiosis.

Conclusion:
Together, these results suggest that a factor secreted by COCs in response to NPPC could suppress GLYT1. These findings provide insight into the signaling mechanisms likely involved in GLYT1 suppression in ovarian follicles in vivo.

Development of a novel cancer therapeutic platform that unleashes the cancer fighting potential of extracellular vesicles

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Background: Cancer remains one of the leading causes of mortality worldwide. However, current chemo and radiation therapies have significant off target effects, resulting in adverse side effects in patients. Recent development of Oncolytic Viruses (OVs) has presented a new method for selectively targeting, and killing cancer cells. These OVs can be further genetically engineered to carry other payloads, amplifying their therapeutic potential. In particular, these payloads can be immunostimulatory in nature by targeting cancer cells that express immune checkpoints, or by infecting these cells and downregulating these inhibitory proteins via delivery of micro RNA in extracellular vesicles. We expect that blocking the expression and function of these immune checkpoints will both re-stimulate cancer-specific immune cells and boost OV therapeutic responses.

Objective: Develop a novel OV system that carries payloads that selectively target cancer cells expressing immune checkpoint proteins, or that carries self-amplifying shRNA cassettes to downregulate the expression of these proteins.

Methods: We are currently testing our immune checkpoint targeting constructs and shRNA cassettes in vitro for their ability to be expressed. We will also evaluate these constructs' efficacy in targeting cancer cells expressing immune checkpoints, or their ability to downregulate immune checkpoint proteins in in vitro culture systems. Once these targets are validated, they will be expressed from an OV platform to test their ability to modulate anti-tumour responses in vitro (organoids and co-culture systems) and in vivo tumour models.

Results: We have generated and validated the functional activity of these shRNA constructs, and we are engineering them into our OV of choice.

Conclusions: Using innovative and new technologies, we can "arm" OVs to not only destroy tumours and stimulate the immune system, but also to deliver microRNA therapeutics via extracellular vesicles.

Chronic desipramine-induced noradrenergic neuroplasticity and recovery of anxiety and depression phenotypes in a fluoxetine-resistant mouse model

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Introduction: Alterations in serotonin (5-HT) have been implicated in major depressive disorder, for which 5-HT reuptake inhibitors (SSRIs) are the first-line treatment. However, many patients fail to respond and are switched to augmentation or other antidepressants like tricyclic-antidepressants (TCAs) on a trial and error basis. To elucidate which strategies can overcome SSRI resistance, we generated the cF1ko mice, in which the repressor Freud-1 is deleted in adult 5-HT cells (Vahid-Ansari et al., 2017). The cF1ko mice have increased 5-HT1A autoreceptor levels, function, and fluoxetine-resistant anxiety/depression phenotype. Here we address the response of cF1ko mice to the TCA desipramine, targeting 5-HT and noradrenaline systems.

Methods: cF1ko mice were treated chronically with desipramine, examined using validated behavioral tests including elevated-plus maze, open-field, novelty-suppressed feeding, forced-swim and tail-suspension. Immunofluorescence for the norepinephrine transporter (NET) detected brain-wide projections of the noradrenaline system. Co-immunofluorescence for FosB/GAD67, FosB/CaMKII/VGluT1-2 was used to detect the brain-wide chronic activity of GABAergic or glutamatergic neurons.

Results: Chronic desipramine treatment reversed the depression/anxiety phenotypes of cF1ko mice associated with the higher activity of noradrenaline cells residing in locus coeruleus comparing to vehicle treatment. Chronic desipramine treatment selectively inhibited the activity of interneurons in the target areas of the medial prefrontal cortex (mPFC) and amygdala (BLA). In addition, successful treatment with chronic desipramine induced recovery in NE projections in mPFC and BLA.

Conclusion: The induction of 5-HT1A autoreceptors in cF1ko mice was associated with global changes in the corticolimbic circuitry implicated in depression and anxiety. In contrast to fluoxetine, chronic desipramine treatment selectively target interneurons associated with reversed NET expression in mPFC and BLA. These results suggest that desipramine may overcome the SSRI resistance phenotype by directly targeting 5-HT-responsive cells. In summary, the cF1ko mice provide a clinically relevant genetic model of SSRI-resistance to investigate the efficacy and mechanisms of alternative antidepressant approaches.

Outcomes after toxic alcohol poisoning: A systematic review

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Background: Toxic alcohol poisonings due to accidental or intentional exposures are challenging to diagnose due to their non-specific presentations and toxin-mediated inebriations of the patient. Although guidelines for evidence-based therapies for methanol and ethylene glycol intoxications exist, variations are noted in treatment modalities across the toxic alcohols. Furthermore, there are no known prior reviews of clinical outcomes amongst this population.

Objectives: To summarize existing evidence on the short and long term outcomes of adult patients with toxic alcohol poisonings that span from methanol to diethylene glycol.

Methods: We conducted a literature search in PubMed, MEDLINE, and EMBASE per pre-specified criteria. Any study type that reported the management and clinical outcomes of toxic alcohol poisonings in patients 18 years or older were included. Toxic alcohols of interest included methanol, ethylene glycol, diethylene glycol, propylene glycol and isopropanol. Citation abstracts were screened and full text articles were reviewed by two reviewers independently. Data were extracted and quality assessments were performed with validated tools.

Results: 1120 citations were identified and 71 studies with 14 observational, 52 case reports/series and 5 abstracts met inclusion criteria. These studies were limited by their small sample sizes and low quality. The in-hospital mortalities for methanol, ethylene glycol studies were up to 36% and 35.3%, respectively. However, post-discharge mortality amongst survivors is seldom reported. High incidences of renal recovery at hospital discharge was reported for ethylene glycol toxicity. Information of renal prognosis post-hospitalization is largely deficient. There is a significant paucity of data on the longterm visual, neurologic and cardiovascular outcomes of patients who survived methanol and ethylene glycol intoxication after hospital discharge. Significant heterogeneity in the eligible citations' types of study, outcomes of interest, duration of follow up and interventions. These precluded meta-analyses of outcomes of interest.

Conclusions: This review highlighted marked deficits in our knowledge of longterm outcomes of toxic alcohol poisonings. Variabilities in reporting of relevant parameters also posed barriers in drawing clinically meaningful conclusions. We hope our summary will stimulate further research towards efforts to understand short and long-term sequelae of toxic alcohols and standardize evidence-based therapies.

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Near-Miss Events for Real-Time Quality Benchmarking of Laparoscopic Liver Surgery

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Background: Conventional measures of the safety of a novel surgical technique rely on generic indicators of morbidity. However, adverse events, especially serious adverse events, may be rare and small studies may be underpowered to detect them. Specific patterns of complications with novel technologies may only become apparent once the technology has already been widely implemented. Near Miss Events (NMEs) may be more common than serious adverse events, and we hypothesize that they can be used for the real-time quality benchmarking of disruptive surgical technologies. NMEs are only now beginning to be defined and used for the purpose of QA in the surgical literature.

Objective: The objective of this study is to generate a classification system for NME. We will use open and laparoscopic liver surgery as a model in which to develop the model.

Methods: A modified Delphi methodology was used to assess surgical-expert opinions on important NMEs. A local panel at The Ottawa Hospital (Ottawa, ON) developed the initial survey and distributed it to a working group on NME in liver surgery formed through the membership of the Canadian Hepato-Pancreatico-Biliary Association. Responses were reviewed and condensed into categories that were redistributed to the survey participants for final ranking.

Results: Twenty-one experts participated in the first iteration and 19 experts participated in the second iteration of the modified Delphi methodology. The top four NME categories identified were: 1) vascular NME 2) laterality and spatial orientation NME 3) oncological NME, and 4) adjacent organ injury NME. In total, consensus was reached on thirteen NME within these categories after the second iteration of the modified Delphi process. A validation step with clinical vignette assessment is currently ongoing.

Conclusions: NME in liver surgery have been defined by a consensus of Canadian expert surgeons for use in real-time quality benchmarking of novel surgical techniques. Next steps will aim to assess the utility of the validated NME QA scoring system in a patient population at The Ottawa Hospital.

Profiling Methylenetetrahydrofolate Reductase (MTHFR) Throughout Mouse Oocyte and Preimplantation Embryo Development

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Background: The global DNA methylation pattern is erased and reestablished during oogenesis and again in preimplantation (PI) embryo development. Understanding where these methyl groups come from and how the process of methylation is regulated is important, as any disruptions could result in detrimental effects. The methionine cycle that produces the cellular methyl pool is linked to the folate cycle. A key enzyme in the folate cycle is Methylenetetrahydrofolate Reductase (MTHFR) which converts 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate. This product is the substrate for only one enzyme, methionine synthase in the methionine cycle, allowing MTHFR to ultimately determine whether methyl groups contribute to the cellular methyl pool or not. MTHFR has two known isoforms, isoform a and isoform b, 77kDa and 70kDa respectively. MTHFR activity has been shown to be regulated by phosphorylation, where the phosphorylated protein is the less active form.

Objective: We are investigating MTHFR in a mouse model, focusing on the oocyte and PI embryos and how MTHFR is changing over the course of this development at the RNA and protein level.

Methods/Results: By western blotting, we have been able to detect three bands between 70-74kDa and have confirmed all as MTHFR by comparisons to tissues from a complete MTHFR knockout mouse model. These bands are present from the GV oocyte through until the blastocyst. The most prominent band out of the three changes depending on the stage of development: the lowest band is prominent in immature oocytes but there is a shift to only higher bands in mature eggs, and a shift back down in PI embryos. With lambda protein phosphatase (LPP) treatment, there is a clear shift to the lowest (~70kDa) band, which indicates that the upward shift in eggs is due to phosphorylation. None of the three bands we observe appear to be as high as the 77kDa protein product of isoform a, and only a band ~70kDa remains after LPP treatment, indicating the likely expression of MTHFR isoform b.

Conclusion: Future work will aim at confirming MTHFR phosphorylation changes and the expression of mRNA for the 70 kDa isoform b. These data will give insight into MTHFR activity and how it may be regulated to contribute to the genomic methylation pattern in oocytes and PI embryos.

Deciphering the functional redundancy of USP4 and USP15

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Background: The deubiquitinating enzymes USP4 and USP15 are encoded by genes that are ohnologues arising from whole genome duplication events early in vertebrate evolution, and the majority of known vertebrate genomes contain a functional copy of both. Both USPs are known to be involved in some of the same signalling pathways such as Wnt/β-catenin, however subfunctionalization has occurred such that they each regulate the stability of distinct substrates. Despite their sequence and evolutionary similarities, the ohnologues may have opposite correlations in overall survival of lung adenocarcinoma patients. Early work with knockout mice has determined that while mice null in one ohnologue display no phenotype, the double null genotype is lethal.

Objective: To determine the extent of functional redundancy of USP4 and USP15 at the organismal and cellular level.

Methods: We are analyzing the progeny of genetic crosses of mice in which one or both ohnologues have been inactivated. The phenotypic effect of deficiency will be determined at the organismal level by IHC and PCR. The cellular substrates and pathways impacted by USP4/15 deficiency will be investigated by western blot and qPCR.

Results: While no mice null in both ohnologues are viable, they do survive until E12.5. Beyond the obvious physical differences in size, the double null embryos have severely underdeveloped livers and have defects in fetal hematopoiesis. Mouse embryo fibroblasts have been derived from embryos of all USP4/15 genotypes. Preliminary qPCR analysis shows potential transcriptional compensation in one ohnologue USP when the related USP is knocked out. Multiple Wnt/β-catenin pathway components are impacted when deficient in one or both USPs.

Conclusion: Defects in the Wnt/β-catenin pathway may be associated with observed impairments in hepatic fetal hematopoiesis in embryos lacking one or both ohnologue USPs of interest. While there is some evidence of transcriptional compensation, a mechanism has yet to be established.

Desipramine, but not fluoxetine, restores norepinephrine innervation in SSRI-resistant mice

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Background: Serotonin (5-HT) is a neurotransmitter that is involved in the regulation of mood. 5-HT signalling is mediated by several receptors in the brain, including the 5-HT1A receptor. Altered regulation of the 5-HT1A receptor expression has been implicated in depression/anxiety. Among the 5-HT1A receptor subtypes, 5-HT1A autoreceptors and heteroreceptors, the former inhibits the firing of 5-HT neurons in the raphe nuclei of the midbrain. Freud-1 is a transcription factor that negatively regulates the transcription of 5-HT1A autoreceptors. We previously showed that Freud-1 knockout mice displayed depression/anxiety phenotypes and were resistant to antidepressant treatment with the selective serotonin reuptake inhibitor, fluoxetine. To determine whether Freud-1 knockout mice were resistant to other treatments, we investigated the effect of desipramine, which targets norepinephrine reuptake. In the Freud-1 knockout mice three-week desipramine treatment improved depression/anxiety phenotypes to levels seen in normal wild-type mice.

Objective: We sought to decipher the molecular mechanisms behind this behavioural recovery. We assessed serotonin and norepinephrine axonal projections in brain regions involved in depression and anxiety, to evaluate whether neurotransmission to these areas was affected.

Methods: We used SERT and NET staining to specifically label serotonin and norepinephrine projections, respectively, in key brain regions involved in depression/anxiety. Regions examined included the medial prefrontal cortex, nucleus accumbens, lateral septum, hippocampus, amygdala and the dorsal raphe nuclei, in sham vs. treated Freud-1 knockout mice.

Results: We found that axonal varicosities were reduced in both neurotransmitter systems, while total axonal volume was unaltered in both systems. Specifically, the nucleus accumbens, entorhinal cortex and basolateral amygdala showed a reduction in noradrenergic varicosity density in the Freud-1 knockout mice compared to controls, and chronic desipramine treatment reversed these deficits to sham levels. The nucleus accumbens, hippocampal CA1, entorhinal cortex, basolateral amygdala and the dorsal raphe nucleus showed reduced serotonergic varicosity density in the Freud-1 knockout mice, an effect which was interestingly not reversed with chronic fluoxetine treatment in these fluoxetine-resistance mice.

Conclusion: We uncovered unique aspects of the mechanism involved in the pathophysiology and treatment of depression/anxiety in Freud-1 lacking mice. Future work will focus on characterizing the nature and level of the synapses formed by the recovered axonal projections in affected areas.

BPTF is essential for murine neocortical development

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Background: Chromatin remodeling complexes modulate DNA accessibility permitting neuronal progenitor cells to proliferate and differentiate to form the mammalian neocortex. In the case of BPTF, the major subunit of a chromatin remodelling complex called NURF (Nucleosome remodelling factor), mutations leading to its haploinsufficiency have been linked to cause a recently annotated human neurodevelopmental disorder called NEDDFL (Neurodevelopmental disorder with dysmorphic facies and distal limb anomalies). Patients with the syndrome are mainly characterized with microcephaly and intellectual disability.

Objective: Here we characterized the role of BPTF during neocortex development using the mouse as a model organism.

Methods: Using Emx-1, a telencephalon specific gene as a driver for the Cre enzyme, we conditionally knocked out (cKO) the BPTF gene. With these animals we analyzed brain development embryonically and postnatally, comparing wild-types, heterozygotes and full BPTF cKOs.

Results: The BPTF cKO animals are viable, born in normal mendelian ratios, survive 9+ months as well as, demonstrate significant forebrain hypoplasia. During cortical neurogenesis, the cKOs show a reduction in intermediate neuronal progenitor (INP) cells, an increase in apoptosis as well as a prolonged cell cycle within proliferating cells. Similarly, the BPTF cKOs demonstrate altered neuronal phenotypes specific to internal pyramidal cells (layer V), with altered CTIP2 and Foxp1 expression. Lastly, RNA-seq data corroborates the phenotypes demonstrated by the BPTF cKOs, mainly gene deregulations involved in CNS development, neuronal differentiation and immune response.

Conclusion: Our results indicate that BPTF is critical for murine telencephalon neurogenesis. The hypoplasia demonstrated in the mouse model can resemble the microcephaly displayed by the human NEDFFL patients. This mouse model serves to highlight critical epigenetic pathways regulated by BPTF specific to the foressbrain; pathways which could also be affecting the human patients and leading into the neural phenotypes observed.

Initial Implementation of Extubation Advisor, a Clinical Decision Support Tool to Improve Extubation Decision-Making in the ICU

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Background: Timely and successful extubation is critical to intensive care yet remains unsuccessful in ~15% of patients. Although a variety of methods to assess extubation readiness have been developed, including the spontaneous breathing trial (SBT), the Rapid Shallow Breathing Index (RSBI), respiratory rate variability (RRV), cough strength and more, no standardized clinical decision-support tool has previously been implemented.

Objective: In this study, we evaluated the feasibility, facilitators and barriers of introducing and implementing Extubation Advisor™ (EA), which combines a respiratory rate variability-derived machine learning model to predict the risk of extubation failure, the rapid shallow breathing index (RSBI), clinical impression of extubation failure risk, and standardized extubation readiness checklist.

Methods: This Phase I mixed-methods observational study includes four data sources: feasibility and predictive model assessments, quantitative questionnaires on data entry process and EA report, and semi-structured qualitative respiratory therapist (RT) interviews. Interviews were audio-recorded, transcribed verbatim and analyzed by a team of researchers with qualitative research expertise. Themes emerged iteratively from coded data.

Results: We enrolled 117 patients (June 2017-October 2018) and included 153/206 spontaneous breathing trials. 85% of 80 extubations succeeded. From the respiratory rate variability-derived model, the incidence of extubation failure was 11% in below-average risk patients and 21% in above-average risk patients. Response rates to surveys were low (21% and 33%). Almost all rated the data entry process average or better. At least 75% rated the EA report average or higher. Several themes emerged from interviews (n=15) including: EA had a well-designed, user-friendly interface with few technological issues; interestingly, we observed that inadequate communication in implementation resulted in RT concerns with the EA's impact on their job security.

Conclusions: EA was perceived by many RTs to have the potential to aid in extubation decision-making and was positively evaluated for its design. However, although it was designed to empower RT expression of extubation risk, this proved threatening to some. This study helps to understand the complex process of bedside implementation of predictive models derived using artificial intelligence.