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

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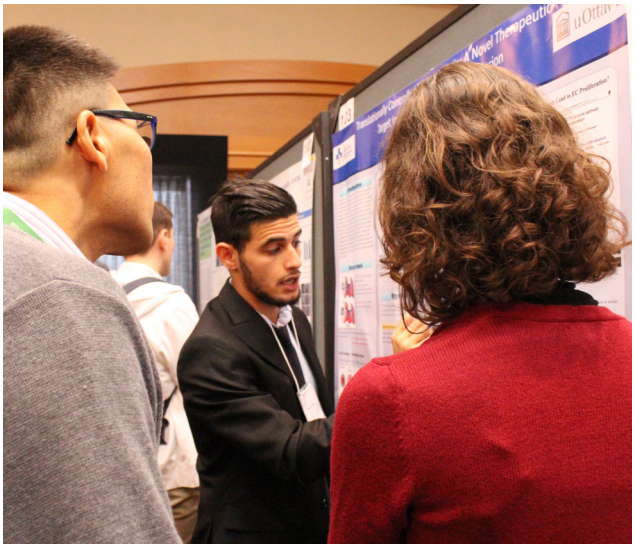
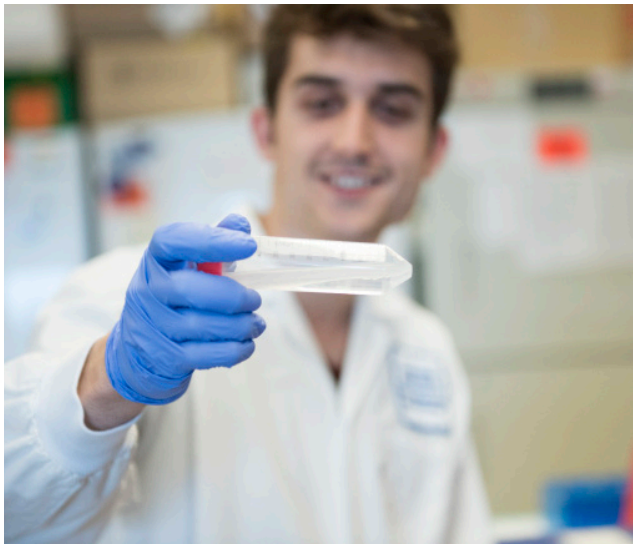
2018 RESEARCH DAY





Program and Abstracts

Thursday, November 8, 2018
7:30 a.m. – 5:00 p.m.

St. Elias Centre
750 Ridgewood Ave.
Ottawa, ON

Research Day is generously supported by:

Research Day Committee

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

Dr. Fraser Scott (Chair)
Dr. Paul Albert (In-coming co-chair)
Dr. Lauralyn McIntyre (In-coming co-chair)
Dr. Dean Fergusson
Dr. Ian Lorimer
Dr. Duncan Stewart
Dr. Jay Baltz
Dr. Ketul Chaudhary
Dr. Marjorie Brand
Dr. Anouk Fortin
Dr. Tim Ramsay
Amelia Buchanan
Emma Grigor
Dr. Angela Crawley
Jennifer Ganton
Dr. Luc Sabourin

Volunteers

Greg Canham
Natasha Hollywood
Jennifer Jean-Louis
Melanie Genereaux
Jennifer Valentino
Wayne Lowe
Terri Van Gulik

WELCOME TO RESEARCH DAY

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.

Research Day also gives us the opportunity to bring world-class researchers to Ottawa to give two keynote lectures.

The first will be given by Dr. Dana Devine, Chief Scientist at Canadian Blood Services. She has had a longstanding research career in blood products, transfusion medicine, platelet biology, complement biochemistry, and coagulation. The title of her talk today is "Improving the quality and safety of blood transfusion products."

The second lecture will be given by Dr. Alexandre Prat, Canada Research Chair in Multiple Sclerosis. His current research interests include the immune roles of the blood-brain barrier, how monocytes and lymphocytes migrate across it and how it is regulated by glial cells. The title of his talk today is "Translational research in MS."

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 Dr. Fraser Scott, the outgoing chair of Research Day, who has worked tirelessly to make this event possible for many years. We also welcome the incoming co-chairs, Drs. Lauralyn McIntyre and Paul Albert, who are always open to input on how to make this inspiring day even better.

Duncan Stewart, MD, FRCPC

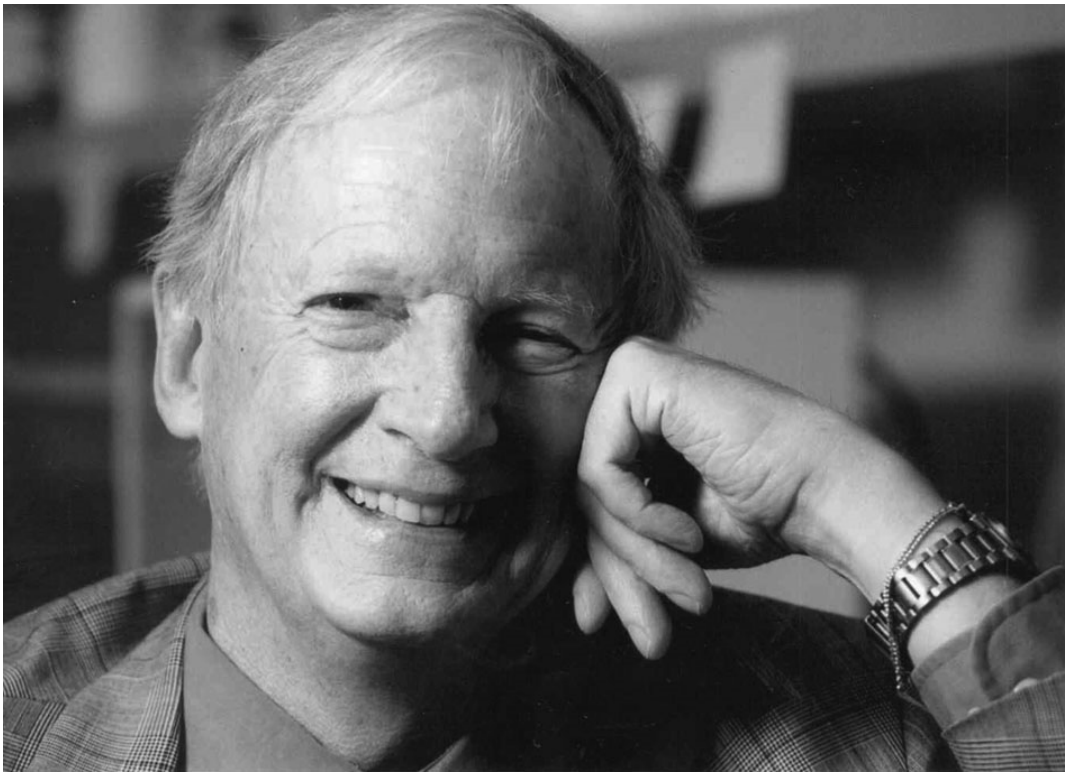
CEO & Scientific Director,
Senior Scientist in the Regenerative Medicine Program,
Ottawa Hospital Research Institute

Executive Vice-President, Research,
The Ottawa Hospital

Evelyne and Rowell Laishley Chair

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

DR. J. DAVID GRIMES LECTURE



Dr. J. David Grimes, MD, FRSPC

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

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

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

DR. J. DAVID GRIMES LECTURE

"Improving the quality and safety of blood transfusion products"

Dr. Dana Devine

Dr. Dana Devine is Chief Scientist at Canadian Blood Services and a professor of pathology and laboratory medicine at the University of British Columbia. She is a a founding member of the university's Centre for Blood Research and Editor-in-Chief of the blood transfusion journal Vox Sanguinis. She has had a longstanding research career in blood products, transfusion medicine, platelet biology, complement biochemistry, and coagulation. In service to the transfusion community, Dr. Devine is a member of numerous advisory committees and boards including the American Red Cross, Blood Systems Research Institute, Bloodworks Northwest, the New York Blood Center and the Australian Red Cross Blood Service. She is also a fellow of the Canadian Academy of Health Sciences.

KEYNOTE LECTURE

"Translational research in MS"

Dr. Alexandre Prat

Dr. Alexandre Prat is a staff neurologist at the CHUM-Notre Dame Hospital (Montréal) and is a full professor of Neurosciences at Université de Montréal. He now holds the Senior Canada Research Chair in Multiple Sclerosis and was inducted to the college of researchers of the Royal Society of Canada in 2015. His current research interests include the immune roles of the blood-brain barrier, how monocytes and lymphocyte migrate across it and how it is regulated by glial cells. The underlying hypothesis of Dr. Prat's work is that deciphering the mechanisms by which the blood-brain barrier controls the passage of cells and molecules to the central nervous should lead to better understanding of diseases such as MS and brain tumors, as well as to the discovery of novel routes for delivery of drugs and chemotherapies.

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 Dr. Velislava Tzaneva and Dr. Sailendra Nath Sarma for their excellent job running the summer student program this year.

Winners of the Dr. Goodman Cohen Summer Student Award

Senior Award

Kaitlyn Rourke (supervised by Jonathan Angel)
"sCD127 secretion in response to IL-7 may be influenced by a single nucleotide polymorphism"

Lubina Nayak (supervised by Rodney Breau)
"Does androgen deprivation improve cancer control in high-risk non-metastatic prostate cancer patients undergoing radical prostatectomy? A systematic review and meta-analysis"

Junior Award

Amit Scheer (supervised by Michele Ardolino)
"Natural killer cells acquire the checkpoint receptor PD-1 by intercellular transfer from tumour cells"

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

Leonard Angka
Meshach Asare Werehene
Ricardo Batista
Anabel Bergeron
Caroline Brun
Valerie Cardin
Pascale Charette
Ketul Chaudhary
Ashley Chen
William Chen
Nicholas Cober
David Cook
Sarah Cummings
Josh Del Papa
Donna Dsouza
Daniel El Kodsi
Peter Feige
Stacey Fisher
Laura Forrest
Ahmad Galuta
Jonathan Hodgins
Victoria Hunniford
Jamieson Taylor
Mahsa Jessri
Lisa Julian
Kaewsakulthong Woratree
Samantha Kornfeld
Alvin Li
Wenshan Li
Marissa Lithopoulos
Anisha Lynch-Godrei
Danijela Maras
Marisa Market
David Massicotte-Azarniouch
Hayley McKay
Thalia Medeiros
Ivana Nad
Vincent Nguyen
Nathaniel Noblett
Iris Perelman
Michael Phan
Adam Pietrobon
Morten Ritso
Chris Rousso
Dina Salama
Reza Salehi
Briana Samson
Teslin Sandstrom
Maude Shaughnessy
Singaravelu Ragunath
Abera Surendran
Zaid Taha
Alvin Tieu
Marie-Ève Wedge
Yunping Xue
Min Zhang

OHRI RESEARCH DAY PROGRAM

7:30 AM **REGISTRATION / POSTER SETUP / CONTINENTAL BREAKFAST**
Sponsored by VWR part of Avantor and Beckman Coulter

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

8:30 AM **Preclinical models in cancer and stem cell biology** (50 Minutes)
(Talks: 9 minutes plus 3 minutes discussion)
Moderators: Serge Neault and David Cook

- **Victoria Allen** (Christina Addison Group) *Towards Diagnostic and Therapeutic Targets for Invasive Lobular Carcinoma*
- **Marie-Ève Wedge** (Carolina Ilkow Group) *Tailoring oncolytic viruses for the treatment of pancreatic cancer*
- **Victoria Hunniford** (Manoj Lalu Group) *Preclinical Multicenter Studies as a Means to Improve Biomedical Research Translation*
- **Morten Ritso** (Michael Rudnicki Group) *Engraftment of Primary Human Muscle Satellite Cells and Satellite-like Cells Derived from Pluripotent Stem Cells*

9:20 AM **Lung, vascular and circulatory disorders** (50 Minutes)
(Talks: 9 minutes plus 3 minutes discussion)
Moderators: Rafael Soares and Charvi Syal

- **Ketul Chaudhary** (Duncan Stewart Group) *Differential effects of female sex hormones on severe pulmonary arterial hypertension induced by VEGFR2 inhibition*
- **Martin Kang** (Bernard Thébaud Group) *Gene Therapy Rescues Respiratory Distress and Improves Survival in a Mouse Model of Surfactant Protein Deficiency*
- **Nicholas Cober** (Duncan Stewart Group) *Microgel encapsulated endothelial progenitor cells provide a portable stem cell niche for treatment of pulmonary arterial hypertension*
- **Iris Perelman** (Dean Fergusson Group) *Patient-Centered Outcomes in the Management of Anemia: A Scoping Review*

10:10 AM **REFRESHMENT BREAK** (15 minutes) Sponsored by Thermo Fisher Scientific

10:25 AM **POSTER SESSION 1** (60 minutes)
Sponsored by STEMCELL Technologies

11:25 AM **DR. J. DAVID GRIMES LECTURE** (35 minutes plus 10 minutes discussion)
Sponsored by MJS BioLynx
Improving the quality and safety of blood transfusion products
Dana Devine, Chief Scientist at Canadian Blood Services, professor of pathology and laboratory medicine at the University of British Columbia.
Moderator: **Dean Fergusson**

12:10 PM **THANK YOU TO OUTGOING CHAIR, WELCOME OF NEW CO-CHAIRS**
(Duncan Stewart, Fraser Scott)

12:15 PM **BUFFET LUNCH/ POSTER SETUP** (55 minutes)

1:10 PM **KEYNOTE LECTURE** (35 minutes plus 10 minutes discussion) Sponsored by 10x Genomics

Translational research in MS

Alexandre Prat, Senior Canada Research Chair in Multiple Sclerosis, neurologist at the CHUM-Notre Dame Hospital (Montréal) and professor of Neurosciences at Université de Montréal.
Moderator: **Michael Schlossmacher**

1:55 PM **POSTER SESSION 2 (60 minutes)**

2:55 PM **REFRESHMENT BREAK** (15 minutes)

3:10 PM **Perspectives in immune modulation of disease** (50 Minutes)
(Talks: 9 minutes plus 3 minutes discussion)
Moderators: Sasha Van Katwyk and Leonard Angka

- **Pascale Charette** (Barbara Vanderhyden Group) *FGL2 as a mediator of immunological preeclampsia and inflammatory lesions of the placenta*
- **Sarwat Khan** (Rebecca Auer Group) *An Interleukin-12-Expressing Oncolytic-Virus Infected Autologous Tumor Cell Vaccine Generates Potent Anti-Tumor Immune Responses*
- **Shaima Kaka** (Angela Crawley Group) *Liver damage severity is associated with sustained dysfunction of circulating CD8+ T-cells following chronic HCV infection cure*
- **Teslin Sandstrom** (Jonathan Angel Group) *HIV-infected macrophages are selectively infected and killed by the oncolytic Rhabdovirus, MG1; a potential tool for viral eradication*

4:00 PM **Neurodegeneration and biology of the nervous system** (50 Minutes)
(Talks: 9 minutes plus 3 minutes discussion)
Moderators: Ahmad Galuta and Bojan Shutinoski

- **Stacey Fisher** (Doug Manuel Group) *Dementia Population Risk Tool (DemPoRT): Predictive Algorithm for Assessing Dementia Risk in the Community Setting*
- **Daniel El Kodsi** (Michael Schlossmacher Group) *The Parkin protein: From its Function in the Brain to the Characterization of a New Mouse Model*
- **Nathaniel Noblett** (Antonio Colavita Group) *The adenylate forming domain protein DIP-2 is an intrinsic repressor of neurite outgrowth and axon regeneration in C. elegans*
- **Faranak Vahid-Ansari** (Paul Albert Group) *Targeting noradrenaline for treatment of anxiety and depression phenotypes in a fluoxetine-resistant mouse model of impaired serotonergic activity*

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 Bio-Rad, CADTH, CCRM and CDRD
Moderators: Duncan Stewart and Fraser Scott

ORAL PRESENTATIONS:

Preclinical models in cancer and stem cell biology (8:30 – 9:20)

Moderators: Serge Neault and David Cook

1-1

Towards Diagnostic and Therapeutic Targets for Invasive Lobular Carcinoma

Victoria Allen[1,2], HuiJun Zhao[1], Dr. Grant Howe[1], Dr. Christina Addison[1,2]

[1]Center for Cancer Therapeutics at the Ottawa Hospital Research Institute
[2]Department of Biochemistry, Microbiology and Immunology, Faculty of Medicine, Graduate and Postdoctoral Studies at the University of Ottawa

Background: Invasive lobular carcinoma (ILC) is in need of improved diagnostic and therapeutic targets due to a lack of valid predictors of metastatic risk or effective treatment options for patients with metastatic ILC given its poor response to chemotherapy. Additionally, ILC is not readily palpable and cannot be detected using standard imaging practices including mammogram or ultrasound, hence is often diagnosed at late stages. microRNA (miRNA) are potential biomarkers of ILC metastasis due to their stability in blood and formalin fixed paraffin embedded tissues. miRNA are involved in numerous biological processes, have modified expression in cancers, and have been linked to invasion and metastasis. miRNA may thus be useful diagnostic or therapeutic markers for metastatic ILC.

Objectives: It is hypothesized that differential miRNA expression will be observed in invasive and non-invasive ILC. It is further speculated that these differentially expressed miRNA participate in the regulation of pathways that modulate invasion and metastasis.

Methods: miRNA levels were measured through qPCR based techniques, while modulation of the cellular levels of miRNA was conducted using hairpin inhibitors and mimics. Subsequent invasion and migration potential was investigated through the use of invasion chambers and Transwells. Potential mRNA targets of the evaluated miRNA were identified using Affymetrix chips, and their expression confirmed through Western blot and qPCR analysis.

Results: A miRNA genome analysis of ILC cell lines identified miRNA with differential expression in invasive compared to minimally invasive ILC cell lines. These miRNA were validated, and miR-23c and miR-23b-3p were found to have significantly increased expression in the invasive compared to minimally invasive ILC cell lines. When the expression of miR-23c and miR-23b-3p was suppressed in invasive cell lines, a significant decrease in their invasive capabilities was observed. In contrast, their overexpression in minimally invasive cell lines tended to increase cell invasive capabilities. mRNA targets of these miRNA have been identified and are under investigation for their potential role in ILC invasion.

Conclusions: This project has identified miR-23c and miR-23b-3p as potential regulators of ILC cell invasion, since they exhibited increased expression in the invasive compared to minimally-invasive ILC cell lines. This project hopes to identify potential therapeutic targets of these miRNA that may act as biomarkers and therapeutic targets of metastatic ILC.

1-2

Tailoring oncolytic viruses for the treatment of pancreatic cancer

Marie-Ève Wedge1,2, Brian Laight[1], Hayley McKay[1,3], Larissa Pikor[1], Adrian Pelin[1,3], Christiano Tanese de Souza[1], John C Bell[1,3] and Carolina S Ilkow[1,3].

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

Background: Pancreatic cancer (PC) is the fourth leading cause of cancer-related death in developed countries and is associated with a very low survival ratio. There has been little progress in the treatment of PC in the last 30 years and current treatment options provide only marginal survival benefit. Recent advances in the use of oncolytic viruses (OVs) as cancer therapeutic agents bring new hope to fight the notorious disease that is PC. OVs are unique cancer-killing bioweapons, since they not only kill specifically tumour cells, but they also trigger protective immune responses that prevent tumour recurrence. Although OVs have shown promising results in certain cancers, some tumours remain resistant to OV therapy due to residual antiviral mechanisms in the cancer cells.

Hypothesis and objective: We hypothesized that the use of small artificially designed RNA sequences, known as artificial microRNAs (amiRNAs), could help target the cellular antiviral components associated with the observed OV resistance of pancreatic cancers. Therefore, this project was aimed at 1) identifying amiRNAs that enhance viral replication and associated cytotoxicity and 2) creating an amiRNA-expressing virus with enhanced PC killing capacity.

Methods: To find such amiRNAs, we passaged a library of viruses encoding ~16,000 unique amiRNAs in several pancreatic cancer cell lines to enrich for sequences that could enhance OV replication.

Results: By using this approach, we were able to identify an amiRNA that improves PC cell killing (amiR-PC) when expressed from an OV. amiR-PC target identification revealed ARID1A as an interesting player in resistance to OV therapy. This target is of particular interest since its downregulation acts in a synthetic lethal fashion with inhibition of the EZH2 methyltransferase. Combining an amiR-PC-expressing OV with a small molecule inhibitor of EZH2 enhances PC cell death. Moreover, we have shown that amiR-PC is packaged in cancer cell-secreted extracellular vesicles which have the ability to reach neighbouring cells to sensitize naïve cells to the EZH2 inhibitor and spread the tumour killing effect throughout the tumour to utterly destroy it. This data ultimately translates to an improvement in tumour debulking and survival of PC tumour bearing mice.

Conclusion: This work is not only broadening our knowledge on the unique PC biology and the resistance of select tumors to oncolytic virotherapy, but it also provides new hope for a cure to the grim disease that is PC.

1-3

Preclinical Multicenter Studies as a Means to Improve Biomedical Research Translation

Victoria Hunniford[1], Dean Fergusson[2,3,4], Agnes Grudniewicz[1,5,6], Casey Landsdell[7], Emma Grigor[4], and Manoj Lalu[2,8,9]; on behalf of the BLUEPRINT Translational Research Group

1. Telfer School of Management, University of Ottawa, Ottawa, Ontario, Canada
2. Faculty of Medicine, University of Ottawa, Ottawa, Ontario, Canada
3. Department of Surgery, University of Ottawa, Ottawa, Ontario, Canada
4. School of Epidemiology and Public Health, University of Ottawa, Ottawa, Ontario, Canada
5. Institute du Savoir, Montfort, Ottawa, Ontario, Canada
6. Bruyère Research Institute, Ottawa, Ontario, Canada
7. The Ottawa Hospital Research Institute, Ottawa, Ontario, Canada
8. Department of Anesthesiology and Pain Medicine, The Ottawa Hospital, University of Ottawa, Ottawa, Ontario, Canada
9. Regenerative Medicine Program, The Ottawa Hospital Research Institute, Ottawa, Ontario, Canada

Background: There is a need to improve translation of basic biomedical discoveries into viable clinical therapies. Multicenter preclinical studies have been suggested as a method to improve reproducibility, generalizability and potential clinical translation of preclinical work. In these studies, multiple independent laboratories collaboratively conduct a research experiment using a shared protocol. The use of a multicenter design in preclinical experimentation is a recent approach and only a handful of preclinical multicenter studies have been published. A systematic synthesis of preclinical multicenter literature will create an evidence map of studies to date, evaluate the design and quality of these studies, and assess their potential effects on 'bench-to-bedside' translation.

Objective: To systematically identify, assess and synthesize published preclinical multicenter studies.
Methods: The review protocol was registered (PROSPERO CRD42018093986). Embase and MEDLINE databases were systematically searched, and articles were screened for preclinical, in vivo, animal studies conducted at multiple centers (i.e. laboratories). Data extracted and synthesized included study methods/design, basic characteristics, outcomes, barrier and facilitators. Study risk of bias, completeness of reporting and the degree of collaboration were evaluated using established methods.

Results: The search identified 3,095 citations with 12 studies meeting eligibility criteria. Conditions investigated included stroke, myocardial infarction, traumatic brain injury, and diabetes. The median number of centers was 4 (range 2-6), the median sample size was 135 (range 23-384). Studies had unclear or low risk of bias across most domains and high completeness of reporting. All interventions assessed were previously shown to have positive effects in single-centered studies. Degree of collaboration was assessed as medium to high. When assessed in multicenter preclinical studies, four interventions had positive results, six had no effect, and two had mixed effects. Facilitators included establishing regular meetings and developing a protocol with input from all centers. Barriers included adhering to the protocol throughout the entirety of the study and consistent funding.

Conclusions: This review demonstrates the feasibility of conducting multicenter preclinical studies as shown through the successful application across a diverse range of models and interventions. Most studies had lower risk of bias and higher completeness of reporting than typically seen in single-centered studies. Only four of the twelve studies produced results consistent with previous single center studies, highlighting a central concern of preclinical research: irreproducibility and poor generalizability of findings from single laboratories. Our review suggests that multicenter preclinical studies provide a method to more robustly assess therapies prior to considering clinical translation.

1-4

Engraftment of Primary Human Muscle Satellite Cells and Satellite-like Cells Derived from Pluripotent Stem Cells

Morten Ritso[1,2], Carole Doré[1], Eve C. Tsai[3,4], William L. Stanford[1,2], Michael A. Rudnicki[1,2]

1. Regenerative Medicine, Ottawa Hospital Research Institute
2. Cellular and Molecular Medicine, University of Ottawa
3. Neuroscience, Ottawa Hospital Research Institute
4. Department of Surgery, Division of Neurosurgery, University of Ottawa

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. Recent breakthroughs in induced pluripotent stem cell (iPSC) myogenic differentiation have provided methods for investigating patient-specific mechanisms of muscle diseases.

Objective

To optimize DMD patient iPSC-derived satellite-like cell differentiation and transplantation into mice for investigating human dystrophic satellite cells *in situ* and *in vitro* on isolated single muscle fibers.

Methods

DMD patient and healthy donor cells were episomally reprogrammed to generate iPSC lines. Four published myogenic differentiation protocols were compared for their efficiency to give rise to 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 with a resident human $Pax7^+$ satellite cell population in mice. Both dystrophic and healthy donor progenitors derived from iPSCs were also able to contribute towards muscle fiber regeneration in transplanted mice, but only healthy donor progenitors gave rise to $Pax7^+$ cells *in situ*. Spontaneous differentiation of neural crest and neuronal lineages was observed in iPSC differentiation protocols. To enrich for myogenic cells, primary donor satellite cell sorting strategies were employed to improve iPSC-derived cell engraftment.

Conclusions

This project improves use of xenotransplant models for investigating human satellite cell biology and anticipates creating human dystrophic muscle progenitors that can be used to test molecular mechanisms discovered in model organisms, as well as testing therapeutic candidates for DMD.

Lung, vascular and circulatory disorders (9:20 – 10:10)

Moderators: Rafael Soares and Charvi Syal

2-1

Differential effects of female sex hormones on severe pulmonary arterial hypertension induced by VEGFR2 inhibition

Ketul R. Chaudhary[1,2], Yupu Deng[1], Kurt Tyson[1], Anli Yang[1], Katelyne Rowe[1], Duncan J. Stewart[1,2]

1. Ottawa Hospital Research Institute, Ottawa, Ontario, Canada.
2. Department of Medicine, University of Ottawa, Ottawa, Canada.

Introduction: Pulmonary arterial hypertension (PAH) is caused by effective loss of the pulmonary microvasculature and shows strong female sex predominance. Inhibition of vascular endothelial growth factor receptor-2 (VEGFR2) by SU5416 (SU), often combined with 3 weeks of chronic hypoxia (CH), causes lung endothelial cell (EC) apoptosis and severe PAH in rats. We have reported that a specific colony of Sprague Dawley (SD) rats is hyper-responsive (HR) to SU and develop severe PAH with single injection of SU, even in absence of CH. Interestingly, there is a bimodal pattern in HR phenotype (responders and non-responders) with strong sex dependence, but with more males than females developing severe PAH after SU alone. Therefore, the goal of this study was to explore the role of female sex hormones in modifying the HR phenotype in HR SD rats.

Methods and Results: SD rats were injected with SU (20mg/kg, sc) or vehicle and right ventricular systolic pressure (RVSP) was measured after 7 weeks. In absence of CH, 72% male and 27% female rats developed severe PAH in response to SU with a similar increase in RVSP in the responder rats (97±18 mmHg and 101±13 mmHg, respectively). Oophorectomy (OVX) resulted in a marked increase in the frequency of response in female rats to 71% (10 of 14). Moreover, estradiol (0.5mg pellet, sc, 60-day release) replacement completely abrogated the HR phenotype in both male and OVX female rats (0% responders). In contrast, progesterone (150mg pellet, sc, 60-day release) treatment inhibited HR phenotype only in OVX female rats but not in male rats. Increased RVSP in male and OVX female HR rats was accompanied by increased cleaved caspase-3 expression and activity in the lungs that was blocked by treatment with estradiol. Furthermore, cell culture studies demonstrated that estradiol reduced EC apoptosis induced by SU, SU with hypoxia (5% O_2) or serum starvation.

Conclusions: These data support a protective role of female sex hormones in a model of severe PAH. While estrogen prevents PAH in both sexes by promoting lung EC survival, we now show for the first time that progesterone acts by a different mechanism, which is female sex-specific. We propose that the very different estrus cycles may account for the apparent paradox between sex predominance of PAH in rodent models and humans, and periodic withdrawal of the protective effects of female sex hormones may predispose to EC injury and apoptosis in human PAH patients.

2-2

Gene Therapy Rescues Respiratory Distress and Improves Survival in a Mouse Model of Surfactant Protein Deficiency

Martin Kang[1,2], Arul Vadivel[1,2], Liqun Xu[1,2], Claudia Milazzo[1,2], Chanele Cyr-Depauw[1,2], Laura van Lieshout[4], Jakob Domm[4], Sarah Wootton[4], Bernard Thébaud[1,2,3]

[1]Sinclair Center for Regenerative Medicine, Ottawa Hospital Research Institute, Ottawa, ON, Canada
[2]Department of Cellular and Molecular Medicine, University of Ottawa, Ottawa, ON, Canada
[3]Division of Neonatology, Department of Pediatrics, Children's Hospital of Eastern Ontario, Ottawa, ON, Canada
[4]Department of Pathobiology, Ontario Veterinary College, University of Guelph, Guelph, ON, Canada

Background: Surfactant is a mixture of lipids and proteins secreted by alveolar type 2 (AT2) cells on the surface of lungs. Surfactant reduces alveolar surface tension and allows proper gas exchange with the environment. Genetic diseases that affect surfactant production manifest as respiratory failure and are usually lethal. Genetic mutations in the *SFTPB*gene impair production of a crucial protein called Surfactant Protein B (SPB). Inherited in an autosomal recessive pattern, SPB deficiency (OMIM#178640) results in death within months following birth. Currently, no treatment exists besides lung transplantation.

Objective: We examine whether adeno-associated virus (AAV)-mediated gene transfer of SPB can rescue respiratory distress and improve survival in a mouse model of SPB deficiency.

Methods: In mice, mutations in both *Sftpb*gene copies results in respiratory failure immediately after birth.To overcome this rapid lethality,we utilized a transgenic mouse model that conditionally expresses SPB under the control of doxycycline. In the absence of doxycycline, these mice suffer progressive respiratory failure and death occurs within 3-7 days. We generated a novel AAV vector with a preference for lung epithelial and AT2 cells and inserted murine SPB. The transgenic mice were administered our AAV-SPB vector through the trachea. They were maintained on doxycycline for 1 month following AAV injection to ensure maximal SPB expression from the vector. Following 1 month, doxycycline was removed and AT2 cell structure, lung function, and survival were assessed.

Results: Following doxycycline removal, untreated or control mice lose SPB expression, show changes in AT2 cell structure, have impairments in lung function, and suffer lethal respiratory failure. AAV-SPB treated mice demonstrate normal AT2 cell structure, and lung function tests are similar to mice on doxycycline. This improvement in function corresponds with an increase in survival.

Conclusion:AAV-SPB gene therapy represents a promising therapeutic strategy to treat genetic SPB deficiency.

2-3

Microgel encapsulated endothelial progenitor cells provide a portable stem cell niche for treatment of pulmonary arterial hypertension

Nicholas D. Cober[1,2], Ketul R. Chaudhary[1], Yupu Deng[1]; Chyan-Jang Lee[1], Katelynn Rowe[1], Ainara Benavente[3], Michel Godin[3], David W. Courtman[1], Duncan J. Stewart[1,2]

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2. Department of Cellular and Molecular Medicine, University of Ottawa
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Background: Late outgrowth endothelial progenitor cells (L-EPCs) represent a uniform, highly endothelial-like progenitor cell population; however, their therapeutic benefits are limited by poor cell persistence. Microencapsulation provides a portable stem cell niche, that can promote cell survival and retention in models of cardiovascular injury. We hypothesize that microencapsulation will increase survival and retention of L-EPCs in the lungs and result in greater therapeutic benefits compared to non-encapsulated cells in a rat monocrotaline (MCT) model of pulmonary arterial hypertension (PAH).

Methods: L-EPCs were encapsulated by vortex-emulsion and assessed for viability, egress, and capsule size. Encapsulated and non-encapsulated L-EPCs were transduced with luciferase and administered to Sprague Dawley rats three days after MCT injection. L-EPCs were tracked *in vivo* by bioluminescence imaging (BLI) for up to 3 weeks post cell injection. At 3 weeks post cell injection, right ventricular systolic pressure (RVSP) and right ventricular hypertrophy were assessed for therapeutic efficacy. Lung vascular remodelling was assessed following H & E staining of lung sections.

Results: Encapsulation of endothelial cells within microgels improved cell survival in response to serum starvation compared to controls (27% vs 71% reduction in absorbance respectively, $p<0.001$). Immediately after cell injection, no significant difference in BLI signal was observed confirming consistent cell delivery between non-encapsulated and encapsulated L-EPCs. However, at 4 and 24 hours post-injection only encapsulated L-EPCs could be detected by BLI (28±12% and 12±8% of baseline signal, respectively; $p< 0.0001$ and 0.05). Importantly, microencapsulation of L-EPCs led to significant improvements in RVSP 3 weeks after delivery compared to MCT alone (56±24 vs. 80±7 mmHg, respectively; $p<0.05$), whereas no improvement in pulmonary hemodynamics was observed with delivery of non-encapsulated L-EPCs (79±14 mmHg). Additionally, improvements in vascular remodelling were also evident with encapsulated L-EPCs, compared to vehicle or non-encapsulated L-EPCs treated rats.

Conclusions: Microencapsulation can increase retention of L-EPCs within the lungs, resulting in an important therapeutic benefit, not seen with non-encapsulated L-EPCs in the rat MCT model of PAH.

2-4

Patient-Centered Outcomes in the Management of Anemia: A Scoping Review

Phillip Staibano[1,2], **Iris Perelman**[1,2], Julia Lombardi[1,2], Alexandra Davis[3], Alan Tinmouth[1,2,3], Marc Carrier[1,3], Ciara Stevenson[1], Elianna Saidenberg[1,2,3]

1. Clinical Epidemiology, Ottawa Hospital Research Institute
2. Faculty of Medicine, University of Ottawa
3. The Ottawa Hospital

Background: Anemia is a frequently diagnosed condition that may be a symptom or complication of many illnesses affecting patients of all demographics. Anemia can lead to both worsened clinical outcomes and reduced quality of life. Patient-reported outcome measures (PROMs) are methodological tools used to capture the impact of disease on patient wellbeing. Use of PROMs in medical research is becoming more common as it is increasingly recognized that disease outcomes of interest to researchers and clinicians are not always consistent with patients' greatest concerns related to their diseases.

Objective: We conducted a scoping review to characterize the studies that have evaluated patient-centered outcomes (PCOs) using PROMs in patients undergoing treatment for anemia.

Methods: We conducted a search of Medline (Ovid), EMBASE (Ovid), PsychINFO, and CINAHL databases for studies published until January 2017 that investigated an intervention to treat anemia in any patient population and used at least 1 PROM to evaluate PCOs. A descriptive synthesis was performed to characterize the PROMs used and to evaluate the quality of PCO reporting.

Results: Of the 3224 studies identified in the initial search, 130 met all eligibility criteria. We found that the population most frequently studied was oncology patients (46.2% of studies). The therapy for anemia evaluated in the majority of studies was erythropoietin-stimulating agents (77.7% of studies). The most commonly used PROM was the Functional Assessment of Cancer Therapy/Functional Assessment of Chronic Illness Therapy tool (46.9%), and the majority of studies used only 1 PROM tool (53.1%). We found significant variability in the quality of PCO reporting across all included studies.

Conclusions: Improved methodological rigor in the assessment of PCOs in anemia management is needed in future studies.

Perspectives in immune modulation of disease (1:10 – 2:00)

Moderators: Sasha Van Katwyk and Leonard Angka

3-1

FGL2 as a mediator of immunological preeclampsia and inflammatory lesions of the placenta

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2. Department of Cellular and Molecular Medicine, Faculty of Medicine, University of Ottawa
3. Interdisciplinary School of Health Sciences, Faculty of Health Sciences, University of Ottawa

Background: Preeclampsia (PE) is a hypertensive disorder affecting 5-8% of pregnancies, caused by faulty placentation. A significant heterogeneity exists in clinical presentation of the disease, fetal health outcomes and placental histopathology, such that no universal treatment or predictive marker are known. Our laboratory has shown the existence of molecular subtypes of preeclampsia, with distinct, divergent histopathological phenotypes and transcriptional patterns. Crucial to placental development is crosstalk between the fetal trophoblast and the maternal immune system. Fibrinogen-like protein 2 (FGL2) is a known mediator of the balance between type 1 and type 2 immunity and a procoagulant, two features that make it a likely player in the pathology of preeclampsia.

Objective: We aim to determine the mechanisms by which high FGL2 expression contributes to the specific features of immunological preeclampsia.

Methods: We stably overexpressed *FGL2* in invasive human trophoblast cell line HTR-8/SVneo and in syncytium-forming trophoblast cell line BeWo to first characterize the effect of FGL2 excess on trophoblast function. We measured chorionic villus FGL2 expression in a cohort of preeclamptic and healthy pregnancies, and compared FGL2 expression with subtype-specific clinical features and histopathological lesions of the placenta. Differential gene expression analysis was performed to better understand the role of FGL2 in transcriptional programs leading to these lesions.

Results: *FGL2* overexpression did not alter proliferation, invasion or migration of HTR-8/SVneo cells. Preliminarily, *FGL2* overexpression resulted in impaired syncytialization of BeWo cells, demonstrated by inhibited expression of syncytium-specific genes after forskolin treatment. FGL2 was most highly expressed in the cytotrophoblast and syncytiotrophoblast of human chorionic villi, and in villous and perivillous leukocytes. In our previously identified molecular subtypes of preeclampsia, we found *FGL2* to be differentially expressed across subtypes, high in "immunological PE" and low in "canonical PE". We found that across all subtypes, genes whose expression best correlates with that of *FGL2* were predominantly involved in immune response. Furthermore, placentas with higher *FGL2*expression were more likely to be affected by inflammatory lesions such as villitis of unknown etiology, massive perivillous fibrin deposition or chronic deciduitis. Significantly upregulated genes in placentas affected by these lesions, including *FGL2,* demonstrated that macrophage signaling and cytokine secretion is a major factor in these lesions.

Conclusions: Overall, these results suggest that *FGL2* excess in the chorionic villi is a consequence of inflammatory signaling, and that *FGL2* possibly mediates trophoblast-immune cell crosstalk and contributes to fibrin deposition.

3-2

An Interleukin-12-Expressing Oncolytic-Virus Infected Autologous Tumor Cell Vaccine Generates Potent Anti-Tumor Immune Responses

Sarwat T. Khan[1,2], Katherine E. Baxter[1,2], Christiano T. de Souza[2], Curtis McCloskey[1,3], Leonard Angka[1,2] Juliana Nguyen[2], Louis Dacquay[2], Ahwon Jeong [2], Meghan Chapados [2], Bryan Lo [2,4], Barbara Vanderhyden[2,3], Michael A. Kennedy[2], Jean-Simon Diallo[1,2], Rebecca C. Auer?[1,2,5,6]

[1]Department of Biochemistry, Microbiology and Immunology, University of Ottawa; [2]Cancer Therapeutics Program, Ottawa Hospital Research Institute; [3]Department of Cellular and Molecular Medicine, University of Ottawa; [4]Department of Pathology and Laboratory Medicine, University of Ottawa; [5]Department of Surgery, University of Ottawa; [6] Department of Surgery, The Ottawa Hospital

Background: Peritoneal carcinomatosis (PC) is one of the major causes of terminal complications arising from gastrointestinal and ovarian malignancies, and current standard of care does not provide satisfactory outcomes. We have shown that an IL-12-expressing oncolytic virus-infected cell vaccine (MG1-IL12-ICV) can prolong survival in murine models of PC in an NK and $CD8^+$ T-cell dependent manner. However, MG1-IL12-ICV enhances survival but does not provide durable cures in more difficult-to-treat models of established disease suggesting the presence of immunosuppressive mechanisms.

Objectives: To characterize the immune response generated by MG1-IL12-ICV to enable a better understanding of the mechanisms contributing to the efficacy of MG1-Il12-ICV.

Method: Changes in the immune profile of the tumor microenvironment (TME) at several time points following vaccination were assessed using a combination of approaches including flow cytometry, cell-based functional assays, gene expression and immunohistochemistry. In addition to phenotypic and genotypic characterization, functional characterization of T-cells, NK cells and antigen-presenting cells were done.

Results: Analyses of the B16F10 peritoneal TME revealed a significant increase in the number of infiltrating $CD4^+$ and $CD8^+$ T-cells and $CD11c^+$ dendritic cells following MG1-IL12-ICV treatment. Analysis of a subset of immune-related genes also revealed significant upregulation of genes involved in antigen presentation (MHC-I and II) and NK and T-cell chemotaxis in the TME of animals receiving MG1-IL12-ICV. Despite this evidence for an ongoing anti-tumor immune response even at later stages of treatment (day 20), median survival is 25 days. Notably, we did not detect significant changes in regulatory T-cells ($CD4^+$ $Foxp3^+$) present in the TME, and MDSCs ($CD11b^+$ $Gr\text{-}1^{hi}$) were reduced following vaccination. However, by day 20, PD-L1 expression was significantly increased on $CD45^+$ subsets in both the peritoneal cavity and tumors. In addition, significant increases in gene expression of PD-L1, PD-1, CTLA-4 and Lag-3 were observed. Furthermore, the increased innate responses (NK and DCs) present after early doses of ICV were no longer observed after subsequent doses, suggesting a need for optimization of dosing strategy or combination with other immunotherapies.

Conclusion: Overall, this study demonstrates that vaccination with autologous cells infected with MG1-IL12 can induce a favorable antitumor immune microenvironment in tumours of the peritoneal cavity and provides avenues that can be explored to further improve outcomes with MG1-IL12-ICV.

3-3

Liver damage severity is associated with sustained dysfunction of circulating CD8+ T-cells following chronic HCV infection cure

Agatha Vranjkovic[1], Felicia Deonarine[1,2], **Shaima Kaka[2]**, Cooper L. Curtis[1,2,3], Angela M. Crawley[1, 2, 4, 5]

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4. Clinical Epidemiology Program, Ottawa Hospital Research Institute
5. Department of Biology, Carleton University

Background

Chronic hepatitis C virus (HCV) infection remains a significant health burden, affecting over 70 million people worldwide – many of whom will silently develop advanced liver fibrosis, resulting in symptomatic liver disease with a high mortality rate and predisposition to end-stage liver disease. Despite the highly successful cure rates of modern direct acting antiviral (DAA) therapies, chronic HCV infection continues to be of concern to the medical community due to treatment failures, relapses and long-lasting associated liver damage. Further, while chronic HCV infection has been shown to disrupt immune functions, it is not yet known whether DAA therapy is sufficient to reverse immune dysfunction.

Objective

We and others have reported generalized dysfunction of important immune response mediators known as cytotoxic CD8+ T-cells in chronic HCV infection. Our research suggests that the degree of liver damage might play a role in CD8+ T-cell dysfunction. We aimed to determine whether liver disease severity is associated with generalized dysfunction of CD8+ T-cells in chronic HCV infection.

Methods

We assessed the distribution of circulating CD8+ T-cell subsets in the blood of healthy controls as well as chronic HCV patients with advanced and minimal fibrosis before and after DAA therapy. We also examined the function of these CD8+ T-cells through the measurement of cytokine production and degranulation markers. In addition, we performed preliminary assessments of CD8+ T-cell proliferation and cytotoxicity through a mixed lymphocyte reaction assay.

Results

We observed shifts in CD8+ T-cell subset distribution in HCV-infected individuals with advanced fibrosis compared to minimal fibrosis or uninfected controls, and this remained unchanged after viral cure. The exaggerated CD8+ T-cell activities in advanced fibrosis were not resolved with DAA therapy. Preliminary assessments further show that CD8+ T-cells from patients following DAA therapy undergo more frequent divisions than at baseline for those with minimal but not advanced liver fibrosis. Preliminary data also show sustained cytotoxic hyperactivity of CD8+ T-cells in advanced liver fibrosis following HCV cure.

Conclusions

We have demonstrated for the first time that the severity of liver fibrosis is associated with a generalized immune dysfunction that is sustained following HCV cure. Understanding clinically relevant immunological changes associated with advanced liver fibrosis in chronic HCV infection is imperative to overcoming the adverse clinical outcomes that are experienced by HCV+ patients receiving DAA therapy.

3-4

HIV-infected macrophages are selectively infected and killed by the oncolytic Rhabdovirus, MG1; a potential tool for viral eradication

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5. Division of Infectious Diseases, Ottawa Hospital-General Campus, Ottawa, ON, Canada

Background: Impairment of the type 1 interferon (IFN1) response, which has recently been observed in latently HIV-infected cells, has the potential to be exploited for therapeutic benefit. This is supported by findings that demonstrate cancer cells with defective IFN1 signalling to be selectively killed by the oncolytic virus (OV) MG1, a recombinant Maraba virus. MG1 may therefore be a useful strategy for the eradication of HIV-infected macrophages, an important cellular reservoir for HIV *in vivo*.

Hypothesis: IFN1 responses are impaired within HIV-infected macrophages and serve as a target for MG1-mediated infection and killing.

Methods: IFN1 responsiveness was first measured by flow cytometry in monocyte-derived macrophages (MDM) infected with the reporter virus, HIV NL4-3 BAL-IRES-HSA. MDM were then stimulated with IFN-alpha and the induction of IFN-stimulated genes (ISG), PKR and ISG15, was measured by qPCR and flow cytometry. To assess OV-mediated killing, Annexin-V staining and Caspase 3/7 activation were measured in MDM by flow cytometry at 24h post-MG1 infection. To confirm MG1-mediated killing, proviral HIV DNA was measured by PCR in HIV-infected MDM cultures, as well as in alveolar macrophages (AM) isolated by bronchoalveolar lavage from antiretroviral therapy (ART) treated HIV-infected individuals.

Results: Induction of PKR and ISG15 was lower in HIV-infected (HSA^+) MDM than in uninfected (HSA^-) cells following IFN-alpha stimulation. Annexin-V staining and Caspase 3/7 activation were significantly greater in HSA^+ MDM than HSA^- MDM at 24h post-MG1 infection. This was paralleled by a decrease in proviral HIV DNA in both MDM and AM cultures, which was not observed when cells were exposed to UV-inactivated MG1. Finally, OV-mediated infection and killing could be reversed by treating MDM with increasing doses of IFN-alpha prior to MG1 infection.

Conclusions: Altered IFN1 signalling in HIV-infected MDM is associated with preferential infection and killing by replication-competent, but not UV-inactivated, MG1. This was confirmed *ex vivo* using AM from ART treated HIV-infected individuals. These findings complement recent observations in latently-infected $CD4^+$ T cells and support MG1 as a novel approach to eradicate the macrophage-based HIV reservoir. As MG1 is currently being used in cancer clinical trials, clinical trials in HIV-infected individuals may be warranted.

Neurodegeneration and biology of the nervous system (4:00 – 4:50)

Moderators: Ahmad Galuta and Bojan Shutinoski

4-1

Dementia Population Risk Tool (DemPoRT): Predictive Algorithm for Assessing Dementia Risk in the Community Setting

Stacey Fisher[1-3], Amy Hsu[1,2], Monica Taljaard[1,3], Doug Manuel[1-5], Peter Tanuseputro[1-3,6-7]

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2. Primary Care and Population Health Research Program, ICES, Ottawa, Ontario
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7. Bruyère Research Institute, Ottawa, Ontario

Background: The burden of disease from dementia is a growing global concern as incidence increases exponentially with age and average life expectancy has been increasing around the world. Planning for an aging population requires reliable projections of future dementia prevalence and resource requirements, however, existing population projections are simple and have poor predictive accuracy.

Objective: To derive and validate the Dementia Population Risk Tool (DemPoRT) to predict dementia incidence in the population setting using multivariable modeling techniques.

Methods: DemPoRT was developed using elderly Ontario respondents of the Canadian Community Health Surveys, representing 98% of the Ontario population (survey years 2001 to 2007, follow-up from 2001 to 2017; 20 506 males and 27 233 females). Incident dementia was identified through individual linkage of survey respondents to population-level administrative health care databases. Using time of first dementia capture as the primary outcome and death as a competing risk, sex-specific proportional hazards regression models were estimated. The pre-specified model predictors capture information on socio-demographic characteristics, general and chronic health conditions, health behaviors and physical function.

Results: There were 1 059 and 2 071 cases of incident dementia, and 120 280 and 171 574 person-years of follow-up, for males and females, respectively. The DemPoRT algorithm contains 29 variables (65 degrees of freedom), is discriminating (C-statistic: males 0.82 (95% CI: 0.80, 0.83); females 0.82 (95% CI: 0.81, 0.83)) and is well-calibrated in a wide range of policy-relevant subgroups including behavioral risk exposure categories, sociodemographic groups, stroke, diabetes and hypertension status.

Conclusions: Health system planning in anticipation of growing dementia prevalence requires reliable projection estimates. DemPoRT is the first and most comprehensive population-based algorithm for predicting dementia incidence, with the potential to improve the ability to answer key policy questions with respect to the future burden of dementia in Canada. Study registration: ClinicalTrials.gov, no. NCT03155815.

4-2

The Parkin protein: From its Function in the Brain to the Characterization of a New Mouse Model

Daniel N. El Kodsi[1,2], Jacqueline M. Tokarew[1,2], Nathalie A. Lengacher[2], Andy Ng[2], Angela P. Nguyen[2], Jasmine M. Khan[2], Lawrence Puente[3], Julianna J. Tomlinson[2], Michael G. Schlossmacher[2]

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Background: Recessively inherited loss-of-function mutations in the *PARK2*gene cause early-onset Parkinson disease. The disease-relevant mechanisms by which Parkin is neuroprotective remain elusive. Furthermore, the lack of symptomatic mouse models for parkin deficiency has impeded progress in PD research and therapeutic advancement. Our studies build on work from our team that Parkin protects against oxidative stress and are informed by what is known about Parkin biochemistry and its age-dependent oxidation seen in human brain.

Objectives: We hypothesize that Parkin neutralizes reactive oxygen species (ROS) produced by mitochondria, thus conferring a previously reported "pro-mitochondrial benefit" (LaVoie et al., 2007). We posit that this is mediated by its abundant redox-sensitive thiol-containing residues. Beyond understanding the mechanisms by which Parkin acts as a redox-stabilizing molecule, our goal was to apply this functional insight to the creation of a new Parkin-linked mouse model that we predict will develop a PD-like phenotype.

Methods: We tested this hypothesis using *in vitro* paradigms, cellular and genetic mouse models and human brain tissue, coupled with assays to monitor ROS levels and markers of oxidative stress, as well as mass spectrometry analysis of Parkin from murine brains post oxidation.

Results: We have modeled the oxidation of Parkin that is observed in human brain using recombinant Parkin proteins and cellular models. Parkin oxidation is reversible, occurs through a direct interaction with oxidants and is dependent on its thiol-containing cysteines, which are oxidized in murine brains post MPTP treatment as detected by mass spectrometry. Upon oxidation, Parkin forms high molecular weight species, which affects its solubility and modulates its E3 ligase activity. These changes confer protection in that cells overexpressing wild-type human Parkin show lower ROS levels as assayed by measurements of H_2O_2. This insight led us to establish an oxidative stress marker phenotype, as previously described by Palacino et al. (2004), which demonstrated significantly increased oxidative damage in lysates of murine brain and heart that lack Parkin.

Conclusion: Parkin protects high energy-dependent organs against oxidative stress-induced damage. This effect is mediated through the oxidation of its thiol groups. Our findings suggest a related function for Parkin in human brain. The creation and characterization of a novel mouse model of recessive parkinsonism, by combining parkin deficiency with sod2 haploinsufficiency, promises to allow for the development of better neuroprotective therapies.

This Work is supported by the Michael J. Fox Foundation.

4-3

The adenylate forming domain protein DIP-2 is an intrinsic repressor of neurite outgrowth and axon regeneration in C. elegans

Nathaniel Noblett[1,2], Zilu Wu[3], Zhao Hua Ding[1,2], Seungmee Park[3], Tony Roenspies[1], Andrew Chisholm[3], Yishi Jin[3] and Antonio Colavita[1,2]

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2. Department of Cellular and Molecular Medicine, University of Ottawa
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Background: Mechanisms that inhibit inappropriate neurite outgrowth in the mature nervous system are likely to play important roles in maintaining normal neuronal function. Many of these mechanisms are being tested as targets for axon regeneration in treating spinal cord injuries. A genetic screen for ectopic neurites in a subset of *C. elegans*neurons identified mutations in a member of the *Disco interacting protein-2* (*dip-2*) family (Carr et al., Plos One, 2016). Subsequent studies showed that *dip-2* is required to establish and maintain neuronal morphology in many neuron types.

Objective: Our objective was to validate the role that DIP-2 plays in maintaining neuron morphology and explore whether the protein was also important in axon regeneration.

Methods: Visualization and scoring of the ectopic neurites were done using *in vivo* florescence and standard genetic methods. Axon regeneration was assessed via laser axotomy (in collaboration with the Jin lab (UC-San Diego)).

Results: We found that *dip-2* mutants display an age-dependent increase in neuronal morphology defects, particularly and increase in ectopic neurite sprouting. Overexpression of DIP-2 suppresses the normal progressive neuronal sprouting observed in aging *C. elegans*. Temporal control of DIP-2 expression shows that DIP-2 is required post-developmentally to maintain neuronal morphology. A CRISPR/Cas9-mediated knock-in of GFP at the endogenous *dip-2* gene revealed expression in many neurons as well as epidermal cells. Cell specific rescue experiments are consistent with DIP-2 acting in a cell autonomous manner to regulate neuronal morphology. In addition to its role in maintaining neuronal morphology by inhibiting ectopic neurite formation, laser axotomy experiments revealed that DIP-2 also acts as an intrinsic repressor of axon regeneration.

Conclusion: Our findings reveal DIP2 family members as potentially new regulators of neuronal morphology maintenance and axon regrowth after injury.

4-4

Targeting noradrenaline for treatment of anxiety and depression phenotypes in a fluoxetine-resistant mouse model of impaired serotonergic activity

Faranak Vahid-Ansari1, Amin Zahrai1, Mireille Daigle1, Paul R. Albert[1]

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Introduction: Reduced activity of the serotonin (5-HT) system is implicated in major depression, which is treated using SSRI antidepressants like fluoxetine (FLX) that boost 5-HT. However, SSRIs mediate remission in only 30% of depressed patients. This resistance may be due to increased levels of 5-HT1A autoreceptors, which negatively regulate 5-HT tone. To model this, we generated the cF1ko mice, in which the 5-HT1A repressor Freud-1 was deleted in adult 5-HT cells (Vahid-Ansari et al., 2017). The cF1ko mice have increased 5-HT1A autoreceptor levels and function, and an FLX-resistant anxiety/depression phenotype.

Objectives: Here, we address whether cF1ko mice respond to chronic DES (desipramine), a tricyclic antidepressant that targets the noradrenaline system and whether DES exerts a different activity pattern compared to chronic FLX treatment.

Methods: The cF1ko mice were treated with DES for >3 wks and examined using validated tests for anxiety (elevated-plus maze, open-field, novelty-suppressed feeding) and depression (forced-swim and tail-suspension) like behaviour. Co-immunofluorescence for chronic activity marker FosB and GAD67, vGluT1-3, TPH/TH was done to detect brain-wide chronic activity of GABAergic, glutamatergic or serotonergic/adrenergic neurons, respectively in FLX-treated vs DES-treated brains.

Results: Unlike FLX, chronic DES treatment promoted recovery from depression and anxiety phenotypes in the cF1ko mice, bypassing the 5-HT system. DES treatment of cF1ko mice activated TH+ cells in noradrenergic locus coeruleus, and significant increases in the activity of GABA and glutamate cells were observed in key areas implicated in anxiety and depression (basolateral amygdala and pre-limbic prefrontal cortex). In contrast, chronic FLX treatment of cF1ko mice reduced the activity of GABA and glutamate cells in the basolateral amygdala and cingulate cortex. while increasing the activity of 5-HT cells dorsal raphe and cells of the entorhinal cortex. Chronic FLX and DES treatments induced similar changes in hippocampal activity.

Conclusion: These findings using the cF1ko mice implicate dys-regulation 5-HT1A autoreceptors in resistance to SSRI treatment. The responsiveness of these mice to DES indicates that SSRI-resistant depression may still response to antidepressants that target other monoamines like noradrenaline rather than 5-HT-targeted treatments. The differential activity changes seen with effective (DES) vs. ineffective (FLX) treatments may suggest a key role for activation of the amygdala and prefrontal cortex in the treatment of anxiety and depression. Future brain stimulation studies can test this hypothesis directly. Supported by CIHR grant.

TRANSFUSION MEDICINE

Immunoglobulin Replacement Therapy: More than Just Restoring Antibodies?

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2. Clinical Epidemiology Program, Ottawa Hospital Research Institute

Background
Hypogammaglobulinemia or low serum immunoglobulin G (IgG) levels either inherited (primary) or acquired (secondary) is associated with increased infection rates. Immunoglobulin replacement therapy (IRT) reduces infection by replenishing the quantitative IgG. However, Ig treatment is also used in many autoimmune diseases. The mechanism of action is thought to be Ig mediated immunomodulation. Innate immune cells have shown to be involved in such mechanism. Weather IRT modulate adaptive immune cells in patients with hypogammaglobulinemia is not known.

Objectives
To determine if IRT has an immunomodulatory effect on cell-mediated immunity and enhances CD4/CD8 T-cell proliferation and function in patients with primary or secondary hypogammaglobulinemia. This study is expected to reveal key changes in T-cell function following IRT that may elucidate our understanding of Ig mediated immunomodulation.

Methods
Forty patients with primary or secondary hypogammaglobulinemia are recruited from the Immunodeficiency Clinic at the Ottawa Hospital General Campus. Blood is drawn twice for peripheral blood mononuclear cells (PBMCs) isolation, before starting IRT and after the patient has been on IRT for at least 8 weeks. CD4 and CD8 T-cell counts will be analyzed by flow cytometry in addition to other cell markers differentiating T-cell state and Treg populations. Cytokine production is measured by ELISA from cultured PBMC supernatant and intracellular cytokine staining. Data regarding IgG level, number and type of infections after receiving IRT is also collected.

Results
IVIG therapy was found to increase CD4 T-cell count and improve CD4/CD8 T-cell ratio in patients with primary but not secondary hypogammaglobulinemia. Treg populations and T-cell activation state do not change significantly post-IRT. CD4+ and CD8+ T-cells in secondary immunodeficiency patients showed significantly higher expression of intracellular IFNy post-CD3/CD28 stimulation (p-value = 0.007) but no change in IL-10 and IL-17 cytokine production in cultured PBMC supernatant.

Conclusions
Our results suggest that IVIG therapy can alter CD4 and CD8 T-cell numbers and function in patients with primary or secondary hypogammaglobulinemia in addition to replenishing IgG level. Experiments assessing cell proliferation and cytotoxicity of T-cells will be conducted to further analyze T-cell subset function. These data are expected to reveal alternate immunomodulatory strategies that may be exploited in the treatment of hypogammaglobulinemia to improve B and T-cell responses, with the possibility of the use of IRT to treat other conditions.

TRANSFUSION MEDICINE

Outcomes of an ED Anemia Management Protocol at The Ottawa Hospital

Anthony Grieco[1],James Worrall[2], Elizabeth Chatelain[3], Malia Murphy[3], Li Liu[3], Guy Hebert[2], Akshai Iyengar[1], Elianna Saidenberg[1], Alan Tinmouth[1]

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2. Department of Emergency Medicine, The Ottawa Hospital, Ottawa, Canada
3. Ottawa Hospital Research Institute, The Ottawa Hospital, Ottawa, Canada

INTRODUCTION: Although patients commonly present to the emergency department (ED) with symptomatic anemia or on the advice of a health-care provider following critical laboratory results, there are currently no guidelines for ED management of anemia. This often results in inappropriate RBC transfusions when alternative therapies, such as intravenous (IV) iron, may be more appropriate.

Given these concerns, an anemia algorithm was introduced at The Ottawa Hospital ED in January 2017, which recommends therapies based on symptoms, anemia severity, and evidence of iron deficiency. Patients may also be referred to the Division of Hematology anemia rapid referral clinic (RRC) for urgent follow-up.

OBJECTIVE: To describe the demographics, clinical findings, and treatment outcomes of patients presenting to the ED with anemia following anemia algorithm implementation.

METHODS: Data was collected from patient charts between January 1 and December 31 2017.

RESULTS: A total of 83 patients were referred to the RRC from the ED.

The median age was 68 years. The median hemoglobin was 73 g/L (range 42 – 101 g/L). The median MCV was 72 fL; 69 % had a MCV less than 80 fL. The median ferritin was 6 mcg/L; 81 % had a ferritin less than 30 mcg/L.

39 patients (47%) were transfused RBCs; 24 received 1 unit, 14 received 2 units, and 1 received 3 units.

55 patients (66%) received any iron therapy, 80 % of which received IV formulation. Of those given IV iron, 82 % had a MCV and ferritin less than 80 fL and 30 mcg/L, respectively.

CONCLUSIONS: Iron deficiency was the most common etiology of anemia identified. Following ED anemia algorithm implementation, more patients were treated with iron than RBC transfusion. In transfused patients, the majority received one unit of blood. These results are congruent with current recommendations from the American Association of Blood Banks and Transfusion Medicine Choosing Wisely Canada, thus indicating that patients are being treated with evidence-based therapy.

FUTURE DIRECTIONS: Confirmation of minimization of ED transfusion using The Ottawa Hospital Data Warehouse is required to prove our hypotheses. Continuous quality improvement is also ongoing, and protocol modifications will be made to ensure adherence with evidence-based management.

DISCLOSURE Nothing to disclose.

TRANSFUSION MEDICINE

Management of Women and Girls with Bleeding Disorders: Review of In- Training Objectives of RCPSC Accredited Training Programs

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Background:

Bleeding disorders can often present either non-specifically or asymptomatically, representing a diagnostic challenge for clinicians. If undiagnosed, these disorders can lead to significant morbidity and mortality for women of all ages. Therefore, it is critical that students receive the appropriate education in this topic, in order to take a careful and detailed medical history and workup to arrive at the appropriate diagnosis.

Objective:

The objective of this project is to identify what core competencies experts in the field of women and girls with bleeding disorders, including hematologists and obstetricians/gynecologists identify as essential. With these core competencies, the objectives of RCPSC accredited training programs will be analyzed to assess which objectives are being achieved, and which objectives needed to be added, to ensure the training of competent physicians in the field of women and girls with bleeding disorders.

Methods

Using a modified Delphi method, we will gather experts from various fields, including hematology, obstetrics, and gynecology, to create a set of core competencies associated with management of women and girls with bleeding disorders. We will obtain objectives from associated RCPSC accredited training programs across Canada, and compare these objectives to the core competencies to assess the education provided in this field. Two reviewers will assess each individual program, with a third to resolve any disputes.

Results

We are currently in the process of analyzing data for this study.

Conclusions

We aim to assess programs to determine how many of the core competencies each one fulfills. We also aim to establish the most commonly and the least commonly addressed core competencies, as well as any commonly addressed topics that fall outside of the core competencies.

TRANSFUSION MEDICINE

Questioning the Therapeutic benefit of Screening for Sickled Hemoglobin in Donated Red Cell Units prior to Exchanges for Sickle Cell Patients

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Sickle cell disease is a genetic disorder resulting from a mutation in the hemoglobin beta chain, which frequently leads to systemic morbidities. To avoid such complications, individuals afflicted with sickle cell disease often require red blood cell (RBC) exchanges. At The Ottawa Hospital, approximately 300 RBC exchanges are administered in a 12 month period. In order to maximize resource efficiency while maintaining patient safety, effective blood product screening protocol must be established. Recently, the importance of screening for the presence of sickle hemoglobin (HbS) in donated RBC units has come into question. This test assures that RBC units used in exchange do not contain any HbS, and therefore theoretically increases the therapeutic benefits of RBC exchanges.

Our objective was to establish if there is a therapeutic benefit of screening for HbS at The Ottawa Hospital prior to RBC exchanges.

Medical records of all patients receiving RBC exchanges from January 2017 to January 2018 were reviewed. During the first six months of this 12 month period, screening for HbS was utilized, while during the last six months, screening was stopped. Pre and post-exchange percentage HbS were calculated for all exchanges done in each of the six month periods. The results were compared for the 293 exchanges completed to determine the importance and benefit of HbS screening.

From January 2017 to January 2018, 36 patients received a total of 293 red cell exchanges. There was no statistically significant difference of percentage change in HbS after screening was stopped.

Although further monitoring should be completed, it has been preliminarily established there is no appreciable benefit for screening for HbS in RBC donations to be utilized for RBC exchanges.

NEUROIMMUNOLOGY

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

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Background: The interest in Leucine-Rich Repeat Kinase-2 (LRRK2) inhibition as a therapeutic agent for Parkinson disease (PD) has been growing steadily. Research in several labs including ours has demonstrated a role for LRRK2 in the innate immune system (Hakimi et al., 2011). The common PD-linked LRRK2 mutation, G2019S, which is associated with increased kinase activity of the protein, confers a protective phenotype against bacterial pathogens in mice (Shutinoski et al., under review). Because kinase function is a major role of LRRK2, inhibiting this activity chronically may put older patients including those with PD at increased risk of virulent infections.

Objectives: We hypothesize that LRRK2 kinase activity is required for its function in the innate immune system and that genetic or pharmacological inhibition of its kinase function will ablate the protective effect of the LRRK2 protein in response to an infection.

Methods: To examine the immunological effects of LRRK2 kinase activity, we used ex vivo as well as in vivo infection models. In the latter, we employed a peripheral (systemic) as well a brain-restricted inoculation paradigm with reovirus-type-3 Dearing (which has tropism for respiratory epithelial cells, intestinal epithelia as well as neurons), and used genetic as well as pharmacological methods to inhibit murine Lrrk2 kinase function.

Results: Using the selective LRRK2 kinase inhibitor, MLi-2 on wild-type bone-marrow derived macrophages, we found no significant difference in cell viability or viral titres after reovirus infection between treated and untreated cells. After intranasal inoculation with reovirus-T3D, we demonstrated that homozygous D1994S kinase-dead mice showed increased survival from encephalitis compared to wild-type and heterozygous mice. Intriguingly, the mortality rate of heterozygous males was identical to that of wild-type animals, whereas heterozygous females behaved similar to homozygous D1994S mice. These findings suggested a sex-difference, which was also seen in the comparison of wild-type vs. *Lrrk2* knock-out mice.

Conclusion: The loss of Lrrk2 kinase activity, as demonstrated genetically in vivo and pharmacologically ex vivo, did not confer an increased risk to a dsRNA virus infection. Moreover, our *in vivo* data suggest that the loss of Lrrk2 kinase function provides relative protection against encephalitis due to reovirus-T3D. Our results will inform the design of preclinical as well as clinical studies for the use of LRRK2 kinase inhibitors as a therapeutic treatment for PD.

NEUROIMMUNOLOGY

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NEUROIMMUNOLOGY

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Microparticles in Hypertension in Pregnancy

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Preeclampsia is a major cause of maternal and fetal morbidity and mortality during pregnancy. Women with preeclampsia are at increased risk of future health complications including renal failure, cardiovascular disease and thrombocytopenia. Alternations to the maternal vasculature are central to the pathogenesis of preeclampsia; however strategies for identifying those at risk of developing preeclampsia are lacking. Microparticles (MPs) are 0.1-1.0 µm vesicles shed from the surface of cell membranes under conditions of stress. Increases in levels of circulating MPs are predictive of cardiovascular morbidity in patients with coronary artery disease or chronic kidney disease. The purpose of this study was to determine whether levels of circulating MPs are increased in those individuals who develop hypertension in pregnancy. We analyzed levels of circulating endothelial and total MPs of women at high risk of hypertension in pregnancy. High risk was defined as at least two of (a) abnormal uterine artery Doppler; (b) abnormal placental biochemistry; (c) abnormal clinical risk factor score; and (d) abnormal placental shape or texture. Plasma samples were collected at 22-26 weeks gestation and prior to any evidence of hypertension in pregnancy. Levels of MPs were assessed by flow cytometry and MPs were identified as 100-1000nm particles with positive staining for Annexin V. Endothelial MPs were further identified by positive staining for CD144. Levels of endothelial and total MPs were increased in those individuals who developed hypertension in pregnancy ($P<0.05$ and $P<0.0001$ respectively). High levels of total MPs (above median value) was associated with earlier delivery (~15 days, $P<0.05$). Taken together these results suggest that circulating MPs may be novel biomarkers that predict risk of hypertension in pregnancy.

Investigating the role of Sox10 as a potential immune modulator

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Introduction: Checkpoint blockade therapy is the most common form of immunotherapy to treat Melanoma patients. Checkpoint blockade activates the immune system by blocking the interaction of inhibitory receptors on cytotoxic lymphocytes. Although these therapies currently exist it is still not fully understood what in the tumor promotes the production of these receptors/ligands.

Objective: Sox10 is a melanoma marker which becomes upregulated as the tumor progresses. We believe that Sox10 is promoting an immunosuppressive phenotype through the upregulation of certain inhibitory receptors against cytotoxic lymphocytes. Therefore we would expect Sox10 to promote tumor growth through the activation of certain transmembrane proteins.

Methods/Results: Melanoma lines were tested for Sox10 expression by western and qPCR. Melanoma cell lines endogenously expressing or lacking Sox10 were injected into C57 mice to observe tumor growth (monitored every 3 days until endpoint, $1cm^3$). What we noticed is that although Sox10 positive lines grow slower *in vitro* they grow faster *in vivo* than the cells not containing Sox10. Further testing revealed a cytotoxic inhibitory protein; Ceacam1 was only expressed in lines containing Sox10 expression. This was confirmed through retroviral overexpression of Sox10 and siRNA knockdown of Sox10. Following this we identified a region on the Ceacam1 promoter that may be a target for Sox10 binding through luciferase assay.

Conclusion: This data suggests Sox10 may play a role in immunosuppression, via the upregulation of Ceacam1 and other immunomodulatory proteins. Further testing is required to determine whether or not Sox10 promotes an immunosuppressive phenotype *in vivo* or whether different factors may be at play.

Loss of the Ste20-like kinase in HER2/Neu-induced mammary tumors accelerates initiation through the AKT-dependent induction of Sox10.

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Background: HER2/Neu is an epidermal growth factor receptor that is overexpressed in approximately 30% of human breast cancers. HER2 expression has been associated with highly aggressive and metastatic breast cancers. Herceptin in combination with chemotherapy is currently the best form of treatment for HER2-positive breast cancers, however, approximately 70% of patients develop Herceptin resistance. Therefore, identifying novel downstream effectors of HER2-mediated tumorigenesis is a critical step in the development of novel therapeutics.

Objective: We have previously shown that the Ste20-like kinase, SLK is activated downstream of HER2 and is required in part for its pro-migratory effects. We propose to further investigate the role of the Ste20-like kinase, SLK in the development and progression of Neu-induced mammary tumor growth.

Methods and Results: Deletion of SLK in an *in vivo* Neu-induced mouse mammary tumor model results in a faster tumor onset and a quicker endpoint. Hyperplastic lesions within the SLK-knockout mammary glands are significantly larger at 16 weeks of age. Using an *in vitro* SLK-knockout Neu-positive mammary tumor cell line, we have identified *Sox10* as one of the most significantly induced genes following SLK-deletion. Sox10 has been shown to be a regulator of mammary stem/progenitor activity and a marker of the Triple-negative breast cancer subtype. Sox10 levels were significantly increased in hyperplastic lesions of SLK-knockout mammary glands. SLK deletion results in an increased mammosphere forming efficiency *in vitro*. Induction of *Sox10* is dependent on PI3K/AKT signaling downstream of SLK-deletion. AKT directly phosphorylates and activates Sox9 which is sufficient for the induction of *Sox10*. Pharmacological inhibition of AKT, using MK-2206, is sufficient to reduce Sox9 activity, *Sox10* expression and mammosphere forming efficiency in SLK-null mammary tumor cell lines. Additionally, we have shown that Sox9 activity and Sox10 levels are positively correlated in human breast cancer samples.

Conclusions: Deletion of SLK significantly increases the activity of both AKT and Sox9. This novel AKT/Sox9 pathway is sufficient to induce *Sox10* expression. The induction of*Sox10* is sufficient to drive an increase in mammary stem/progenitor cell activity and drive tumor growth both *in vitro* and *in vivo*. Inhibition of AKT is sufficient to reduce the activity of Sox9 and downregulate Sox10. Therefore, we predict that combination therapy of HER2-positive tumors with both Herceptin and MK-2206 may result in increased clinical efficacy by simultaneously repressing both SLK- and Sox10-dependent signaling pathways.

Can an Arginine Enriched Diet Prevent Surgery Induced Metastases?

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Background: Surgical resection of primary tumours for curative intent, while often necessary, is also associated with an increase in metastatic disease recurrence. The intense stress that ensues following surgery leads to a rapid drop in plasma arginine - an amino acid important for immune cell function. We have previously shown that NK cells are severely suppressed following surgery. Therefore, we hypothesize that supplementing arginine perioperatively will improve immune cell function thus reduce postoperative metastases.

Objective: Investigate the beneficial role of Arginine in the perioperative period.

Methods: To assess the role of Arginine perioperatively, we fed C57Bl6 mice either an arginine-enhriched diet or a control diet for 14 days before I.V. B16F10lacZ injection and surgery (laparotomy and nephrectomy). Lung metastases were quantified 3 days after surgery. Blood amino acid levels were measured by liquid chromatography tandem mass spectrometry. Immunophenotyping was performed by flow cytometry. We also treated mice with an *in-vivo* Arginase-1 inhibitor to determine whether Arginase-1 was responsible for the drop in arginine after surgery.

Results: The arginine-enriched diet fed mice had a 1.7 fold increase in blood arginine compared to control diet mice (161.2µM vs 278.8µM, ****p<0.0001). Following surgery there was a significant reduction in postoperative B16F10lacZ lung metastases (135 vs 41 metastases in control vs arginine diet fed mice following surgery, n=14/group; ***p<0.0003). Interestingly, increased pre-operative arginine did not have an effect on post-operative NK cell dysfunction. To understand the cause of Arginine depletion following surgery we assessed the expression of Arginase-1 (a major arginine metabolizing enzyme) in myeloid cells after surgery. Surgical stress led to a >2-fold increase in polymorphonuclear myeloid derived suppressor cells (CD11b+Ly6CdimLy6Ghi) which had increased expression (2.36 fold, **p<0.01) and enzymatic activity (2.54 fold, *p=0.013) of Arginase-1. Treating mice with an inhibitor of Arginase-1 prevented the post-operative drop in arginine and also increased basal arginine levels higher than that attainable by an Arginine-enriched diet alone. Unexpectedly, the Arginase-1 inhibitor could not protect mice from post-operative lung metastases despite the high preoperative arginine levels (599µM). These results indicate that increased perioperative arginine, in the absence of Arginase-1 activity, cannot protect against lung metastases.

Conclusion: Perioperative arginine supplementation has been used pre-clinically and clinically to reduce post-operative infections and improve patient recovery. The results from this murine study reveal an underappreciated role for Arginase-1 and its downstream metabolites in regulating the metastatic cascade after surgery.

The Exosome-Mediated Autocrine and Paracrine Role of Plasma Gelsolin (pGSN) in Ovarian Cancer Chemoresistance

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Background: Ovarian Cancer (OVCA) is the most lethal gynecological cancer, due predominantly to late diagnosis, recurrence and chemoresistance. Although combined surgical debulking and chemotherapy is an important treatment strategy, chemoresistance remains a major challenge to successful long term therapeutic success. OVCA is considered to have a cold tumor microenvironment (TME) hence patients are unresponsive to immunotherapy although melanoma and lung cancer patients respond well. There is thus an urgent need for alternative immunotherapies that target novel pathways responsible for the coldness of OVCA. Plasma gelsolin (pGSN), a soluble protein which is also carried by exosomes; however, its role in chemoresistance is yet to be explored. We have demonstrated previously that elevated circulatory levels of pGSN in head-and-neck and oral squamous carcinoma cell patients are significantly associated with chemoresistance and tumour recurrence respectively. These findings led us to hypothesize that exosomal pGSN contributes to OVCA coldness by regulating OVCA responsiveness to CDDP and T cell modulation.

Objective: To determine if and how OVCA cell-immune cell interactions regulate chemosensitivity and how deregulation of these interactions modulate the tumour microenvironment (TME) as well as tumor chemosensitivity.

Methods: Western blot (WB) and ELISA were used to assess the protein contents. Cell death was examined morphologically by Hoechst nuclear staining. Exosomes were isolated by ultra-centrifugation and characterized by nanoparticle tracking analysis, transmission electron microscopy and WB. IF was used to examine co-localization of pGSN and tumour infiltrated lymphocytes (TILs) and data analyzed by Visiomorph.

Results: Exosomal pGSN, up-regulates HIF-1α–mediated pGSN expression in chemoresistant OVCA cells in an autocrine manner and confers cisplatin resistance in otherwise chemosensitive OVCA cells. Immunolocalization studies on 213 high grade serious ovarian tumors indicated co-localization of pGSN with CD8+ T cells and that the overexpression of pGSN, hinders the prognostic impact of infiltrated CD8+ T cells; this correlated with poor overall survival (OS) and shorter disease free survival (DFS). We observed that increased pGSN levels positively correlate with activated caspase-3+ TILs; patients with high activated caspase-3+TILs have poorer OS and shorter DFS. Exogenous pGSN reduces FLIP content, activates caspase-8 and caspase-3 in cytotoxic lymphocytes without affecting FADD content in vitro suggesting that pGSN induces T cell death via caspase-8 activation.

Conclusion: We have demonstrated for the first time that pGSN secretion is involved in exosome-mediated signaling in the OVCA microenvironment, resulting in TILs apoptosis and chemoresistance. This presents pGSN as new target for immunomodulation in chemoresistant OVCA patients.

Contributors to the pain experience: A comparison between women with and without endometriosis

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Background: Chronic pelvic pain (CPP) in women remains a neglected reproductive health morbidity, and one of the leading presenting complaints in a gynecology clinic in Canada. The interplay between physical, psychological and social factors in conjunction with multiple possible etiologies for CPP encourages a holistic care approach to these patients. Endometriosis is one the of most common causes of CPP, yet little is understood as to why some women experience pain whereas others remain asymptomatic. Contributors to the pain experience in vulnerable women need to be elucidated within a gynecological clinic with proper referral to additional services as needed.

Objectives: The objective of this study was to identify the contributors of pain among women with and without endometriosis, among those admitted for gynecological surgery at The Ottawa Hospital.

Methods: A retrospective analysis was done in women presenting for gynecological surgery for non-malignant indication between April 1, 2016, and March 31, 2017 at a tertiary care centre in Ottawa, Canada. Patients were stratified by women without endometriosis and women with endometriosis (International Classification of disease [ICD10] = N80). CPP was defined as pain and other conditions associated with female genital organs and menstrual cycle (N94), and pelvic and perineal pain (R10.2). Pearson Chi-square test was employed to assess the significance of pain contributors in each group. Statistical significance was established at a $p<0.05$.

Results: A total of 1069 women were included in the study. 117 (13.0%) of 897 women without endometriosis had CPP, while 83 (48.3%) of the 172 women with endometriosis had pain complaints. Among women without endometriosis, patients with CPP were more likely to have had a history of a previous laparoscopy, abnormal uterine bleeding, fibromyalgia, depression, history of physical/sexual abuse, and smoking, and were less likely to have had a vaginal prolapse ($p<0.05$ for all comparisons). Among women with endometriosis, patients with CPP were less likely to have uterine fibroids and be undergoing a hysterectomy ($p<0.05$ for both comparisons).

Conclusion: Important differences exist between women presenting for gynecologic surgery with chronic pelvic pain compared to women without chronic pelvic pain. Although endometriosis is often an important confounder in such studies, these differences are seen in women without endometriosis.

Developing a faster mouse model of Leber's hereditary optic neuropathy with functional and histological deficiencies in retinal ganglion cells

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Leber's hereditary optic neuropathy (LHON) is a rare inherited disease that affects approximately 1 in 50,000 people worldwide. Patients often suffer through a series of various differential diagnoses until they are finally diagnosed with LHON. A mitochondrial DNA mutation, most commonly in the ND4 gene, results in the production of a mutant Complex I subunit involved in oxidative phosphorylation. These mutations lead to an increase in the production of reactive oxygen species, and ultimately, retinal ganglion cell (RGC) apoptotic death. Although there are current LHON animal models, they are not without their limitations. Histological and functional changes consistent with LHON develop in these models after a long time. The goal of this project is to accelerate the onset of LHON in mouse models. We aim to accomplish this by introducing a mutant human ND4 gene to RGCs in mice with a background of increased oxidative stress. Three different mouse models of genetically induced oxidative stress will be used. These mouse models are heterozygous for the mitochondrial superoxide dismutase 2 gene ($Sod2^{+/-}$), have a parkin null genotype ($Par^{-/-}$) or have a combination of the two genes ($Sod2^{+/}/Par^{-/-}$). Mutant ND4 will be introduced on each of these genetic backgrounds and the results obtained will be statistically compared against wild type mice. We will test the function of RGCs using electroretinogram measurements at various time points in the study and at the final time point, the mouse retina will be harvested to test for histological differences.

Developing an oncolytic prime-boost vaccine targeting a self antigen for the treatment of pancreatic cancer

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Background: Pancreatic cancer affects 4400 Canadians each year and with 5yr survival rates <8%, there is an unmet need for new therapeutic approaches for treating this deadly disease. In addition to their inherent tumoricidal capacity, evidence indicates oncolytic viruses are potent vaccine vectors and as such are the ideal immunotherapeutic treatment for pancreatic cancer.

Objective: Understand how neoadjuvant administration of a prime-boost oncolytic vaccine targeting a pancreatic tumor associated antigen (pTAA) could potentiate pancreatic tumor specific immune responses to improve patient prognosis.

Methods: The oncolytic virus encoding a pTAA (OVpTAA) was created and its efficacy was assessed in a heterologous prime-boost paradigm. A priming Adenovirus expressing the pTAA was injected 7 days before boosting with the OVpTAA in mice depleted of regulatory T cells. The immune response was subsequently examined 7 days after by (i) quantifying the CD8 T cells responsive to stimulation with published epitopes, (ii) the T cell cytotoxic response was measured using an *in vivo* cytotoxicity assay, (iii) recruitment of immune cells to implanted tumors, and (iv) survival in CD8 T cell depleted animals.

Results: A measurable CD8 T cell response against various peptides was achieved only when regulatory T cells were depleted before prime-boost vaccination. The number of responsive CD8 T cells response was comparable to that observed by an established whole cell vaccine approach. The prime-boost vaccination was also able to effectively stimulate cytotoxicity of pulsed target cells suggesting CD8 T cells were able to effectively recognize and kill expressing targets however this response did not translate to an effective protection against Pan02 challenge, a result that is not attributed to a lack of MHC I expression. In addition, vaccination did not provide protection from two other pTAA expressing cell lines. Furthermore, CD8 depletion studies revealed tumor growth was not reliant on CD8 responses in the prime-boost model suggesting an impairment in the recruitment or activation of specific CD8 T cells. Notably, removal of tumor cell implants after vaccination revealed that CD8 T cells were recruited to the tumor, in similar numbers as the whole cell vaccination, however were lacking in other cell population recruitment.

Conclusion: A heterologous prime-boost vaccine platform has the potential to stimulate an effective immune response, however our studies indicate that an anti-pTAA immune response does not correlate with control of tumor growth suggesting other mechanisms of immune evasion or targeting may be more relevant to anti-tumor immunity.

Improving Oncolytic Virotherapy Using Vanadium-Based Compounds

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BACKGROUND & OBJECTIVE
Sarcomas are lethal cancers that stem from mesenchymal tissues. Survival rates of sarcoma patients with metastatic disease remain very low despite aggressive therapy.Immunotherapy is a robust treatment paradigm that has improved the prognosis of a broad range of malignancies such as melanoma, lung and renal cell cancers. However, it has not yet translated into meaningful outcomes in the clinic for sarcoma patients. Oncolytic virotherapy (OV) employs viruses with engineered oncotropism to selectively target and kill cancer cells as well as stimulate an antitumor immunity. Despite promising results achieved with OVs, some of which are approved (Imlygic) or in advanced stages of clinical development, heterogeneity of response and resistance to monotherapies remains a clinical challenge. Our group has discovered small molecule enhancers of OVs – these molecules, described as viral sensitizers (VSe), suppress the antiviral response thereby improving the efficacy of OVs. Most recently, our lab reported that vanadium-based protein tyrosine phosphatase (PTP) inhibitors improve viral oncolysis as well as long-term antitumor immunity and survival when used in conjunction with oncolytic virus VSV?51 in syngeneic glioma and carcinoma tumor models. Thus, we sought to evaluate the impact of the combination of VSV?51 and vanadate in the context of sarcoma.

METHODS
The combination therapy was tested *in vitro* using a panel of canine, murine and human sarcoma cell lines where viral spreading as well as cytotoxicity were assessed. Viral infection was also measured by microscopy in *ex vivo*-treated human patients biopsies (liposarcoma and sarcoma) and human tumor xenografts. Murine intraperitoneal and subcutaneoussyngeneic models of sarcoma were also used to monitor OV infection (quantified using an *in vivo imaging system*), survival and tumor progression.

RESULTS
Upon infection, vanadate enhanced viral cell-to-cell spread in canine, murine and human sarcoma cell lines. Vanadate synergistically decreased cell viability upon VSV?51 infection. Furthermore, vanadate sensitized patient biopsies and human osteosarcoma xenografts (primary and metastatic tumors) to VSV?51 infection without causing infection of normal tissues. The combination therapy significantly improved tumor control and survival in murine models of sarcoma.

CONCLUSION
Overall, this project supports the translational potential of vanadium compounds paired with oncolytic virotherapy for the treatment of local and metastatic sarcomas. Future experiments will aim at testing alternative delivery approaches as well as delineating this VSe's mechanism of action.

A defect in innate viral sensing may limit the efficacy of an autologous infected cell vaccine in a murine model of Acute Myeloid Leukemia

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Background

Despite aggressive therapeutic interventions, most patients diagnosed with acute leukemia will succumb to their disease in 5 years. Although promising clinical results have been achieved with a number of immunotherapeutic approaches including CAR-T, bispecific T cell engagers and checkpoint blockade, these are not effective in all patients, can be associated with significant toxicity and may not provide long term protection. Our group has recently shown that a vaccination strategy incorporating autologous tumor cells infected ex vivo with an oncolytic virus can elicit a robust and durable anti-tumor response in a murine model of acute lymphoblastic leukemia (L1210). However, this approach is relatively ineffective in a model of acute myeloid leukemia (C1498).

Objective

To identify factors which may contribute to the observed differences in the vaccines' efficacy.

Methods

L1210 and C1498 cells were infected with MG1-GFP virus at MOI:10 in 10% FBS DMEM media. After 4hrs, infection was confirmed by GFP expression and cell pellets and supernatants were collected. Total RNA was extracted from infected and uninfected L1210 and C1498 cells and reverse-transcribed into cDNA to be used in qPCR assays. Fold change in gene expression was quantified using the Livak method (using two reference genes, β actin and GAPDH). Alternatively, gene expression was qualified as expressed/not expressed as compared to the same reference genes. Supernatants were used for quantification of cytokine production by ELISA.

Results

Here we report the presence of a robust anti-viral immune response following infection of L1210 cells characterized by the upregulation of IFNβ, IL-6 and RANTES. In contrast, infection of C1498 cells does not result in the production of antiviral, immune modulatory cytokines despite expression of ssRNA sensors and adaptors including TLR7, MyD88, TRAF6, RIG-I, STING. Further examination into the expression of of other members of the ssRNA sensing pathway and downstream signaling molecules (e.g. IRF7) is in progress.

Conclusions

In consideration of these findings, we hypothesise that an intact viral sensing mechanism is a key contributor to the immunogenicity of the ICV and will be directly tested using a TLR7 deficient L1210 cell line. This study provides important insight into the mechanisms contributing to the immunogenicity of the ICV platform which can be leveraged to guide the planned clinical trial.

Cilia-mediated Hedgehog signaling regulates satellite cell function

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Satellite cell function and regenerative myogenesis are tightly regulated by a number of extrinsic factors. Among them, we identified Sonic Hedgehog (Shh), a well-characterized activator of the cilia-mediated Hedgehog (Hh) signaling. Shh is expressed at day 3 following a cardiotoxin-induced muscle injury. Using myofiber culture, we showed that treatment with Hh signaling agonists activates *Myf5* expression in the committed satellite cell following an asymmetric stem cell division. By contrast, inhibiting primary cilia assembly using siRNA for *Ift88* represses Hh signaling, consequently decreasing *Myf5* expression and asymmetric divisions. The primary cilium regulates the processing of Gli3, the primary transcriptional repressor of Hh signaling. Using mice allowing for tamoxifen-inducible genetic deletion of *Gli3* in satellite cells, we found that tamoxifen-treated *Pax7*$^{CreER/+}$*:Gli3*$^{fl/fl}$ mice show an increased number of satellite cells in uninjured muscle, suggesting that the derepression of Hh signaling through *Gli3* deletion promotes satellite cell activation. In early stages of repair, *Gli3* deletion drastically increases the number of Pax7-expressing cells, while decreasing myogenin-expressing cell number. Consistent with these results, *Gli3*-deficient satellite cells and myoblasts proliferate faster, while their differentiation is delayed. Following repair, tamoxifen-treated *Pax7*$^{CreER/+}$*:Gli3*$^{fl/fl}$ mice display increased muscle mass due to myofiber hypertrophy and increased numbers of centrally located nuclei per regenerated myofiber, indicating increased satellite cell fusion. Moreover, the regenerated muscles from *Pax7*$^{CreER/+}$*:Gli3*$^{fl/fl}$ mice are stronger compared to the *Pax7*$^{CreER/+}$ littermate controls. Altogether, our data strengthen the idea that cilia-mediated Hh signaling contributes to satellite cell activation, proliferation and commitment to the myogenic lineage and its activation improves muscle regeneration.

Right ventricular bioenergetics adaptation to pulmonary arterial hypertension in the Sugen5416-chronic hypoxia rat model

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Introduction: Pulmonary Arterial Hypertension (PAH) is a progressive and incurable disease of the microvasculature of the lung. In PAH, arterial remodeling and loss of effective microvasculature is accompanied by an increase in pulmonary vascular resistance. Right ventricular (RV) remodeling in response to pressure overload can be either adaptive, with RV hypertrophy, or maladaptive, with chamber enlargement and decrease in function leading to heart failure. We have previously reported differential expression of genes involved in biogenesis and energy metabolism in two rat strains that exhibit either adaptive (Sprague-Dawley; SD) or maladaptive (Fischer) RV remodeling in response to pressure overload. Alterations in RV energy metabolism have been previously reported in PAH and metabolic modulators have been shown to have beneficial effects; but the mechanisms underlying the transition to right heart failure in PAH remain unclear. The objective of this study was to explore the role of alterations in mitochondria function and energy metabolism in a model of maladaptive RV remodeling in response to pressure overload.

Methods and Results: Severe PAH was induced by a single subcutaneous injection of SU5416 in age-matched SD or Fischer rats (150-250g), followed by a 3-week exposure to chronic hypoxia (SUHx). Both SD and Fischer rats exhibited similar degree of RV hypertrophy (Fulton index) in response to SUHx, despite significantly higher right ventricular systolic pressure (RVSP, right heart catheterization) in the Fischer rats (81±2 vs. 111±4 mmHg, respectively; $p<0.001$). Mitochondria function was measured 4 weeks post SUHx in permeabilized cardiac fibres isolated from the RVs. Mitochondrial respiration in the presence of respiratory chain substrates was similar between strains and was not significantly altered by SUHx, whereas mitochondrial ROS production was decreased similarly in both strains in response to severe PAH, consistent with depressed mitochondrial oxidative capacity in RV hypertrophy. Interestingly, RV cardiomyocytes from Fisher, but not SD, SUHx rats showed a significant decrease in mitochondrial content (Citrate synthase: 0.98 vs. 1.82 mU/mg protein) and in respiration supported by palmitoyl-CoA, consistent with a strain-dependent difference in bioenergetics and fatty acid metabolism in severe PAH.

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

Identification of synthetic lethal interactions in acute myeloid leukemia

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Acute myeloid leukemia (AML) is a disease characterized by the expansion into the bone marrow and blood of abnormally differentiated hematopoietic cells called blasts. While AML is the most common form of adult leukemia in Canada, the five-year survival rate is disappointingly below 20%. Dismal outlook among patients is the result of vast heterogeneity in the molecular etiology of AML rendering a single treatment regimen unlikely to suit all those affected. By using a unique panel of molecular prognostic indicators identified by our lab, we have performed a whole genome CRISPR/Cas9 synthetic lethal screen in matched AML cell-lines to identify new drug targets to treat refractory AML. This strategy combines high throughput DNA sequencing with the lentiviral delivery of Cas9 and pooled short guide RNA cassettes representing knock-out every gene in the genome. Drop-out of viral integrations from sequencing data are then compared between matched cell-lines to identify genotype specific liabilities.

So far, matched THP1 myelomonocytic blasts have been engineered to express Cas9 and have been transduced with the dual vector GeCKOv2 library which includes 123411 unique sequences targeting over 20000 genes. This approach will not only generate a low incidence of off-target effects, but a decreased false negative rate due to high specificity and elimination of variable knock-down efficiencies which have been problematic in past shRNA-based synthetic lethal screens. In THP-1 blasts, 628 gene targets have been identified. Prospective targets will then be validated by genetic and pharmacologic inhibition in primary bone-marrow aspirates from both healthy and diseased patients.

Role of ATRX in neuronal circuitry and behavior

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Background:
ATRX is a nuclear protein part of the SWI/SNF chromatin remodeler family. It forms a complex with DAXX to facilitate the deposition of histone variant H3.3 into repetitive DNA sequences. In mice, Atrx is expressed throughout the brain with its greatest expression in postmitotic cells. Brain-specific deletion of Atrx using Foxg1-cre and Nestin-cre results in embryonic lethality suggesting a role for Atrx in neuronal survival. Their brains have smaller cortices and no dentate gyrus. Mutation in ATRX has been linked to the X-linked neurodevelopmental disorder, Alpha Thalassemia X-linked Intellectual Disability Syndrome, or ATR-X syndrome. Patients with ATR-X syndrome have severe psychomotor, physical and hematological abnormalities. Previous mouse models have failed to delineate ATRX's role in neuronal function and maturation due to early postnatal lethality. Here, we want to dissect how the loss of ATRX leads to altered neuronal circuitry and intellectual disability, using the Emx1-cre model.

Objective:
1. Identify the role of ATRX in hippocampal neurogenesis
2. Determine if ATRX mediates the balance of inhibitory and excitatory signals
3. Determine the behavioral impact of ATRX ablation in the cerebral cortex

Results:
Unlike Atrxf/y::FoxG1-Cre conditional KO mice which die at birth, Atrxf/y::Emx1-Cre (Atrx cKO) mice had a normal lifespan, reduced body weight, and cortical thickness. Examination of the hippocampus (HPC) at P0 showed a disorganization of the neuronal layer in all the subregions including the dentate gyrus, with an increase in Ki67-positive cells. At P10, the localization of the neuronal layer in CA1, CA2 and CA3 regions is normal but thinner. However, the dentate gyrus remains disorganized and showed cell accumulation at the apex. Further investigation is required to determine the different cell populations within the HPC that are affected by ATRX ablation.
Behavioral tests were used to characterize the learning and memory capacity of Atrx cKO mice. However, we discovered a hyperactivity phenotype and multiple seizure-like episodes which prevented the assessment of the learning and memory capacity of these mice.

Conclusions:
Our preliminary results indicate that ATRX ablation at a later embryonic time (E10.5 vs. E8.5) allows the cKO mice to survive beyond the prenatal stage. The cKO animals show a reduction in cortical thickness and altered histogenesis of the hippocampus, similar to more severe models. The prolonged survival allowed us to characterize the behavioural phenotype and Atrx loss is associated with a hyperactivity and an increased incidence of seizure-like episodes. Further studies are required to link the altered behavior to the disorganization within the hippocampus.

Identifying Barriers and Enablers to Recruitment, and Participation in, an Early Phase Clinical Trial of Chimeric Antigen Receptor T-cell Therapy for Hematologic Malignancies

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Background: Cancer clinical trials sometimes fail to accrue sufficient patients. Identifying barriers and enablers to participating prior to conducting a clinical trial may help to inform protocol and training adaptations needed to better support recruitment to, and participation in, the eventual trial. The Theoretical Domains Framework (TDF) summarizes 33 behaviour change theories into 14 broad domains that can be used to identify and explore barriers and enablers to trial participation.

Objective: Getting Better Outcomes with Chimeric Antigen Receptor T-cell Therapy (GO CART) is a program of research undertaken to inform an early phase clinical trial for hematologic malignancies. In preparation for this early phase CAR T-cell therapy trial, we sought to identify potential barriers and enablers to hematologists' *screening* patients, and potential barriers and enablers to patients' *participation* in such a trial.

Methods: We used a qualitative approach for this study. Guided by the TDF, we developed topic guides for hematologists and patients and conducted a directed content analysis. The most relevant barriers and enablers to participating in and recruiting to the trial were identified based on frequency, relevance, and conflicting beliefs across TDF domains.

Results: We interviewed 15 hematologists (median years' experience = 12) from across Canada and 13 patients (median age = 56, 38.5% women) from two provinces.
Hematologists expressed an interest in knowing more about CAR T-cell therapy safety and efficacy (knowledge). They emphasized the need for clear trial information and eligibility criteria (knowledge) and were concerned about the cost and feasibility of the trial (environmental context and resources). While physicians saw it as their role to screen patients for trials (social/professional role & identity), the division of responsibility varied across sites.
Patients wished to learn more about CAR T-cell therapy safety, efficacy and trial logistics (knowledge). They wanted trial information directly from their hematologist or oncologist and valued their opinion most (social influences). Most were primarily motivated by personal health benefits (beliefs about consequences) and many indicated that having transportation and accommodations covered would help during trial participation (environmental context and resources).

Conclusions: Both hematologists and patients require clear current evidence about trial safety and efficacy. The type of content, how it is presented, and by whom, will impact patient intentions to participate. Involving hematologists in recruitment procedures and streamlining screening and recruitment processes may help ensure better trial accrual. This study demonstrates the value of conducting implementation interview studies in preparation for conducting clinical trials.

Targeting asymmetric cell division to restore muscle stem cell function in Duchenne muscular dystrophy

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The regulation of muscle stem cell division ensures a balance between symmetric cell divisions to maintain stem cell numbers and asymmetric cell divisions to generate myogenic progenitors for muscle repair. In *mdx* mice, a mouse model of Duchenne muscular dystrophy (DMD), muscle stem cells are ineffective at establishing cell polarity and hence unable to undergo proper asymmetric cell division. We identified the p38-gamma MAP kinase and the arginine methyltransferase Carm1 as part of a signaling cascade that regulates the epigenetic activation of muscle stem cell commitment downstream of the dystrophin glycoprotein complex. Specific phosphorylation of Carm1 by p38-gamma prevents Carm1 nuclear translocation, where it is required to methylate Pax7, resulting in the recruitment of the MLL trithorax histone methyltransferase complex and subsequent histone H3K4 tri-methylation at the *Myf5* promoter. Importantly, this regulation is lost in muscle stem cells of *mdx* mice, resulting in the hyper phosphorylation of Carm1, reduced methylation of the chromatin at *Myf5*, and ultimately reduced levels of *Myf5* transcript. siRNA-mediated knock down of p38-gamma results in enhanced levels of *Myf5* in *mdx* cells. Thus, preventing the negative regulation of Carm1 in dystrophic satellite cells may rescue the inefficient generation of myogenic progenitors observed in *mdx* mice. To test this hypothesis, we have employed CRISPR/Cas9 to introduce mutations at the Carm1 phosphorylation site in order to generate Carm1 mutant *mdx* mice. These findings will provide insights into the mechanisms of stem cell dysfunction in DMD and aid in the design of therapeutic strategies to stimulate endogenous muscle repair.

Evaluation of markers of immunogenic cell death following infection with an oncolytic Maraba virus. Immunogenic cell death in the context of cancer immunotherapy.

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The efficacy of oncolytic virotherapy is due in part to the generation of a potent immune response to tumor antigens. However, the factors which favor the development of tumor specific immune responses by infected cells are not well understood. The literature indicates that the immunogenicity of dying tumour cells is dependent upon externalization of calreticulin and the release of ATP and High Mobility Group Box 1 (HMGB1). Here we report that infection with an oncolytic Maraba virus in vitro results in an externalization of Calreticulin as detected by flow cytometry and immunofluorescence. In contrast, we could not detect any ATP release following infection by flow cytometry or fluorescent microscopy. This unexpected finding suggests that ATP release is not a contributing factor in the development of the anti-tumor response following infection with an oncolytic Maraba virus. Future studies investigating strategies aimed at modulating ATP release from infected cells should be explored to determine whether providing an additional immunogenic signal that can enhance the effectiveness of this virus.

Breastfeeding after a caesarean delivery on maternal request: a systematic review

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Background: The rate at which caesarean deliveries on maternal request are being performed has risen in countries worldwide. Caesarean delivery may be associated with a decrease in the rate and duration of breastfeeding. Given the increase risk of breastfeeding problems and the benefits of breastfeeding for the infant, the lack of focused data for women undergoing a caesarean delivery on maternal request limits the quality of counseling that healthcare professional can provide.

*Objectiv*e: The objective of this study was to systematically review the literature to assess the association between a caesarean section delivery on maternal request and breastfeeding.

Design/Methods: Literature searches were performed using Medline, EMBASE and Cochrane databases. Articles published before July 2017 that reported breastfeeding outcomes associated with caesarean delivery on maternal request were included. The extracted data were analyzed independently by two reviewers. A risk of bias assessment was conducted.

Results: A total of six articles met the inclusion criteria for qualitative analysis and three were included in the meta-analysis. Compared to women who delivered vaginally, women who delivered by a caesarean under maternal request breastfed for a shorter period of time postpartum. However, initiation of breastfeeding was not delayed. Breastfeeding complications and feeding problems were experienced in a higher proportion of women who had a caesarean delivery by maternal request.

Conclusion: Caesarean delivery on maternal request shortens the duration of breastfeeding and increases the likelihood of breastfeeding complications postpartum. Future research for long term outcomes associated with caesarean delivery on maternal request is warranted.

Identification of Small Molecule Modulators of Satellite Stem Cell Asymmetric Division using a Novel In-niche High Content Analysis Platform

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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 have developed a high content analysis platform using myofibers derived from Myf5-Cre R26YFP mice to screen small molecule compounds previously characterized in clinical trials. Using this platform, we have identified several novel modulators of asymmetric stem cell division. We hypothesize that the restoration of satellite cell asymmetric self-renewal and cell polarity pathways will restore the muscle regeneration capacity in mdx mice. Here we show preliminary data validating and characterizing shortlist targets that affect stem cell asymmetric division. With our findings, we aim to characterize additional molecular signaling pathways that participate in muscle regeneration and uncover novel therapeutic targets for the treatment of DMD.

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Transcriptional dynamics of the epithelial-mesenchymal transition in ovarian cancer

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Background

The epithelial-mesenchymal transition (EMT) is a phenotypic switch, where epithelial cells lose defining characteristics, such as stable cell-cell junctions, and transition towards a mesenchymal state, capable of migration and tissue invasion. It is critical for many biological processes, including embryo development and tissue homeostasis. The EMT is also thought to promote the progression of a variety of cancers, including ovarian cancer, where it is associated with metastasis, chemoresistance, and reduced overall survival. Ovarian cancer is currently the most lethal gynecological malignancy, and developing a comprehensive understanding of the EMT in this context would provide valuable insight into the progression of the disease. Much of the previous work has focused on the signalling cascades involved, and, despite the ubiquity of this process, relatively little is known about the transcriptional and epigenetic determinants coordinating it.

Objective

The objective of this study is to map transcriptional dynamics throughout the EMT in ovarian cancer, and to determine which components are critical for acquiring traits associated with cancer progression.

Methods and Results

To map the transcriptional events coordinating the EMT, we generated single-cell RNA sequencing (scRNA-Seq) libraries from the OVCA420 high-grade serous ovarian carcinoma cells that were treated with transforming growth factor beta-1 for 0, 1, 3, or 7 days. After sequencing and filtering, our dataset comprised expression profiles of over 10,000 single cells. Principal component analysis revealed a time-dependent structure in the data, supporting that we successfully captured cells along various stages of the EMT. In order to infer the transcriptional dynamics throughout the process, we leveraged the cells' asynchronous response, using pseudotime analysis to map a transcriptional trajectory through the data and ordering cells based on their position along this trajectory. We then identified differentially expressed genes throughout the trajectory. Clustering these genes based on their pseudotemporal dynamics identified several waves of transcriptional events, including a transient repression of cell cycle genes that is restored towards the end of the trajectory. This repression is followed by the activation of EMT-associated genes, and interestingly, many components of cancer-related pathways are activated following this.

Conclusions

Here, we have generated a comprehensive map of transcriptional dynamics that occur throughout the EMT in ovarian cancer, resolving waves of transcriptional events that were previously uncharacterized. This will provide insight into the progression of the disease and may identify novel therapeutic targets. These findings may also be generalizable to other contexts involving the EMT.

Identifying small molecules that restore tumor-directed NK cell cytotoxicity

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Background

Natural Killer (NK) cells are cells of the innate immune system that can recognize and kill malignant cells such as cancer cells. Recognition of cancer cells occurs when activation receptors on the surface of NK cells engage with their ligands on cancer cells. This engagement will trigger activation of the NK cell resulting in the release of inflammatory cytokines and cytotoxic granules. NK cells found in the tumor microenvironment often cannot conduct their effector functions due to the expression of inhibitory checkpoint receptors. We have previously shown that NK cells express the checkpoint receptor Program Death Protein 1 (PD-1). Engagement of PD-1 with its ligand PDL-1 results in suppression of NK cell activity. However, the mechanism of PD-1-mediated inhibition in NK cells remains unclear. Here, we propose using an unbiased drug screen to uncover how PD-1 signals in NK cells.

Objective

The goal of this study is to identify small molecules that can restore NK cell cytotoxicity by supressing the inhibitory function of PD-1.

Methods

The Prestwick Chemical Library will be used in this study to test the ability of small molecule drugs to restore NK cell cytotoxicity. NK92 and K562 cells were transduced to express PD-1 and PDL-1 respectively. NK92 cells expressing PD-1 will be pretreated with compounds from the Prestwick Chemical Library, followed by co-incubation with K562-GFP target cells expressing PDL-1. Loss of GFP fluorescence will be used as a readout for NK cell cytotoxicity.

Results

We have generated the two cell lines needed for the drug screen: NK92 cells stably expressing PD-1 and K652 cells expressing PDL-1. NK92-PD-1 and K562-PDL-1 were incubated without drug pre-treatment to first evaluate NK92 cytotoxicity in this system.

Next Steps

We will conduct a series of killing assay experiments to determine the ideal NK92-effector to K562-target ratio and drug concentration to use in the library screen. Following the library screen, drug hits that were identified will be further investigated to determine the molecular mechanism in which NK cell cytotoxicity was restored.

Overcoming Chondroitin Sulfate Proteoglycan-Mediated Inhibition of Oligodendrocyte Differentiation and Myelination

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Background: In multiple sclerosis, oligodendrocyte precursor cells (OPCs) migrate to lesion sites to repair myelin. Throughout disease progression, the ability to repair such damage diminishes considerably. This is thought to be a consequence of lesion-associated inhibitory factors, including chondroitin sulfate proteoglycans (CSPGs), which perturb OPC maturation into myelinating oligodendrocytes. While these factors are known to inhibit the differentiation of the OPC, the mechanism driving this signal remains unclear.
Objectives: The current study aims to characterize the oligodendrocyte response to CSPG exposure, as well as explore the molecular pathways involved in CSPG-mediated inhibition of oligodendrocyte differentiation.
Methods: We investigated signaling proteins previously implicated in CSPG-mediated inhibition of neuronal morphology to determine if the same pathways are involved in the CSPG-oligodendrocyte interaction. A primary oligodendrocyte cell culture system was used for most experiments, with readouts tailored to OL morphological and molecular differentiation. Based on results from the *in vitro* studies, an inhibitor of rho kinase was tested in the cuprizone mouse model, which serves as a model of demyelination.
Results: We have validated the impact of CSPGs on oligodendrocyte maturation, such that exposure to CSPGs dramatically impedes morphological complexity of the cell. We show that GSK-3ß signaling is likely not crucial in mediating the effects of CSPGs in oligodendrocytes. Contrastingly, pharmacological inhibition of either rho kinase or non-muscle myosin II improved the morphological perturbations of oligodendrocyte differentiation in the presence of CSPGs. Based on these results, it was important to test the potential benefits of rho kinase inhibition *in vivo*. To this end, we employed the chronic cuprizone model of demyelination, which has been shown to result in an accumulation of CSPGs, and incomplete remyelination. The rho kinase inhibitor treatment did not appear to improve the extent of remyelination during the recovery phase of this model.
Conclusion: This study reveals targets involved in CSPG-mediated inhibition of oligodendrocyte growth and highlights previously unappreciated differences between oligodendrocyte and neuronal responses to the same cue. Unsurprisingly, the reductionist approach of determining signaling pathways for a single cue does not translate well to the more complex *in vivo* environment, where there are a number of different inhibitory cues present at the lesion site. Future studies will shift focus to a promising micro-RNA candidate that has been shown to improve OL differentiation and myelination in a number of contexts, and may be more successfully translated to an *in vivo* environment.

Characterization of SLK-deficient Mouse Embryonic Fibroblasts (MEFs)

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Background: The Ste20-like kinase (SLK) has been shown to be expressed in all cell lines and tissue. SLK plays a role in the induction of apoptosis, cytoskeletal dynamics and cell migration. However, the exact signaling mechanisms have yet to be elucidated.

Objective: This project will characterize the effect of SLK deletion on proliferation, migration and apoptosis in Mouse Embryonic Fibroblasts (MEFs) in tissue culture. Our main question is whether a SLK knockout behaves similarly to the SLK knockdown.

Methods: A proliferation assay and flow cytometry have been used to assess cell growth and the proportion of cells in the various phases of the cell cycle. Westerns blots of Cyclin markers will be also provide further validation. A Boyden chamber migration assay will be used to measure cell motility. Western blot analysis was used to evaluate focal adhesion turnover. To assess the localization of cytoskeletal protein during migration, a scratch wound assay was performed along with immunofluorescence. SLK has been shown to play a role in apoptosis. Therefore, an Alamar blue apoptosis assay was conducted. Apoptotic markers were blotted for through Western Blot to further validate any differences.

Results: Surprisingly, SLK-null cells were shown to proliferate faster than wild-type MEFs. Migration appears to remain unchanged through Boyden chamber assays; however higher levels of pFAK suggest impaired focal adhesion turnover in the SLK-deficient cells. Indeed, phospho-FAK appears to be persistent in SLK-null cells compared to wild type cells. Although SLK has been shown to induce apoptosis, cell death was unaffected in SLK(-/-) cells. Interestingly, SLK appears to be breaking down during apoptosis induced by Staurosporine. Whether this is actively playing a role in cell death remains to be investigated. To check whether the breakdown was specific or not, other proteins were surveyed. To check whether this was a general effect of apoptosis, other drugs and cell lines were assessed.

Conclusion: ?Overall, our data show that the genetic deletion of SLK increases cell growth but does not significantly affect cell migration. Interestingly, focal adhesion turnover appears to be delayed in SLK-deficient cells. Although SLK was suggested to play a role in apoptosis, SLK deletion does not affect the cell death response to various triggers. As the loss of SLK may induce compensatory mechanisms, a future direction will be to study the effect of expressing a kinase inactive SLK in SLK-null cells.

Lung protective effect of hUC-MSCs isolated from preterm delivery in neonatal lung injury.

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Complications of preterm birth are the most frequent cause of death in children less than 5 years of age. Bronchopulmonary dysplasia (BPD) is the most severe complication of extreme prematurity. This chronic lung disease occurs following ventilator and oxygen treatment for acute respiratory distress. BPD results in impaired lung growth with life-long complications such as asthma, pulmonary hypertension and emphysema. Currently, there is no effective treatment for BPD. We and others provided proof of concept for the therapeutic potential of human umbilical-cord mesenchymal stromal cells (hUC-MSCs) in BPD. Considering that BPD is a disease of prematurity, therapeutic potential of hUC-MSCs isolated from preterm delivery (hUC-PMSCs) is of major interest for the development of cell based-therapy for BPD. To determine whether hUC- PMSCs can exert a therapeutic effect in BPD, hUC-PMSCs isolated from 24 weeks gestational age were administered intratracheally at postnatal day 4 (P4) in rat pups exposed to 85% oxygen from P0 to P14 (BPD model). Survival and body weight were monitored throughout the experiment. At P21, lung function (flexivent), lung structure (mean linear intercept, MLI) and right ventricular hypertrophy (Fulton index) were assessed. Our results show that hyperoxia significantly reduced survival, body weight, lung compliance and increased MLI and Fulton index compared to normoxia control pups. Treatment with hUC-PMSCs increased survival and body weight compared to hyperoxic control pups. In addition, hUC-PMSCs significantly improved lung compliance and significantly decreased MLI and Fulton index when compared to control hyperoxic pups. Thus, hUC-PMSCs can provide lung protective effects in a rat BPD model.

Regulation of Muscle Stem Cell Polarity During Muscle Regeneration

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Background

Dchenne Muscular Dystrophy (DMD) is a severely debilitating childhood disease marked by muscle weakness and degeneration. Currently there exists no cure for DMD. DMD symptoms are in part due to dysfunction of the muscle stem cells (satellite cells). Our lab has previously shown that muscular dystrophy can be ameliorated through treatment with the protein Wnt7a. Wnt7a/Fzd7 signaling drives the symmetric division of muscle stem cells through the planar cell polarity (PCP) pathway, stimulating skeletal muscle repair and growth. 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 muscle stem cell division. Our lab has demonstrated that Dvl2 (a phospho-regulated protein) is an essential component of Wnt7a/Fzd7 signaling, in which it interacts with Fzd7 to transduce an intracellular signal. Furthermore, NuMA1 is an effector protein required for PCP-specification of centrosome orientation that is polarized in activated muscle stem cells.

Objective

This study aims to identify the mechanisms underpinning muscle regeneration driven by Wnt7a-mediated muscle stem cell division. Specifically, we wish to identify the key protein-protein interactions that occur upon Wnt7a stimulation of muscle stem cells, which we predict require Dvl2 and NuMA1.

Methods

Ex vivo muscle stem cells were analysed via proximity ligation assay (PLA) to identify whether Dvl2 and NuMA1 interact during Wnt7a stimulated mitosis. We predicted that p38 MAP-Kinase, recently shown to be required for symmetric muscle stem cell division, mediates Dvl2 function. This interaction was explored using *in vitro* kinase assay and LC-mass spectrometry. In-progress work includes investigating the effects of silencing p38 on Wnt7a-mediated symmetric stem cell expansion as well as the ability of Dvl2 to interact with NuMA1.

Results

Through PLA we demonstrated that Dvl2 interacts with NuMA1, which is enhanced through Wnt7a treatment. In vitro kinase assay and LC-mass spec revealed that Dvl2 is phosphorylated by p38 at S358. In vitro studies to examine the effects of ablating Dvl2 phosphorylation at S358 in satellite cells are underway to determine the functional consequences of Dvl2 phosphorylation at this site.

Conclusions

Our results support the requirement of a p38-Dvl2-NuMA1 signaling axis during symmetric muscle stem cell division, a key process during muscle regeneration. The findings from this study yield critical information regarding Wnt7a's mechanism of action, thus aiding in the development of Wnt7a-based therapeutics and potentially revealing new therapeutic targets for DMD.

Expression of the p14 Fusion Associated Small Transmembrane Protein Improves the Oncolytic Efficacy of a Conditionally Replicating Adenovirus

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Background

Recent advances in the field of oncolytic virotherapy have sparked a number of studies aiming to design oncolytic adenovirus (Ad) that can specifically replicate in and kill cancer cells. Unfortunately, Ad has been shown to have difficulty spreading throughout a significant proportion of the tumor mass following intratumoral injection. Multiple studies have highlighted the effectiveness of viral fusogenic membrane glycoproteins (FMG) in improving the efficacy of oncolytic Ad through cell to cell fusion within the tumor and the generation of multinucleated syncytia. However, the size of typical viral FMGs makes their inclusion into an oncolytic Ad genome difficult due to viral DNA packaging size constraints and prohibits the use of other genes used to "arm" Ad, such as the tumor suppressor p53 or immune-stimulating cytokines. The p14 Fusion Associated Small Transmembrane (p14 FAST) protein is a non-structural viral fusogenic protein originating from reptilian reovirus, which is significantly smaller than typical FMGs.

Objective

Our objective is to evaluate whether expression of p14 FAST from an oncolytic Ad vector can enhance virus spread and efficacy in a novel immune-competent mouse of cancer.

Methods

We have expressed the p14 FAST protein in a conditionally replicating Ad (CRAdFAST) and tested its anti-cancer capacity in both immune competent and immune deficient mouse models of cancer.

Results

In the A549 human lung adenocarcinoma xenograft model of cancer, p14 FAST expression significantly improved CRAd's oncolytic ability *in vitro* and *in vivo*. In the CMT-64 cell line which is syngeneic with C57bl/6 mice and therefore an immune-competent model, CRAdFAST induces a significant reduction in growth rate, but does not induce tumor regression.

Conclusions

CRAdFAST leads to significant reduction in tumor growth rate in an immune-competent mouse model of cancer. A known defect in antigen processing through the TAP1 protein in CMT-64 cells may hinder antigen presentation and thus reduce the capacity for CTLs to target tumor cells. We are in the process of designing a CRAdFAST/TAP1 virus in order to address this common tumor immune-escape mechanism.

Synthetic-lethal genome-wide CRISPR knockout screen reveals known and newly identified vulnerabilities of TSC2-null neural crest cells

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Background: Lymphangioleiomyomatosis (LAM) is a low grade, but progressive neoplasm of the lung that affects only women. LAM is characterized by the formation of thin walled cysts throughout the lung interstitium, caused by the abnormal proliferation of smooth muscle-like cells (LAM cells). These cysts eventually rupture, leading to parenchymal destruction and eventual respiratory failure. LAM results from inherited or spontaneous mutations in the *TSC1* or *TSC2* genes, with mutations in TSC2 resulting in more severe symptoms. Loss of functional TSC2 results in the inability to regulate mTORC1 signaling, leading to uncontrolled growth and proliferation of tumor cells. Rapamycin is the current FDA approved therapy for LAM, and although it is beneficial to patients, it is merely tumoristatic. New treatments are desperately needed. A major impediment is that the cell of origin for LAM is unknown; however, the expression of melanocyte markers in LAM tumors suggests the cell of origin is neural crest-like.

Objective: Through transcriptomic analysis of neural/neural crest differentiation combined with genome-wide CRISPR/gRNA synthetic lethal screen, we aim to identify LAM-specific pathways contributing to disease etiology and identify novel targets for more effective therapies.

Methods: Four unique TSC2-null pluripotent cell lines were generated using CRISPR/Cas9 genome editing. RNA-seq time course samples were collected during differentiation from pluripotency to neural crest cells (NCCs). Genome-wide CRISPR knockout synthetic lethal screen (SLS) was performed on NCCs using the human GeCKO v2 pooled gRNA library.

Results: The TSC2-null cells generated in this study no longer express functional TSC2, display aberrations in mTORC1 signaling, and express LAM-associated markers. RNA-seq analysis of neural/NCC differentiation reveals maximal differential gene expression following neuralization, prior to NCC specification. Differentiated TSC2-null NCCs exhibit differential gene expression of known pathways dysregulated in LAM. Analysis of the SLS identified multiple essential genes involved in pathways previously targeted in LAM. Additional essential genes in TSC2-null NCCs were identified as novel targets, including multiple microRNAs.

Conclusions: TSC2-null NCCs serve as an accurate model of LAM. Our data suggest TSC2 plays a critical role during NCC differentiation, in which aberrations in LAM-associated pathways are precipitated, offering insight into the origin of LAM. This study provides gene-level resolution within pathways already suspected as being lethal to LAM cells, allowing precise targeting strategies to be developed. Importantly, additional genes were identified that are essential to TSC2-null cells, opening the door to novel treatment strategies for LAM.

The influence of individual characteristics and organizational context on the use of research evidence by nurses in residential long-term care settings

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Background: Little scientific attention has been focused on understanding the use of research evidence in long-term care settings and almost none on regulated nurses' use of research in nursing homes.

Objective: We examined the influence of individual characteristics and organizational context features on nurses' self-reported use of research evidence in residential long-term care (LTC).

Methods: A cross-sectional analysis of survey data (N=756 nurses (registered nurses and licensed practical nurses) working in 89 residential LTC facilities in Western Canada) collected in the Translating Research in Elder Care program was conducted. Generalized Estimating Equation modeling was used to identify which individual characteristics and organizational context features significantly predict ($p<05$) three kinds of self-reported research use: instrumental (the direct application of research findings to practice), conceptual (using research findings to change thinking), and persuasive (using research findings to make a point or convince others).

Results: There were no significant differences in mean research use scores by nursing role. Predictors of instrumental research use at the individual level were: past presentation of research ($\beta = 0.604$), problem solving ability ($\beta = 0.344$), and attitude towards research ($\beta = 0.290$); and at the organizational level were: availability of structural and electronic resources ($\beta = 0.146$), engaging in formal interactions ($\beta = -0.117$), and better perceptions of organizational slack-staff ($\beta = -0.167$). Predictors of conceptual research use at the individual level were attitude towards research ($\beta = 0.476$), self determination (empowerment) ($\beta = 0.168$), and job efficacy (burnout) ($\beta = 0.141$); and at the organizational level were: structural and electronic resources ($\beta = 0.117$). Predictors of persuasive research use at the individual level were: attitude towards research ($\beta = 0.336$), belief suspension ($\beta = 0.176$), organizational citizenship behaviour ($\beta = 0.215$), self-determination (empowerment) ($\beta = 0.242$), and job efficacy (burnout) ($\beta = 0.140$); and at the organizational context level were: availability of structural and electronic resources ($\beta = 0.132$), evaluation ($\beta = 0.205$), and better perceptions of organizational slack-time ($\beta = -0.232$).

Conclusions: Conceptual research use and persuasive research use by nurses in LTC were most strongly influenced by individual characteristics, while instrumental research was predicted equally by individual and organizational variables. A more positive attitude towards research (individual-level) and better access to structural and electronic resources (organizational-level) were the only variables that predicted all three kinds of research use.

Protracted endothelial cell apoptosis leading directly to microvascular loss is a major mechanism resulting in severe pulmonary arterial hypertension in the rat SU5416-Hypoxia model

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Introduction: Evidence from experimental models has implicated endothelial cell (EC) apoptosis as a central trigger for pulmonary arterial hypertension (PAH). While the mechanism is still unclear, EC apoptosis is thought to indirectly induce dysregulated vascular cell growth leading to pulmonary arterial narrowing and occlusion. However, recent studies have suggested that complex arterial remodelling may be a consequence, rather than a cause, of sustained elevation in pulmonary arterial pressures.

Hypothesis: EC apoptosis results in the direct loss of microvasculature through degeneration of distal arterioles as a primary mechanism of increased in pulmonary arterial pressures and resistance in PAH.

Methods and Results: Sprague Dawley rats were subjected to 3-week chronic hypoxia (CH; 10% O_2) with or without a single subcutaneous injection of the VEGFR2 antagonist, SU5416 (SU; 20 mg/kg). EC apoptosis was markedly increased in the pulmonary microvaculature at 3 days post SU and persisted for at least 7 weeks post SU as assessed by immunohistochemistry, western blotting and cleaved caspase 3 activity assay. Marked increases in right ventricular systolic pressure (RVSP) were seen as early as 4 weeks post SU (84 ± 15 mm Hg) with a non-significant progression at 7 weeks (94 ± 8 mm Hg). The lung microcirculation was assessed by microCT (SkyScan 1272, Bruker, Belgium) at 4 and 7 weeks post SU after perfusion with barium at the relevant arterial pressures. Vascular volume was markedly reduced from 4.2 ± 5.4x10^{10} μ^3 in control rats to 1.6x± 0.18x10^{10} μ^3 and 2.1 ± 4.2x10^{10} μ^3 at 4 and 7 weeks post SU, respectively ($p<0.001$). Functional arterial loss was nearly exclusively restricted to the small arterioles (<200μ), and was closely correlated with the increases in RVSP at 4 weeks ($r=0.66$; $p<0.02$); whereas evidence of widespread occlusive arteriopathy was seen only after the 7-week time-point. Moreover, there was a sharp threshold for increase in RVSP, with an inflection point at ~80% loss of microvascular volume.

Conclusions: Inhibition of VEGFR2 resulted in persistent EC apoptosis, which was associated with early loss of lung microvasculature, occurring before the appearance of complex arterial remodelling. Moreover, severe PAH was only manifested in animals with >80% loss of lung microcirculation. These results support degeneration of fragile precapillary arterioles, occurring as a direct result of EC apoptosis, as a primary mechanism for the hemodynamic abnormalities in severe PAH.

*Equal contribution to the work.

Optogenetic stimulation of adult-generated cells to promote stroke recovery

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Background
Following a stroke there is a significant increase in the number of dividing progenitor cells (PCs) that ectopically migrate to the infarct. The functional significance of the PCs during stroke recovery is controversial, in part because most of the cells that endogenously migrate are unable to survive, and may not receive activation or integrate into cortical networks.
Objective
This study will determine if enhancing the survival of the PCs and/or optogenetic stimulation of the PCs, is sufficient to improve stroke recovery using the inducible BAX (iBAX) transgenic mouse model.
Methods
A photothrombosis stroke model was used to produce reproducible cortical infarcts and unilateral behavioral impairments in adult mice. One week after stroke, tamoxifen (TAM) treatment was given to recombine the nestin-expressing PCs and their progeny in : 1) iBAX mice to remove *Bax* specifically in the PCs; or in 2) Nestin Cre^{ERT2}-ChR2-YFP (iBAX-CHR2) transgenic mice to remove *Bax* and express channelrhodopsin (CHR2) in the PCs. Mice that expressed CHR2 received daily high frequency optogenetic stimulation or sham stimulation for 5 weeks following TAM treatment. Recovery was assessed by behavioural performance on the cylinder, adhesive and horizontal ladder to quantify deficits at 1, 4, 8 and 12 weeks after stroke.
Results
Increasing PC survival after stroke in the iBax mice increased the number of PCs that migrated to the peri-infarct region. The majority of the PCs had neuronal phenotypes, yet there was no change in long-term recovery from the behavioural deficits up to 12 weeks post stroke. Preliminary analysis of the nestin-Cre^{ERT2}-ChR2-YFP mice that we generated revealed significant behavioral deficits post stroke and histological assessments suggested daily stimulation can activate PCs surrounding the infarct during recovery. Ongoing analysis is determining if the optogenetic stimulation for 5 weeks improved behaviour during stroke recovery.
Conclusions
Removing Bax from PCs improved the survival of the cells and increased the proportion of cells that had a neuronal phenotype, with no significant impact on stroke recovery. These findings suggest strategies that enhance the survival of the PCs by preventing cell death and making more neurons, alone, will be insufficient to promote recovery. Ongoing work is determining whether the addition of optogenetic stimulation of the surviving PCs will alter recovery, which has clinical implications due to the growing use of non-invasive brain stimulation to promote recovery.

Effectiveness of a Patient Surgical Notepad on Patient Experience, Adverse Events and Hospital Readmissions in Patients Undergoing Elective Orthopaedic Surgery

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Background and Objectives : A recent study conducted on inpatients at The Ottawa Hospital revealed communication discrepancies between patients and most-responsible physicians. As such, a surgical notepadaimed to actively engage orthopaedic patients in their care was created and implemented on the inpatient units. The objectives of the study were to determine whether a surgical notepad could improve patient experience, reduce 30-day adverse events and hospital readmissions in patients undergoing elective orthopaedic surgery.

Methods: Members of the care team were asked to go over any questions that the patients had during each bed-side visit. Also, a pre-discharge checklist prompted the patients to ensure they understood their prescriptions/medications (including any side effects), how to care for their wound/dressings, permitted activity levels, time/place of their follow up visit, how to contact their surgeon, and what to do/who to contact for any problems or issues that arise after they have gone home. Space was provided for patients to take notes. Patients were asked to take home their Notepad after discharge for reference. The Notepad was designed to address the common problems reported from inpatients, including lack of empathy from staff, lack of discharge instructions planning, post-operative dressing issues, and pain control inconsistencies.

Results: Interrupted-time series analyses were performed to determine the influence of the surgical Notepad on patient experience. We tracked the average of each question of interest over 24 two-week periods. The intervention (ie. The Notepad) was introduced at period 13. Interrupted time series analyses revealed no significant impact of Notepad introduction at period 13 on all three MD questions ($p > 0.05$). The percent of patients selecting the top best answer (top box scores) also did not change at period 13.

Conclusions: Continuous engagement from surgeons and patients is critical to the success of an intervention aimed at improving communication in a large tertiary care institution. Communication between patients and physicians/surgeons requires ongoing efforts. Although the intervention did not lead to statistically significant improvements in CPES-IC questions, further work is warranted to better understand whether such a tool could benefit patients longer-term.

WITHDRAWN

The Effects of High Dose Radiation on Juvenile Mouse Muscle Development and Progenitor Cell Populations

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Background: Radiation induced fibrosis (RIF), characterized by muscle atrophy and fibrosis, is a debilitating late-effect of radiation therapy that affects up to 80% of childhood survivors. Radiation depletes myogenic stem cells (i.e. satellite cells), which results in impaired muscle development, adaptation, and fibrosis. Fibro/adipogenic progenitors (FAPs) are non-myogenic cells that contribute to satellite cell commitment to the myogenic lineage in healthy conditions, but their dysregulation is associated with fibro/fatty tissue accumulation in muscle. The effects of juvenile radiation exposure on muscle development and stem/progenitor cell populations has not been investigated.

Objective: The purpose of this study was to examine the effects of juvenile radiation exposure on mouse muscle morphology and stem/progenitor cell populations throughout a time course of development.

Method: Using a within design, one lower limb of 5-6-week-old male CBA mice was exposed to a single dose of 16 Gy radiation (IR) while the other limb served as the non-irradiated control (CON). Mice were sacrificed at 3, 7, 14, and 56 days post-radiation for analysis of muscle morphology and stem/progenitor cell content.

Results: Average gastrocnemius/soleus myofiber cross-sectional area (CSA) was reduced (main effect of radiation, $p<0.05$ vs. CON). The reduced CSA in IR was accompanied by an increase in the proportion of small fibers at 3 and 14 days post-radiation in IR ($p<0.05$ vs. CON), and a reduction in the proportion of large fibers at 3 days post-radiation in IR ($p<0.05$ vs. CON). The number of Pax7+satellite cells was not different between IR and CON; however, there was a significant reduction in the number of differentiated myoblasts (Pax7-MyoD+) in IR compared to CON at 7 and 14 days post-radiation ($p\leq0.05$). FAP content was reduced at 14 days post-radiation in IR compared to CON ($p<0.05$).

Conclusions: Our findings indicate that juvenile radiation decreases muscle size, reduces content of differentiated myoblasts, and reduces FAP content. These findings could have implications for late effects of radiation therapy on muscle health in juvenile cancer survivors.

The BEACON Study: Protocol for a cohort study as part of an evaluation of the effectiveness of smartphone-assisted problem solving therapy in men who present with intentional self-harm to Emergency Departments in Ontario.

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Background

Every year, approximately 1,600 individuals present to Ottawa region Emergency Departments (ED) for self-harm. Provincially, there are roughly 16,000 unique presentations to the ED for self-harm. Care received in the ED is highly variable and when it does result in a connection to psychological services, these are often not covered by OHIP. Presentation to the ED with an episode of self-harm is associated with an increased chance of dying by suicide (1.2% in women; 2.7% in men) in the following year. Despite men only accounting for 40% of presentations for self-harm, nearly two-thirds of deaths by suicide after visiting an ED for an episode of self-harm are men. Blended therapy is the use of electronic resources to support traditional face-to-face psychotherapy. This approach may improve access to and engagement with care within a high-risk population.

Objective

The objective of this study is to evaluate a blended therapy approach, using a novel smartphone application, in men who present to Ontario EDs with self-harm.

Method

We have developed a platform comprised of a web-based clinician dashboard and patient-facing smartphone application to support delivery of Problem-Solving Therapy in 350 adult men who presented to the ED with self-harm. Seven (7) hospitals across Ontario have been randomized to receive the intervention, while fifteen (15) hospitals across Ontario will continue to deliver standard of care.

Anticipated Results

A pilot study we conducted using a beta-version of the application showed good engagement with both the face-to-face therapy sessions use of the application, while highlighting areas for improvement. Based on the pilot study, we expect a decrease in suicidality and depression scores as well as improvements on quality of life measures, with greater increases for those who attend more sessions of therapy. We expect that that the severity of suicidality at six weeks will decrease proportionally with the number of PST sessions completed by participants, with participants having completed three sessions or more of smartphone-assisted PST experiencing less suicidality. We also expect that participants who complete three or more sessions of smartphone-assisted PST sessions will experience improvements in quality of life, amelioration of depression, anxiety and PTSD symptoms and a reduction in health care costs.

Conclusion

Our study has the potential to increase engagement and improve outcomes in a high-risk population, men who self-harm, while also decreasing the economic burden on the healthcare system in Ontario utilizing a novel smartphone application to support Problem-Solving Therapy.

WITHDRAWN

EGF Stimulates Asymmetric Divisions in Muscle Stem Cells and Enhances Regeneration of Dystrophin-Deficient Muscle

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Background

Dystrophin loss in Duchenne Muscular Dystrophy (DMD) leads to progressive and severe degeneration of skeletal muscle that is exacerbated by reduced rates of asymmetric division of muscle stem cells (satellite cells), reduced progenitor generation, and impaired regeneration. In-niche screening of *Myf5-Cre/ROSA26-eYFP* myofibers against well-characterized library of compounds that have defined therapeutic targets, we have identified compounds modulating activation/proliferation kinetics of satellite stem cells and their committed progeny. We identified epidermal growth factor receptor (EGFR) and Aurora kinase A (Aurka) as regulators of muscle stem cell asymmetric division.

Results

In resting tissue, we found EGFR polarized to the basal surface of satellite cells while ex vivo stimulation of myofibers with EGF promotes apico-basal alignment of Aurora kinase A at mitotic centrosomes resulting in a 2.5-fold increase in asymmetric satellite stem cell divisions. Ex vivo perturbation of EGFR or Aurora kinase A by pharmacological inhibition or siRNA knockdown reduced the capacity of satellite stem cells to perform asymmetric divisions, defaulting to symmetric divisions that expand the stem cell pool. Importantly, inhibition of Aurora Kinase A abrogated EGF stimulation of asymmetric division rates. EGF treatment of dystrophin-deficient satellite cells from *mdx* mice similarly increased asymmetric division rates, resulting in increased progenitor formation and augmented regeneration. EGF treated *mdx* tissue show significant increase in myofiber formation without hypertrophy and significantly improved muscle strength, suggesting activating EGFR polarity pathway leads to a rescue in dystrophin-deficient satellite cell function. Pharmacological inhibition of this pathway could be used to therapeutically modulate stem cell numbers and enhance muscle regeneration.

Transvaginal ultrasound characteristics differentiating surgical presentations of endometriosis

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Background: Endometriosis is the presence of endometrial tissue outside of the uterine cavity, which can present with a variety of different histologic and phenotypic subtypes. Ultrasound is the first line investigation, but these can be limited in accuracy for diagnosing deep infiltrating disease. Advanced endometriosis transvaginal ultrasound (AETU), performed by an experienced clinician, can improve detection rates of deep endometriosis by incorporating real-time standardized evaluation.

Objective: To determine which clinical characteristics and findings by AETUs can significantly differentiate the surgical presentation of endometriosis.

Patients and Methods: Six-hundred and forty-three AETUs were performed on 614 patients at The Ottawa Hospital by two expert imagers (MF or VDZ) between January 2014 and December 2017. Of these, 187 patients underwent an endometriosis related surgical procedure after their AETU and were included in this study. Patient demographics, clinical presentation, findings on AETU, and surgical diagnosis were systematically collected from patient charts.

Patients were grouped into three categories based on surgical presentation: patients with deep endometriosis – **DeepEndo**; patients with superficial – **SupEndo;** and patients that did not have excision of or were not suspected to have endometriosis – **NoEndo**. Comparisons in categorical variables were evaluated using Chi-square tests and adjusted residuals with significance accepted at absolute z-score>1.96. One-way ANOVA's and Kruskal-Wallis tests evaluated differences among respective continuous and ordinal variables at the $p<0.05$ level.

Results: Of 60 cases reviewed, 23 patients were surgically confirmed to have DeepEndo, 17 with SupEndo, and 20 with NoEndo. Groups did not significantly differ in age, parity, or gravidity; however, DeepEndo (23.3±3.7kg/m^2) had significantly lower BMI than NoEndo (29.7±4.3kg/m^2; p=0.011) and SupEndo (28.2±6.6kg/m^2; p=0.030).

No significant associations in clinical presentation (symptoms and nodularity) were observed across groups. However, endometriomas were more likely to be suspected on AETU in cases of DeepEndo (77.3%) compared to NoEndo (26.3%) and SupEndo (31.3%) (Main Effect p=0.002). Similarly, a greater association of AETU-suspected POD obliteration and ovarian fixation was observed in cases of DeepEndo (7.0%; 85.0%) compared to NoEndo (22.2%; 52.9%) and SupEndo (36.4%; 50.0%) (p=0.010; p=0.047).

Conclusion: Preliminary results demonstrate significant associations between observations on AETU and the surgical presentation of endometriosis. Frequencies of suspected endometriomas, POD obliteration, and ovarian fixation were more likely to be observed in cases of DeepEndo than SupEndo or NoEndo, corresponding with previous studies. AETU allows real-time standardized evaluation of endometriosis, and shows potential in assisting with the identification of important disease characteristics and diagnosis of deep endometriosis prior to surgery.

GREB1 expression in reproductive tissues and ovarian cancer

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Background: Estrogenic hormone replacement therapy is a known risk factor for ovarian cancer, but the underlying mechanisms are unknown. Estrogen (E2) accelerates tumour initiation and progression in mouse models of ovarian cancer. We have shown that Growth Regulation by Estrogen in Breast Cancer 1 (GREB1) is highly upregulated by E2 in these tumours. GREB1 is required for E2-driven proliferation of several hormone-responsive cancer cell lines, and is a transcriptional cofactor with ESR1, but may have additional functions.

Objective: This project explores the clinical relevance of GREB1 by assessing its expression in human ovarian cancers and cell lines, compared to normal tissues. It also investigates possible correlations between ESR1, GREB1, and patient outcomes.

Methods and results: To determine if GREB1 mediates E2-signaling, we evaluated GREB1 levels in normal human and murine E2-responsive tissues. Nuclear GREB1 is expressed in ovarian surface epithelium, fallopian tube epithelium, and stromal cells of the ovary, fallopian tube, and uterus. Strong cytoplasmic expression was seen in endometrial epithelium. This suggests a function for GREB1 in normal reproductive processes. GREB1 is E2-induced in the PEO1 ovarian cancer cell line, but not in the OVCA433 cells, despite their expression of ESR1.

In human ovarian cancers, mRNA expression of *GREB1* and *ESR1* positively correlate but, unlike breast cancer, GREB1 and ESR1 protein in tissue microarrays do not correlate. The majority of ovarian cancers express GREB1 (80-100%), with a trend for higher levels of expression in the serous and endometrioid subtypes. Analysis of high-grade serous cancers (HGSOC), the most common subtype, shows no correlation between GREB1 and ESR1, and no correlation between GREB1 expression and overall survival (O.S.). However, when patients are segregated by stage, GREB1 expression correlates with increased O.S. in stage 1 patients, with a similar trend in stage 2 patients. Further, in ESR1+ tumours, GREB1 absence correlates with improved progression-free survival (P.F.S.). When patients are segregated by response to chemotherapy (P.F.S. >12 months), absence of GREB1 correlates with increased P.F.S. in responders.

Conclusion: These data suggest that, in HGSOC, GREB1 may have stage-specific activity and it may be a biomarker of poor prognosis in patients whose tumours express ESR1. The frequent expression of GREB1 in ESR1-negative tumours indicates that GREB1 can be regulated by factors other than E2. The high prevalence of GREB1, a growth promoting factor, in reproductive tissues and ovarian cancers warrants further investigation of its function in these tissues.

Identifying Barriers and Facilitators to Improving Reporting and Reducing Risk of Bias in Preclinical Studies

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Background: Use of rigorous study design methods and maneuvers (such as randomization and blinding) and their transparent reporting in publications are two key strategies proposed to improve the reproducibility of preclinical research. To promote use of these methods, the National Institutes of Health (NIH) has released a set of Principles and Guidelines. Despite endorsement by journals and funding agencies, assessments by our research group and others suggest uptake has been low.

Objective: This study aims to identify barriers and facilitators to the use of the NIH Principles and Guidelines for preclinical research by preclinical scientists.

Methods: We addressed this objective by conducting a qualitative interview study. Semi-structured interview guides were developed from the Theoretical Domains Framework (TDF). The TDF is a validated, consensus-based framework, which encompasses 14 domains (synthesized from 84 theoretical constructs). Application of this framework to our study ensures that a broad and comprehensive range of theories relevant to behaviour are considered. Interview transcripts were coded in duplicate using directed content analysis.

Results: To date, interviews with 6 cancer researchers and 12 cardiovascular researchers have been completed. From a preliminary analysis of 10 interviews we see that scientists are highly motivated to apply the methods outlined by the NIH. The majority noted benefits to their application, including potentially improved quality, reproducibility and translation of preclinical research. Most researchers expressed having access to a statistics department or working with a biostatistician better enables application of some methods.

However, researchers also noted that these guidelines can result in increased sample sizes, expenses, time, and can require several personnel to operationalize. While not always felt to be a personnel barrier, it was noted that new investigators and small labs may find the financial implications of the guidelines to be an obstacle. A few researchers also mentioned concerns about using large numbers of animals (based on sample size calculations).

Furthermore, our interviews have helped to identify several proposed strategies to improve routine use of the guidelines (e.g. a universal training platform, harmonization of guidelines across institutions and journals etc.), as well as several items that could be addressed through workshops (e.g. methods for randomization and blinding).

Conclusions: Upon completion of this study, we plan to develop educational tools and outreach activities for preclinical scientists to help address the identified barriers. Obtaining perspectives from both investigators and trainees will help to ensure a wide range of potential barriers and facilitators are considered.

Crosstalk between the Planar Cell Polarity Pathway and Hedgehog signaling Regulates Satellite cell Fate

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Muscle stem cells, known as satellite cells, are regulated by a variety of extrinsic factors. Amongst them we have identified the ligands Wnt7a and sonic hedgehog.

Wnt7a activates the planar cell polarity (PCP) pathway to drive symmetric satellite cell expansion. Conversely, sonic hedgehog is an activator of cilia mediated hedgehog signaling (Hh) signaling, which holds roles in cell cycle regulation and myogenic differentiation. Crosstalk between these pathways has been well characterized during development, and is likely to be conserved in muscle regeneration. Accumulating evidence suggests the PCP pathway influences primary cilia formation, an organelle required for proper Hh signal transduction. Indeed, Wnt7a treatment on cultured myoblasts increases the presence of primary cilia. Using myofibers culture, we demonstrate that Wnt7a increases myogenic differentiation. Additionally, removal of primary cilia through a small interfering RNA for IFT88 impedes Wnt7a mediated differentiation suggesting crosstalk between the PCP pathway and cilia mediated Hh signaling is responsible for this response. We have identified the downstream PCP effectors, Inturned and Fuzzy as the main candidates responsible for this crosstalk. Knockdown of either Inturned or Fuzzy significantly reduces primary cilia number. Additionally, knockdown of Inturned reduces Wnt7a mediated differentiation further supporting its role in crosstalk between these pathways. Overall, our data suggests that crosstalk between the PCP pathway and Hh signaling regulates the differentiation of satellite cells.

FGL2 is a Mediator of Surgery-Induced Tumour Metastasis

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Background: The tumour microenvironment (TME) in ovarian cancer is infiltrated by populations of highly suppressive immune cells, such as regulatory T cells (Tregs), which allows the tumour to escape the immune system. Fibrinogen-like protein 2 (FGL2) is a Treg effector protein that promotes immunosuppressive activity and high levels are associated with poor overall survival in several malignancies. Using the TCGA database of 374 ovarian cancers, we found that FGL2 expression correlates with expression of markers for immunosuppressive cells (FOXP3, TIM3, CD39, LAG3) and for immune checkpoint inhibitor targets (PD1, PD-L1, PD-L2, CTLA-4).
Objective: Given that increased numbers of Tregs in ovarian cancers predict poor patient survival, we hypothesize that high FGL2 expression correlates with poor survival and promotes tumour progression in ovarian cancer by fostering an immunosuppressive environment.
Methods: Patient samples (FFPE tumours and ascites fluid) were assayed by immunohistochemistry and ELISA for the presence of FGL2. Mouse models of ovarian cancer (intra-bursal/intra-peritoneal) were used to assess the effect of FGL2 in the cancer cells vs. in the TME on tumour progression and survival. Intravenous injection of B16 melanoma cells, followed by nephrectomy, is a well-established model of surgery-induced metastases, in which the effect of FGL2 was assessed by quantification of lung metastases.
Results: FGL2 is present at variable levels in epithelial and stromal compartments of ovarian tumours, and in patients' ascites fluid. FGL2 levels in ascites ranged from 14 to 222 ng/ml, as determined by ELISA. To understand if FGL2 expression affects tumour progression in ovarian cancer, ID8 ovarian tumours were generated in FGL2 knockout (*fgl2*$^{-/-}$) mice vs. FGL2 overexpressing (*fgl2*^{tg}) mice. FGL2 was undetectable in the ascites fluid of *fgl2-/-* mice, averaged 15.1 ± 1.2 ng/ml in wild-type mice, and increased nearly 5-fold in *fgl2*^{tg} mice (72.1 ± 4.9). However, the presence of FGL2 in the TME had no effect on rate of tumour progression or survival. Similarly, intra-peritoneal tumours generated by ID8 cells with/without FGL2 expression had no effect on tumour progression or survival, T-cell infiltration, or vascularization (CD31). Interestingly, in a model of surgical stress metastasis, the absence of FGL2 reduced the number of metastases in the lungs.
Conclusion: The presence of FGL2 in ovarian cancer cells vs. the TME did not affect the rate of tumour progression; however, FGL2 contributes to the mechanism by which cancer cells metastasize to the lungs. Elucidating this mechanism may identify strategies to reduce metastases in cancer patients undergoing surgery.

A Translational Assessment of Adult Human Spinal Cord Neural Stem/Progenitor Cell Behaviour

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Background: The mammalian spinal cord harbors neural stem and progenitor cells (NSPCs) that are activated following traumatic injury. NSPCs can be utilized to promote regeneration in animal models through the regulation of their proliferation and differentiation behaviour. However, it is unclear how efficiently adult human spinal cord NSPCs can be modulated towards similarly beneficial fates.

Objectives: To compare the *in vitro* proliferation and differentiation fates of primary adult human and rat spinal cord NSPCs under identical conditions and to direct their fate using signaling factors.

Methods: Thoracic spinal cord was obtained from adult humans (n=15) and rats (n=10) and cultured using the neurosphere assay to expand NSPCs. Primary derived NSPCs were assessed for spontaneous differentiation with serum supplementation (1%) and for proliferation with mitogen treatment (epidermal growth factor, basic fibroblast growth factor2). To direct differentiation, NSPCs were treated with retinoic acid (RA), platelet derived growth factor (PDGFα), and bone morphogenic protein-(BMP4) to induce neurons, oligodendrocytes and astrocytes, respectively. NSPCs were treated for 7 or 14 days, fixed, and characterized by immunocytochemistry (β-III tubulin, GFAP, O4, Sox2, BrdU). BrdU was added 24 hours prior to fixation to track proliferation.

Results: Upon spontaneous differentiation, rat NSPCs favored a glial phenotype (74.6±6.7%) consisting mostly of astrocytes (71.0±4.2%) while human NSPCs formed mostly neurons (68.5±16.9% for NSPCs) with little gliogenesis (<2%). Mitogen stimulation increased proliferation of human (n=3) and rat (n=5) NSPCs similarly by 2.6±0.6 and 4.1±1.2 fold, respectively, after a 14 day treatment. Neuronal differentiation of human and rat NSPCs could be enhanced with RA treatment, PDGFα only increased oligodenrocyte differentiation of rat NSPCs, and BMP4 increased astrocyte differentiation of human NSPCs at 100ng/mL and rat NSPCs at 40ng/mL only after 14 days of treatment.

Conclusion: When cultured identically, adult human and rat spinal cord NSPCs possess distinct differentiation profiles and respond differently to external cues relevant to regeneration. This information is important for the translation of regenerative strategies targeting endogenous human spinal cord NSPCs.

Investigating the Role of VGF in Post-stroke Recovery

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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 stroke recovery and rehabilitation. VGF (non-acronym), a neuropeptide that is processed into several secreted peptides, has been identified as a post-stroke repair molecule playing a role in neurogenesis and the modulation of neuroinflammation. Our preliminary work has demonstrated the chemoattractant properties of VGF and Its ability to recruit immune cells (ICs) through C3a receptor (C3AR) signalling as well as its ability to influence neural stem cell (NSC) migration in the peri-ventricular region post-stroke. These findings suggest that an upregulation of VGF in the peri-infarct region could activate neurotrophic factors and complement signalling pathways, thus promoting post-ischemic migration of NSCs and ICs resulting in the clearance of damaged tissue, alteration in the peri-infarct microenvironment and ultimately improving functional recovery through the optimization of these repair processes.

Objective

In the present study, we assess the requirement for VGF and its secreted peptides during post-stroke neurogenesis and behavioural recovery.

Methods

Using a photosensitive dye and a collated beam of light, we induced stroke in the left frontal cortex of wild type and VGF cKO mice. All groups were further subdivided into mice that received adenovirus expressing VGF or no other treatment. Following stroke, the cylinder and horizontal ladder behavioral tests were performed to measure sensorimotor deficits over a four week period. Finally, mice were sacrificed and used to assess the levels of neurogenesis and neuroninflammation at timepoints ranging from 1-28 days post-stroke.

Results

We have determined that TLQP-21, a secreted peptide of VGF, recruits immune cells through C3AR. We have also determined that the loss of VGF in neural precursor cells results in decreased functional recovery as well as a decrease in NSC migration post-stroke.

Conclusion

We have used gain- and loss-of-function experiments to demonstrate that VGF and its derived peptides can improve recovery following photothrombotic stroke in the sensorimotor cortex. Preliminary results indicate that VGF peptides increase mobilization of NSCs from the ventricular zone. Further investigation is required to define the precise mechanism underlying the functional recovery and to fully determine the effectiveness of VGF as a relevant therapeutic for post-stroke recovery in the future.

Is histopathology required for the diagnosis of endometriosis?

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Background
Endometriosis is a disease of the female reproductive system involving the proliferation of glands and stroma outside the uterine cavity and affecting structures such as ovaries, pelvic peritoneum, cervix, vagina, recto and vesicovaginal septea; affecting approximately 5-10% of women of reproductive age. Visual laparoscopy followed by histopathological confirmation is the gold standard to establish the diagnosis of endometriosis. However, in many cases, endometriosis is treated with electrosurgical ablation rather than excision, and histopathology is not available. While endometriosis if often diagnosed by laparoscopic visualization alone in the absence of histopathology, it is unclear whether this approach is sufficient for accurate diagnosis of the disease.

Objective
The purpose of this study was to compare the diagnosis performance of the surgeon's laparoscopic visualization with histopathologic diagnosis of endometriosis.

Methods
A retrospective chart review was performed for women who underwent a gynecological surgery at The Ottawa Hospital between April 1, 2016 to March 31, 2017. Women were included if they had laparoscopy for endometriosis and concurrent biopsy for histopathological diagnosis. Information was extracted for the age, endometriosis associated symptoms, site and staging of endometriosis and surgical procedures. After describing frequency distribution of pertinent variables; sensitivity, specificity, predictive values and accuracy were calculated for comparison between diagnosis based on laparoscopy only with laparoscopy plus histopathology.

Results
Among 1069 women who were admitted for non-malignant gynecological surgical indication, 96 women met our inclusion criteria for having laparoscopic biopsy with histopathologic report for suspected endometriosis. Among this cohort, mean age was 40 years (SD 7.2 years), while 29.2% had dysmenorrhea and 41.7% had abdominal/pelvic pain. More common sites of endometriosis were ovarian endometriomas (31.3%), deep infiltrating endometriosis (25.0%), especially in the rectovaginal septum (14.6%) and the uterosacral ligament (19.8%) and finally, endometriosis on the uterus, the uterine surface/serosa (21.9%), the pelvic peritoneum (14.6%) and the bowels (16.7%). Endometriosis was diagnosed in 79/96 women by surgeon during laparoscopy and was confirmed by histopathology in 64 of these 79 women. The sensitivity and specificity for laparoscopic visualization of endometriosis were 90.1% and 40.0% respectively, while the positive and negative predictive values were 81.0%, and 58.8%, respectively.

Conclusion
Surgeons have a high probability of detecting endometriosis when it is present; while misdiagnosis is more likely when endometriosis is absent. The probability of endometriosis is high when surgeons have made a diagnosis by visualization alone. However, when endometriosis is not visualized by the surgeon, misdiagnosis is possible.

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Comparison of Weight-Based and Lymphocyte Count-Based Dosing of Anti-Thymocyte Globulin in Matched Unrelated Donor Stem Cell Transplantation

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Background: Graft-versus-host disease (GVHD) is a significant detriment to patient health following allogeneic stem cell transplant. Anti-thymocyte globulin (ATG) is used to reduce the incidence and severity of GVHD, yet optimum dosing has yet to be determined. We have previously demonstrated that ATG doses of 2.5 mg/kg as part of transplant conditioning can reduce the incidence of GVHD in unrelated donor transplants. Recent literature suggested weight-based dosing of ATG is not ideal and that absolute lymphocyte count (ALC) based dosing could lead to more optimum exposure of the drug.

Objective: We sought to determine if weight-based versus ALC-based dosing differs in clinical outcomes in our patients.

Methods: We conducted a retrospective single-center study analyzing all patients at TOH who received a matched unrelated donor (MUD) stem cell transplant for any indication between 2009 and 2014. Patients received a total pre-transplant dose of 2.5 mg/kg of rabbit ATG (Thymoglobulin®). Univariate and multivariate analysis were used to determine if patient or transplant related factors, including weight, ALC, and total ATG administered, impacted GVHD, relapse, or mortality.

Results: 119 patients who underwent MUD transplant with ATG were identified. The median age was 50 years, 64% male, and the mean weight was 80.5 kg. The most common diagnosis was AML (42.8%). The mean ALC on day -2 was 2.15 x 10^9/L. The mean total dose of ATG received was 201.2 mg (SD 51.4).The incidence of acute and chronic GVHD was 37.0% and 20.2% respectively.

If patients received ALC-based dosing according to the published calculation (Admiraal,R et al., Lancet Haematology 2017) the mean dose would have been 1151 mg, over five times the administered weight-based dose. In a multivariate Cox model, the total weight-based dose of ATG (in increments of 25mg) administered was not associated with GVHD (HR=1.08, 95%CI 0.96-1.2), relapse (HR=1.10, 95%CI 0.94-1.27) or mortality (HR=1.06, 95%CI 0.89-1.25). Similarly, the pre-transplant ALC (in increments of 25x10^9/L) was not associated with GVHD (HR=1.06, 95%CI 0.77-1.45), relapse (HR=1.01, 95%CI 0.76-1.34) or mortality (HR=1.12, 95%CI 0.86-1.47).

Conclusions: The standard practice of weight-based dosing has recently been challenged by better understanding of the mechanism of ATG. At TOH with ATG administered at 2.5mg/kg, GVHD rates are acceptable and ALC is not associated with outcomes, suggesting ALC based dosing may not be needed. Prospective comparisons of the 2 dosing strategies are still needed.

Modulating EGFR signaling to stimulate muscle repair in Duchenne Muscular Dystrophy

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Background: Duchenne Muscular Dystrophy (DMD) is a lethal genetic neuromuscular disorder caused by the absence of dystrophin, a structural protein linking the muscle fiber cytoskeleton to the extracellular matrix. In DMD, fibers are extremely fragile and vulnerable to mechanical stress, which leads to continuous cycles of degeneration and regeneration and progressive loss of muscle function. Although DMD has been long thought as a myofiber-specific disease, our laboratory recently described it as a more complex disease involving muscle stem cell activity. Therefore, we undertook a small molecule screen to identify new regulators of muscle stem cell function and identified epidermal growth factor receptor (EGFR) that modulates the balance between satellite cell commitment and self-renewal. As EGFR stimulation increases myogenic commitment at the expense of stem cell expansion, we hypothesized that EGFR inhibition enhances satellite stem cell self-renewal and results in improved muscle regeneration.

Objective: To identify EGFR inhibitors that stimulate symmetric expansion of muscle stem cells.

Methods and Results: Our goal is to characterize the in vivo activity of candidate compounds on the satellite cell regenerative program and to assess the longer-term effect on ameliorating dystrophic changes in mdx mice. We first will perform short-term experiments where drugs will be directly injected into regenerating muscles following acute injury to identify candidates that act similarly in vivo in wild type and mdx mice. We will second perform long-term experiments where we will deliver candidate drugs systemically, and evaluate functional and histological changes to the mdx muscles over time. These experiments will identify which drugs work best for preventing degeneration of dystrophic muscles. In addition, to examine the satellite cell-specific role of EGFR for regeneration in vivo, we generated wild-type and mdx mouse models using an inducible Pax7CreER allele to delete EGFR specifically in satellite stem cells. Preliminary phenotypic analysis will be presented.

Conclusion: We hope that the small molecule inhibitors will stimulate satellite stem cell function which then will ameliorate the DMD phenotype by enhancing the regeneration function of satellite cells.

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Clinical Manifestations of Twenty-Two Patients Testing Negative for Anti-Nuclear Antibodies but Positive for Extractable Nuclear Antigen Antibodies.

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Background: Approximately one in fifteen Canadians suffer from autoimmune disease. Clinicians use autoantibody testing to help identify and diagnose autoimmune conditions. In general, patients with a positive ENA test also have a positive ANA test. However, there is a subset of patients that test negative for ANA.

Objective: The aim of this study is to analyze the clinical features of these patients to note if certain autoimmune conditions are more prevalent in this subgroup of patients. We hypothesize that these patients are more likely to have a specific autoimmune condition and this pattern of autoantibody testing could aid clinicians in diagnosis.

Methods: A retrospective chart review was completed at The Ottawa Hospital (TOH) on patients with a positive ENA test but negative ANA test between January 2014 and January 2018. Inclusion criteria included age greater than 18, testing done at TOH, and complete chart documentation. Exclusion criteria included pregnant females at the time of testing and incomplete charting. A total of 22 patients were included for review: we extracted data related to age, sex, indication for testing, physician department, ENA specific positive antibody types, clinical manifestations, and final diagnosis. In those cases, the Hep-2 ANA slides were reviewed to identify any additional patterns. This study is qualitative and descriptive in nature.

Results: A total of 26 patients (22 females, 4 males) were identified as having a negative ANA test but a positive ENA test. Four patients were excluded due to missing clinical information. Testing requisitions were most frequently ordered by rheumatologists. The most common ENA specific auto-antibody present was Anti-Ro52/TRIM 21 with 14 patients testing positive. A wide variety of clinical manifestations were documented, but as expected 17 of 22 patients exhibited signs and symptoms of connective tissue disease. The most prevalent final diagnoses were anti-synthetase syndrome (8 patients) and systemic lupus erythematosus (6 patients). Review of the ANA slides showed the majority were negative for any staining (65%) corresponding to low ENA positivity. Significant cytoplasmic staining was seen in 23% of the slides corresponding to high titer Ro-52 ENA.

Conclusion: In summary, our chart review of patients testing ANA negative but ENA positive, showed that over 60% of patients had a specific diagnosis, lupus or anti-synthetase syndrome. In this subset of patients, if ANA is negative but clinical suspicion is high, further testing is warranted. Due to the relatively small sample size, further study is required to delineate the relevance of these findings.

A tailored viro-immunotherapy combination approach for the treatment of drug resistant breast and ovarian cancers

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Background: Hereditary breast and ovarian cancers make up 5-10% of breast cancer 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) are a type of drug that targets the DNA repair pathway; many of these drugs are undergoing clinical trials and some are presently in use for cancers harbouring mutations in their DNA repair machinery, such as hereditary breast and ovarian cancers. Unfortunately, numerous patients become resistant to PARPi, leaving limited options for further treatment. Oncolytic or "cancer-killing" viruses are an innovative approach for treating even the most complex cancers. Oncolytic viruses target and destroy cancer cells, leaving normal cells unharmed, all while activating a patient's own immune system to fight the cancer. 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.

Objective: The aim of this project is to overcome PARPi resistance by engineering cancer specific viruses that will target genes relevant to the DNA repair pathway, sensitizing them to PARPi treatment in a synthetically lethal manner.

Methods: I have cloned shRNA sequences targeting components of the DNA repair pathway into the genome of an oncolytic rhabdovirus (a non-targeting control sequence has been inserted into the same virus previously). Prior to encoding shRNA sequences into the virus, validation of these targets was performed using siRNA transfection. Downregulation of targeted genes are being assessed via qPCR and Western Blot analysis following transfection and infection. Cell viability following treatment with PARPi +/- viruses will be assessed by Alamar Blue and Crystal Violet assays.

Results: I have demonstrated downregulation of siRNA targeted genes using qPCR analysis (Western Blot analysis is ongoing). Validation of the newly engineered viruses to show downregulation of their specific gene targets is currently underway. I have measured changes in cell viability for five different human/mouse cell lines following treatment with varying concentrations of two different PARPi to establish a dosing scheme for combination therapy with viral infection.

Conclusions: This project is in the early stages of development; therefore, no conclusions may be made thus far. I predict that validated cell lines and patient samples with intact DNA repair pathway machinery and/or those with previously demonstrated PARPi resistance will be sensitized by infection with targeted oncolytic viruses, resulting in enhanced cell death after combination therapy.

Impairment of cell lineage specification, mitochondrial function and autophagy signaling underlie development of tumor phenotypes in a human stem cell model of Tuberous Sclerosis Complex

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Background: Tuberous Sclerosis Complex (TSC) is a multi-system genetic disorder characterized by the development of low-grade but debilitating tumors in the brain, lungs, kidney and skin. TSC is caused by spontaneous or inherited germline mutations in the *TSC1* or, more commonly and with greater phenotypic severity, *TSC2*gene. We have established a human stem cell modeling platform for the multi-system tumorigenic cell types that develop in TSC using CRISPR-Cas9 genome engineering and directed differentiation into the neural and neural crest (NC) lineages, putative TSC cell types of origin. While dysplastic cells observed in patient tumors exhibit features of aberrant differentiation, mitochondria and vesicle accumulation, and cellular stress, the underlying mechanisms are largely unknown. Uncovering these mechanisms is necessary to develop effective treatments for TSC patients.

Objective: Investigate the mechanisms underlying the lineage-specific development of tumorigenic phenotypes in *TSC2*$^{-/-}$ stem cells.

Methods: Four pairs of isogenic control and *TSC2*$^{-/-}$ pluripotent stem cell lines were generated and differentiated using dual SMAD pathway inhibition through the neural and neural crest lineages. Neural and NC stem cells were expanded in culture to model tumor cell progression. RNA samples were harvested at multiple time-points for temporal RNA-sequencing analysis during lineage development. Cell lines were analyzed at developmental and progressive time-points for cell fate markers by immunocytochemistry and by live cell stains to identify markers of autophagy, lysosomes, mitochondria and reactive oxygen species (ROS). Seahorse extracellular flux assays was used to measure metabolic potential of control and *TSC2*$^{-/-}$ cell lines.

Results: Upon differentiation into neural and NC lineages *TSC2*-null cultures demonstrate a striking and progressive increase in mTORC1 activation, cell size, ROSbiomass accumulation (mitochondria, lysosomes, autophagosomes), and markers of aberrant cell fate that characterize phenotypes observed in the mesenchymal andlow-grade brain tumors observed in TSC patients. This typifies dramatic functional deficits in the lysosomal-autophagy pathway and in cellular energetics, highlighting a preference for glycolytic metabolism. Further neuronal differentiation establishes *TSC2*-null cultures with aberrantly high glial content and excitatory synaptic activity,reflective of TSC epileptic phenotypes.

Conclusions: Our findings, including kinetic RNA-sequencing analysis, indicate that *TSC2*$^{-/-}$neural/ NC cells exhibit highly aberrant lineage induction propensities, a heightened epithelial-to-mesenchymal transition, aberrant autophagy signaling and accumulation of defective mitochondria. We have established the first humanized model of the multi-system tumors in TSC and are further investigating the contribution of these mechanisms to the development and survival of aberrant TSC cells, focusing on identifying putative therapeutic avenues.

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Evaluation Mutation Detection Using Different Publicly Available Mutation Callers

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Recent studies employing next generation sequencing (NGS) technologies have identified specific genetic mutations that predict therapeutic efficacy with targeted treatment in many forms of cancer, including non-small cell lung cancer. To detect mutations, several tools have been recently introduced in the literature. However, it remains unclear which mutation callers are best, particularly for targeted DNA sequencing data. According to the Cancer Genome Atlas benchmark studies, as well as many other studies, there are substantial discrepancies between the mutations called with each tool. We have investigated the performance of five recent mutation callers (SAMtools, Mutect2, VarScan2, VarDict and Pisces) using different statistical metrics, including precision and sensitivity. We have used two sets of data, a synthetic-DNA dataset containing 397 known mutations, and TruSight Tumor 170 test data from replicates of a well-characterized Coriell cell line (GM12878) sequenced on an Illumina NextSeq. We initially observed large discrepancies between the variant sets called, but many disagreements turned out to be due to different default values for different built-in parameters—such as minimum allele frequency, minimum map quality, etc. Discrepancies are also partially based on the algorithms and model settings unique to each mutation caller. When matching parameters between programs as far as possible, performance was much more similar, although VarDict remained the best performer. We recommend standardizing the parameter values for different mutation callers to ensure fair comparisons, while we continue to investigate reasons for disagreements.

Risk Factors for Blood Transfusion in Women Undergoing Gynecological Surgery

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Background: Perioperative blood loss is a common complication in gynecological surgery known to increase the risk of receiving blood transfusion. Along with the usual risks of transfusion, transfusing women of child-bearing potential incurs a risk of alloimmunization, which may lead to hemolytic disease of the fetus and newborn. Therefore, identifying modifiable risk factors that can predispose patients to receiving a transfusion is of significant importance.

Objective: The objective of this study is to identify demographic, medical, and surgical factors that may predict the need for blood transfusion in patients undergoing gynecological surgery.

Methods: We performed a retrospective chart review of patients admitted to The Ottawa Hospital for gynecological surgery for non-malignant indications between April 1st 2016 and March 31st 2017. Data collected included patient diagnosis, intervention, demographics, preoperative blood work, medications, medical history, and previous transfusions before April 1st 2016. Patients requiring transfusion during admission were contrasted with patients not requiring transfusion.

Results: We identified 1069 patients who underwent gynecological surgery between April 1st 2016 and March 31st 2017. The mean age was 46.12□14.51 years; mean hemoglobin and hematocrit were 126.01□15.75 g/L and 0.38□0.04 L/L, respectively. Fifty (4.7%) patients received blood transfusion, the majority of which were red blood cell (RBC) transfusion (45 of 50, 4.2% of entire cohort).

Among patients requiring RBC transfusion, the mean age was 38□11 years; the mean hemoglobin and hematocrit were 101.82□22.94 g/L and 0.32□0.06 L/L, respectively. Patients had an increased risk of requiring RBC transfusion if they had preoperative anemia (RR: 8.37, 95% CI: 4.48-15.64), had previously received a transfusion (RR: 2.30, 95%CI: 1.01-5.24), or had a diagnosis of abnormal uterine bleeding (RR: 2.27, 95% CI: 1.12-4.58).

Several surgical risks for transfusion were identified. These included vaginal/hysteroscopic myomectomy (RR: 6.17, 95% CI: 1.79-21.22), abdominal salpingectomy (RR: 2.39, 95% CI: 1.30-4.39), and abdominal oophorectomy (RR: 3.02, 95% CI: 1.60-5.67). After controlling for preoperative anemia, only abdominal oophorectomy showed statistically significant association with RBC transfusion.

Conclusion: Preoperative anaemia is an important risk factor for needing RBC transfusion in the perioperative period. Patients at elevated risk of preoperative anemia and blood transfusion are those with abnormal uterine bleeding, submucosal fibroids requiring hysteroscopic myomectomy, ectopic pregnancy requiring abdominal salpingectomy, and pelvic masses requiring abdominal oophorectomy. Where bleeding is not immediately life-threatening and cancer is not suspected, such patients should be offered pre-operative patient blood management to avoid incurring risk of transfusion.

Poster Number: 63

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Antimicrobial peptide LL-37 and its truncated forms, GI-20 and GF-17, exert spermicidal effects and microbicidal activity against Neisseria gonorrhoeae

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Background: LL-37 exerts microbicidal effects on a number of sexually transmitted infection (STI) pathogens. Recently, we have demonstrated that LL-37 is also a spermicide (Srakaew *et al*., 2014). Hence it is attractive to develop LL-37 into a vaginal microbicide/spermicide. This potential, however, is challenged by its high synthesis cost. Searching for truncated LL-37 peptides with LL-37 equivalent potency is one solution to this challenge. Two truncated LL-37 peptides, GI-20 and GF-17, have been shown for their microbicidal activity against select microbes.

Objective: Determine whether 1) GI-20 and GF-17 have the same spermicidal effects as LL-37. 2) LL-37 and its truncated peptides have any adverse immunological/histological effects on female reproductive tract. 3) LL-37 and its truncated peptides have microbicidal action against STI pathogen, *Neisseria gonorrhoeae*.

Methods: The plasma membrane integrity, motility and ability to fertilize eggs *in vitro* of peptide-treated sperm were assessed as described by Srakaew *et al*., 2014. Sperm with or without peptide were transcervically injected into naturally cycling mice, and the success of *in vivo* fertilization was scored by formation of 2-4 cell embryos 42 h afterwards. Female reproductive tract tissues of peptide injected mice were then assessed histologically for any damages. Vaginal lavages of females transcervically injected with LL-37 were immune-assayed for pro- and anti-inflammatory cytokines by the Bioplex system. Antimicrobial effects (MIC/MBC) of each peptide were determined following standard methods.

Results: Like LL-37, treatment of sperm with GI-20 and GF-17 resulted in dose-dependent elevations of sperm plasma membrane permeabilization and immotility, reaching the maximum at 18 and 3.6 µM for human and mouse sperm, respectively. Mouse sperm treated with 3.6 µM GI-20 or GF-17 did not fertilize eggs either *in vitro* or *in vivo*. Reproductive tract tissues of female mice pre-exposed to 3.6 µM GI-20 or GF-17 and 18 µM LL-37 remained intact. LL-37 (36 µM) did not markedly induce the release of either pro-inflamatory cytokines (IL-1a, b, IL-6, TNF-a, MCP-1 and MIP-2) and anti-inflammatory cytokine (IL-10) in the vaginal lumen. At 1.8-7.2 µM, LL-37, GI-20 and GF-17 exerted bactericidal effects on *N. gonorrhoeae*.

Conclusion: The spermicidal efficacy of GI-20/GF-17 was the same as LL-37. All three peptides did not cause histological damages to the female reproductive tract, and cytokine levels were not elevated by LL-37 uterine exposure. All three peptides were effective antimicrobials against *N. gonorrhoeae*. With lower costs to synthesize GI-20/GF-17, these two peptides are therefore attractive to be developed into vaginal spermicides/microbicides.

MiR-145-5p regulates oligodendrocyte differentiation by targeting myelin gene regulatory factor – insights into remyelination failure in progressive multiple sclerosis

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Background/Objective: 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 to identify targets involved in this process, to better understand how high miR-145-5p may contribute to remyelination failure in pMS.

Methods: CNS cell RNAseq database Brain RNAseq and miR targeting algorithm TargetScan were analysed to predict miR-145-5p targets in differentiating OLs. MiR-145-5p targeting was assessed by dual luciferase assay. Lentivirus and small RNAs were used to overexpress and knockdown miR-145-5p in primary OPCs and OLs. Transduced cells were assessed for morphological and molecular changes by immunofluorescence. Molecular changes were further assessed by qRT-PCR.

Results: Expression of 15 OL-specific miR-145-5p targets was assessed following both overexpression and knockdown of miR-145. Amongst these, myelin gene regulatory factor (MYRF) was the most enriched factor in differentiating OLs by ~400X. MiR-145-5p knockdown in OPCs led to spontaneous differentiation, underpinned by increased expression of MYRF as well as extension of more complex branches. MiR-145-5p knockdown OLs displayed enhanced differentiation evidenced by increased expression of MYRF and greater myelin membrane area. The MYRF 3'UTR is directly targeted by miR-145-5p, and simultaneous knockdown of both miR-145-5p and MYRF blocked spontaneous differentiation of OPCs. Conversely, differentiating OLs overexpressing miR-145-5p showed severe defects in branching and myelin protein expression, including MYRF, and increased apoptosis. Finally, these defects could be rescued by concurrent overexpression of pro-myelination miRNA miR-219.

Conclusions: Taken together, these data suggest that miR-145-5p negatively regulates OL differentiation through direct targeting of MYRF. Downregulation of miR-145-5p is required for OL differentiation, but expression of miR-219 can compensate to push differentiation forward. The overabundance of miR-145-5p in the lesion microenvironment may be a factor in the OL differentiation block in pMS, and we highlight miR-219 as a relevant therapeutic target to help overcome this aspect of remyelination failure.

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. A 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 labeling (PL) using MyoD would allow for identification of histone modifications associated with muscle enhancers in an unbiased manner. Using a MyoD-TurboID fusion protein for proximity ligation, target histones were biotin labeled, 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.

Mechanisms of Periostin acquired expression in 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 depleted 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, dissecting this supplement into individual components is ongoing. Using size exclusion and ion exchange chromatography we are able to get a narrower pool of proteins in order to identify the component responsible for the repression of Postn by mass spectrometry. Using Q-PCR we can assess the mRNA 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 to prevent them from expressing Postn.

External Validation of The Ottawa Troponin Protocol

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Background:
Chest pain is the second most common emergency department presenting complaint among adults. Patients with chest pain are at risk for acute coronary syndrome which encompasses unstable angina, ST-elevation myocardial infarction (STEMI) and non-ST-elevation myocardial infarction (NSTEMI). The diagnosis of NSTEMI requires patients to be on a monitored bed and undergo serial TnI investigations. Our team previously developed "The Ottawa Troponin Protocol" (OTP) for NSTEMI diagnosis using serial TnI 3 hours apart. This protocol will allow for early disposition of patients to either referral to consultants or be discharged home safely.

Objective:
The primary objective of this study is to validate the diagnostic accuracy of "The Ottawa Troponin Protocol", in the subgroup of patients who present with any one of the TnI values above the 99th percentile.

Methods:
This study was a Health Records review conducted at the Civic and General Campuses of The Ottawa Hospital from August 2017 – December 2017. Adults (≥18 years) who presented to the ED who had serial TnI (at least two values 3-hours ± 15 minutes apart) performed for diagnosis of NSTEMI and at least one TnI value >45ng/L were included in the study. Patients who presented with cardiac arrest, STEMI, unstable angina or those with TnI values <45ng/L were excluded. The primary outcome was death and NSTEMI adjudicated by two blinded investigators within 30 days of the index ED visit. Data that was collected include demographics, medical histories, ED management, length of stay, TNI values and times of measurement, disposition, and outcome. We used descriptive statistics and test diagnostic characteristics to analyze our data.

Results:
We screened 53,077 patients, of whom 1,220 patients were included in the final study. 637 patients in that cohort who had more than 1 TnI value and that wasn't diagnosed with unstable angina were eligible for validation of the OTP. 109 patients were diagnosed with NSTEMI within 30 days of the index ED visit. The results of the validation showed that the OTP did not miss any patients diagnosed with NSTEMI. The sensitivity and the specificity of the OTP was 100% and 33% respectively.

Conclusions:
Our results show that the OTP is diagnostically accurate in ruling out NSTEMI in patients presenting to the ED with symptoms concerning of an NSTEMI. By using the OTP, this will allow for early referral to cardiology or medicine for management, early discharge home, and reduce ED waiting times.

The role of peptidylarginine deiminases in endothelial cell function

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Background: Vascular damage, which often stems from endothelial dysfunction, results in the thickening and stiffening of blood vessels, plaque formation and reduced blood flow. Peptidylarginine deiminases (PADs) are calcium-dependent enzymes responsible for the irreversible conversion of L-arginine to L-citrulline in protein. PADs have been linked to the development of a number of inflammatory diseases such as rheumatoid arthritis, lupus, multiple sclerosis and Alzheimer's. NET formation, which is mediated by PAD4, has been associated to the development of such pathologies. Whether PAD4 has a direct effect on the vasculature is not known.

Objective: This project aims to look into the role of PAD4 in endothelial cells to evaluate its effect on vascular function.

Methods: We have generated an endothelial-specific deletion of PAD4 in mice through a Cre-LoxP system. PCR analysis confirmed a lack of transcripts for PAD4 in knockout mice. Endothelium-dependent vasorelaxation was measured in both WT and KO mice.

Results: A decrease in vasorelaxation was observed in PAD4 knockout mice($P=0.0836$). A similar response was observed in resistance vessels following treatment with a pan-PAD inhibitor ($P=0.0542$).

Conclusions: Preliminary results suggest that PAD4 could potentially play a role in endothelial cell function.

WITHDRAWN

Examining caregiver distress among older adults with care needs: a population-based study

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Introduction: With an aging population, 2.2 million Canadians are projected to require informal care by 2031. About 80% of unpaid care is provided by informal caregivers. The negative consequences of caregiving, such as caregiver distress, is well documented. However, past studies often investigate caregivers of specific patient profiles (eg. dementia patients) rather than examining caregiver distress longitudinally or on a population level. Not only were sample sizes small and generalizability reduced, it was impossible to determine whether predictors of distress are the diseases or underlying symptoms or functional consequences. Furthermore, factors that are cross-sectionally associated with prevalent caregiver distress may not be the causes of distress due to the possibility of reverse causality.

Objective: This study uses population-level, longitudinal data to examine the current landscape of caregiver distress and the development of distress among caregivers of older adults in Ontario with care needs. Specifically, the objective is to describe the health profiles of senior care-recipients based on their caregivers' distress status and to identify predictors of both prevalent and incident caregiver distress.

Method This open cohort study uses the Resident Assessment Instrument for Home Care (RAI-HC) data in Ontario. 526644 adults over 50 years of age who have completed an initial RAI-HC assessment between Jan 1, 2007 and June 30, 2015 and reported having a primary caregiver were identified. Subsequent assessments that occurred within one-year of baseline were examined for changes in caregiver distress status. The main outcome measures were reports of caregiver distress at baseline (i.e. prevalent distress) and development of caregiver distress among caregivers who were non-distressed at baseline (i.e. incident distress). Descriptive and bivariate analyses were performed examining patient socio-demographic characteristics and health status/care use as well as caregiver/caregiving variables based on prevalent and incident caregiver distress. Two logistic regressions were also performed using patient and caregiver/caregiving variables as covariates and prevalent or incident caregiver distress as outcomes.

Results: Of the 526644 older adults, 24.3% reported their caregivers being distressed at baseline. Of the 223987 (42.53%) who had subsequent assessments within the year, the caregivers of 163093 were non-distressed at baseline. 16.6% of initially non-distressed caregivers became distressed within one year.

Results from the two logistic regression models are pending but will be ready for presentation. Covariates that are predictive of both prevalent and incident caregiver distress would provide strong evidence for causality.

Conclusion: The conclusion is pending but will be ready for presentation.

Validation of the PREDIGT Score for the Incidence of Parkinson's Disease

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Background: Fifty-five years after the concept of dopamine replacement therapy was introduced, Parkinson disease (PD) remains an incurable progressive neurological disorder. The inability to predict time and risk of PD incidence in healthy adults is a limitation in disease-modifying drug development, since ≥ 60% of dopamine neurons died by the time of clinical diagnosis. We recently designed an incidence prediction model founded on the concept that the pathogenesis of PD is a complex, multifactorial disease. This model considers five factors to determine cumulative incidence rates for PD: (1) DNA variants (*D*); (2) Exposure history to select environmental factors including xenobiotics (*E*); (3) Gene–environment interactions that initiate pathological tissue responses (*I*); (4) gender (*G*); and (5) time (*T*) encompassing ageing-related changes, latency of illness and propagation of disease. The proposed formula for calculating cumulative incidence rates for PD (P_R) is P_R (%) = (E + D + I) * G * T.
Objective: To validate the P_REDIGT model and have the preliminary assessment of the model's predictive accuracy.
Methods: We began to validate this model using baseline data from two longitudinal, nested-control cohorts: (1) the Parkinson's Progression Marker Initiative (PPMI) study for 492 patients with PD and 241 controls without neurological disease; and (2) the De Novo Parkinson (DeNoPa) study for 159 PD subjects and 110 age-and sex-matched healthy controls. Risk/protective factors were selected based on previous meta-analyses and the concept of PD pathogenesis as a multifactorial disease
Results: In the cohort of PPMI subjects, an initial assessment of the P_REDIGT model to distinguish subjects with PD from healthy controls generated an area under the curve (AUC) of 0.862 (95% CI: 0.833 – 0.892) with a sensitivity of 0.845 (95% CI: 0.809 – 0.882) and specificity of 0.671 (95% CI: 0.600 – 0.741) with an optimal cut-off of 59. In the DeNoPa cohort, the P_REDIGT model distinguished PD subjects from healthy controls at an area under the curve (AUC) of 0.769 (95% CI: 0.699 – 0.839) with a sensitivity of 0.779 (95% CI: 0.702 – 0.855) and specificity of 0.667 (95% CI: 0.544 – 0.789), with an optimal cut-off of 81.72.
Conclusions: The initial results validate the P_REDIGT model with a very good AUC. Work is currently ongoing to use additional cohorts (e.g. Epipark, PROBE, and PARS) for further validation studies and to calibrate/optimize the P_REDIGT formula and related coeffcients.

This work is supported by Parkinson Canada and the M.J. Fox Foundation.

Validity of a model to predict the risk of developing atrial fibrillation after major non-cardiac thoracic surgery

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Background: Post-operative atrial fibrillation (POAF) is the most common sustained arrhythmia following non-cardiac thoracic surgery and is associated with increased morbidity, mortality, and length of stay. Effective prophylactic therapies have been developed but are not recommended for low-risk patients due to potential side effects. Thus, there is a need to risk-stratify patients preoperatively to optimize prevention. Multiple factors that predispose a patient to developing POAF have been identified and integrated into prediction models, such as one developed and internally validated by Passman et al[1]. However, none have been externally validated.

Objectives: The primary aim of this study was to evaluate the validity of Passman's prediction model for POAF in patients undergoing non-cardiac thoracic surgery. The secondary aim was to assess the incidence, severity and risk factors of POAF at the Ottawa Hospital, the largest single thoracic oncology practice in Ontario.

Methods: A retrospective cohort analysis was performed using data collected from 2122 non-cardiac thoracic surgical patients at the Ottawa Hospital between 2008 to 2017. Demographic and surgical data as well as the incidence of complications were analyzed and compared to Passman's development sample. POAF risk was estimated in our population using Passman's prediction rule and then compared to the incidence and proportion of patients who developed atrial fibrillation (AF) in each risk category.

Results: There were no significant differences in baseline demographics and surgical factors between our validation sample and Passman's derivation population. The overall incidence of POAF in our population was 8.3%. The majority of the POAF cases occurred within 72 hours after surgery and were classified as grade 2 POAF (requiring only pharmacotherapy for management). The proportion of individuals who developed POAF increased incrementally with increased risk score derived from Passman's prediction model (0% with a risk score of 0 developed POAF vs 16.77% with a risk score of 6).

Conclusions: We have externally validated a prediction model for POAF developed by Passman and colleagues. Using preoperative heart rate, age and gender, we have demonstrated the model's ability to identify patients who are at increased risk for developing POAF. This prediction model is effective at identifying patients at risk of POAF who may benefit from targeted prophylactic POAF therapy and post-operative monitoring.

1. Passman RS, Gingold DS, Amar D, et al. Prediction rule for atrial fibrillation after major noncardiac thoracic surgery. *Ann Thorac Surg*. 2005;79(5):1698-1703. doi:10.1016/j.athoracsur.2004.10.058

Investigating heterogeneity in the muscle satellite stem cell population

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Background: Muscle stem cells provide the regenerative potential for skeletal muscle throughout adult homeostasis, while deficiencies in its maintenance may be the etiology of muscle diseases, such as Duchenne Muscular Dystrophy. Understanding the regulators of this stem cell population is therefore essential for cell therapies. One such regulator is Pax7, which uniformly marks muscle stem cells and is essential for muscle regeneration. However, the bulk population of Pax7+ muscle stem cells is highly heterogeneous. For instance, our laboratory has shown that within the Pax7+ population, the majority expresses Myf5 (Pax7+/Myf5+), while a small subset (Pax7+/Myf5-) does not and retains greater stem cell characteristics. We are interested in understanding whether these two populations are functionally distinct and contribute to different muscle types. Finally, we want to uncover the regulators that control each population and their lineage choices by using single cell sequencing technologies.

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

Methods: Muscle stem cells were sorted by fluorescence-activated flow cytometry (FACS) from *Myf5Cre;ROSA-nTnG* and *Myf5CreER;ROSA-mTmG* mice and captured with the 10X Genomics Platform. In parallel, molecular biology techniques were used to validate and further understand the lineage differences.

Results: We have successfully captured and sequenced >1000 muscle stem cells and have begun to bioinformatically characterize and stratify the populations. In parallel, we have conducted functional analyses on the Myf5+ and Myf5- populations with *in vitro* differentiation experiments, isolated myofiber culturing to assess for division kinetics, and an *in viv*o regeneration time course. Here, I will present preliminary data suggesting that a Pax7+/Myf5- lineage exists and is primed towards making fast (high glycolytic) myotubes.

Conclusion: Within the bulk population of muscle stem cells, some are functionally distinct and are primed for a different route of differentiation.

Comparison of the uniform field electroretinogram and the pattern electroretinogram to checkerboard and bar gratings

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Background
The pattern electroretinogram (PERG) is frequently employed for investigation of retinal dysfunction but is limited by requirements of steady fixation and refractive correction. The uniform field electroretinogram (UF-ERG) to stepwise luminance modulation of low temporal frequency permits delineation of retinal responses to increments and decrements of luminance that are otherwise indistinguishable in PERG and does not impose fixational or refractive constraints. Separate analysis of ON- and OFF- retinal responses provides valuable diagnostic utility in many retinal pathologies.

Objective
The purpose of the current study was to compare the electroretinal response associated with the uniform-field electroretinogram (UF-ERG) to that of the pattern electroretinogram (PERG) to checkerboard and bar grating stimuli in visually normal human subjects.

Methods
UF-ERG and PERG to bars and checkerboard were recorded in 18 patients (36 eyes) of mean age 45 years (range 20-75). UF-ERG was recorded to the increment and decrement of a 200ms duration luminance modulation. Luminance onset and offset UF-ERG responses were averaged to produce a simulation of the PERG response. PERG was recorded to checkerboard and bar grating stimuli of 0.9 cycles per degree at 98% contrast and 2 reversals/second. The mean amplitude and implicit time for the P_{50} and N_{95} potentials of actual and simulated PERG responses were recorded for each eye in the cohort.

Results
The simulated PERG waveform resulting from arithmetic averaging of the UF-ERG to luminance increment and decrement was characterized by prominent positive and negative components resembling those of the P_{50} and N_{95} PERG potentials. Implicit timing of the P_{50} potential was lengthened in the actual PERG to bars and checks relative to that of the simulation ($P < 0.05$, $P < 0.001$). Amplitude of the N95 potential was greater in the PERG to bars than in the PERG to checks ($P < 0.05$) or the simulated PERG ($P < 0.001$). All other comparisons between testing modalities were statistically non-significant.

Conclusions
The UF-ERG to light onset and offset can be reliably recorded in visually normal human subjects and closely mirrors actual PERG recordings. The UF-ERG simulated PERG recapitulates the actual PERG response to checkerboards better than that to bar-grating stimuli.

Neural Progenitor Cell Impairment and Vascular Remodelling in a Mouse Model of Neonatal Chronic Lung Disease

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Background: Preterm birth (<37 weeks gestation) is an urgent healthcare concern for the global community. Annually, a staggering 15 million infants are born preterm. The most common complication of preterm birth is a chronic lung disease called bronchopulmonary dysplasia (BPD). BPD is characterized by an arrest in lung growth. Infants with BPD are at a high risk of adverse neurodevelopment. Neural progenitor cells (NPCs) have not yet been investigated in BPD.

Objective: To determine whether NPCs, as well as the brain vasculature, are impaired in a mouse model of neonatal chronic lung disease, and whether this impairment leads to significant adverse neurodevelopment.

Methods: Mice were exposed to 85% oxygen from postnatal day (P) 0 to P14, to mimic the supplemental oxygen administered to preterm infants. NPCs were isolated at both P14 and 1 year of age. Cell-autonomous self-renewal was tested using successive neurosphere assays. Furthermore, the brain vasculature of neonatal mice was assessed by measuring vessel length, branching, tortuosity, as well as baseline and whisker-evoked cerebral blood flow responses. At 1 year, the DigiGait test, the rotarod test, the light-dark test, the Morris Water Maze, and a fear conditioning assessment were used to examine neurodevelopment.

Results: At both P14 and 1 year, NPCs from hyperoxia-exposed mice formed significantly fewer neurospheres than those isolated from control mice, demonstrating significant impairments in self-renewal. Neonatal hyperoxia-exposure also led to a significant reduction in vascular growth within the brain, as measured by smaller vessel length, fewer branching points, and a lower tortuosity compared to control mice. Hyperoxia-exposed mice also had significantly higher baseline cerebral blood flow levels and when stimulated, showed a significant deficiency in their ability to increase cerebral blood flow to the cortex. Moreover, 1 year after hyperoxia-exposure, mice showed long-term, significant adverse neurodevelopment, including motor coordination impairments, hypoanxiety, and learning and memory deficits.

Conclusions: Together, these results show that NPCs and the brain vasculature are functionally impaired in a neonatal mouse model of chronic lung disease, ultimately leading to long-term adverse neurodevelopment. Gaining a better understanding of the mechanisms involved in BPD-associated adverse neurodevelopment, will aid in the development of new potential therapies, to help reduce the life-threatening complications of this vulnerable neonatal patient population.

WITHDRAWN

Evaluating gastrointestinal morphology and enteric nervous system viability in the dystonia musculorum mouse model of 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 diarrhea, and abdominal pain. Similarly, a mouse mutant known as *dystonia musculorum* (*Dst*^{dt}) also arises due to mutations in the dystonin gene. In our studies, we have also come to recognize certain gastrointestinal pathologies present within this mouse model. At the level of gross morphology, we have observed discolouration of the gastrointestinal tract, as well as an accumulation of gas, which often causes distension of the ileum, cecum, and colon.

Methods:

As HSAN-VI and *Dst*^{dt} are primarily sensory neuron disorders, we hypothesize that the underlying cause for these gastrointestinal defects is due to impairments in the enteric nervous system. Here we aim to assess gut morphology by hematoxylin and eosin staining, evaluate the myenteric and submucosal plexuses by wholemount immunofluorescence staining, and assess function by GastroSense 750 tracking as well as gut absorption by plasma analysis.

Results:

In late stage *Dst*^{dt} mice (postnatal day 15) we have observed small, but significant reductions in ileum villi width and crypt length, as well as decreased smooth muscle wall thickness at all levels of the gastrointestinal tract. Despite the reduced muscle size, motility as assessed by tracing of the fluorescent GastroSense 750 marker appears to be normal in *Dst*^{dt} mice. Investigation of the enteric nervous system also reveals no difference in markers for cell death, and total number of neurons per ganglia in the myenteric plexus is unchanged.

Conclusion:

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

IL-1ß and TNF-a Stimulates Adipocyte-Derived TSLP Secretion via Different Signaling Pathways

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BACKGROUND/OBJECTIVES: Thymic stromal lymphopoietin (TSLP) is a cytokine that plays a role in regulating inflammatory responses. It has been implicated in platelet activation and atherosclerosis. We have recently shown that TSLP is a novel adipokine that is upregulated in TSH-stimulated adipocytes. TSLP is upregulated by TSH partially through a protein kinase A (PKA)-dependent pathway, as inhibiting PKA with H89 inhibited 66% of this response. We aimed to determine if other inflammatory factors can stimulate adipocyte-derived TSLP secretion and elucidate the signaling pathways that regulate them.

SUBJECTS/METHODS: Adipocytes were differentiated from stromal preadipocytes that were isolated from adipose tissue of surgical patients. Adipocytes were treated with IL- IL-1β, TNF-α, IL-6, IFNγ, and IL-4 for 24 hours. The media was collected and TSLP in the media was measured by ELISA. Inhibitors of the NF-κb pathway (sc-514) and MAPK pathways (SB203580, UO126, and SP600125) were used to investigate the signaling transduction routes used by these factors for adipocyte-derived TSLP secretion.

RESULTS: Similar to TSH, IL-1β and TNF-α significantly stimulated adipocyte-derived TSLP secretion at 24 hours. However, TSLP secretion was not observed when treated with IL-6, IFNγ, and IL-4. IL-1β dose-responsively stimulates TSLP protein secretion in differentiated adipocytes, with 20 ng/mL IL-1β resulting in a 16-fold increase ($P<0.01$). Inhibiting ERK1/2 with UO126 inhibited ($P<0.05$) TSH-stimulated TSLP secretion by 68%. An inhibitor of IKKβ, SC-514, blocked ($P<0.01$) IL-1β-stimulated TSLP secretion by 69%. NF-κb and MAPK inhibitors had no effect on TNF-α stimulated adipocyte-derived TSLP secretion.

CONCLUSIONS: TSLP is a novel adipokine that is upregulated by inflammatory factors in addition to TSH in human adipocytes. These studies are beginning to reveal the signaling pathways that regulate adipocyte-derived TSLP.

Does a peptidase control oolemma-zona pellucida detachment in murine oocytes?

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Background: Increasing use of assisted reproductive technologies means that oocytes spend more time exposed to stressors in suboptimal culture conditions. Before ovulation is triggered, the oocyte is firmly attached to its extracellular matrix shell, the zona pellucida (ZP). During the first four hours of meiotic maturation, the oocyte detaches from the ZP and begins independently regulating its volume. How this occurs mechanistically remains unknown. We hypothesize that oolemma-zona release is mediated by a peptidase that cleaves ZP proteins remaining in their transmembrane form.

Objective: We aim to identify a peptidase responsible for ZP detachment.

Methods: Candidate peptidases were identified by comparing a published RNAseq dataset for MII mouse oocytes (GSE70116), with those listed in the MEROPS peptidase database and whose Gene Ontology terms indicated localization in the plasma membrane or extracellular space. Inhibitors were tested to determine their effect on oolemma-zona release. An osmotic shock assay was used to detect progressive ZP detachment. Live cell imaging of maturing oocytes confirmed cell detachment and recorded volume changes in living oocytes. Protein expression and localization was assessed using immunofluorescence. Fluorescently tagged proteins were expressed using cRNA injections. Activation of independent cell volume regulation in oocytes was assessed using [^{3}H]-glycine uptake to detect GLYT1 activity, an oocyte-specific cell volume regulatory mechanism activated during meiotic maturation.

Results: The gamma secretase system was identified as a candidate by the in-silico screen. A gamma secretase inhibitor, BMS299897, delayed ZP detachment and reversibly maintained germinal vesicle stage oocytes in meiotic arrest. Live-cell imaging showed the expected release from the ZP and decrease in cell volume in control oocytes, but ZP release and the onset of cell volume decrease was markedly delayed in BMS299897-treated oocytes. The GLYT1 transporter, which normally becomes activated in oocytes to regulate cell volume during meiotic maturation, was suppressed by BMS299897. It has been reported that the protein kinase GSK3beta is a downstream target of gamma secretase. In the presence of BMS299897 GSK3beta becomes phosphorylated and inhibited. Exploration of the putative ZP protein cleavage site is ongoing and includes the use of ZP3 overexpression as well as cleavage site-mutated ZP3 mRNA (collaboration with Jurrien Dean, NIH). A monoclonal antibody targeting distal to the putative cleavage site will directly demonstrate cleavage.

Conclusions: We hypothesize a model where a gamma secretase mediated signaling pathway, involving GSK3beta, regulates detachment and osmotic regulation in the oocyte. Advancing understanding of these cellular processes should help improve in-vitro outcomes.

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Avoidant Attachment and Health Self-Efficacy Relate to Psychological Distress and Quality of Life in Treatment-Seeking Adults at a Rehabilitation Centre

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Background: Some individuals have difficulty adapting to a chronic illness or disability (CID), that is, they experience psychological distress (PD) and poor quality of life (QoL). The attachment behavioural system guides responses to internal (e.g., illness symptoms) and external (e.g., uncertainty) signals of danger. Health self-efficacy is important in predicting adaptation to CID.

Objective: Examine the cross-sectional relationships between attachment anxiety and avoidance, health self-efficacy, PD, and QoL among treatment-seeking adults with a CID.

Methods: Patients completed reliable and valid questionnaires assessing attachment anxiety and avoidance, health self-efficacy, PD, and QoL. This study was approved by the institution's research ethics board. Two hierarchical regressions were conducted with PD and QoL as the dependent variables. In each regression, age and biological sex were entered in step 1, followed by anxious and avoidant attachment in step 2, and health self-efficacy in step 3.

Results: Participants included 54 adults (70% female), with a mean age of 47 years (*SD* = 12). Approximately 80% (*n* = 43) of the sample reported clinically significant levels of PD. Mean QoL was low, falling almost 2 SDs below the mean of the general population and a sample of patients with cancer. Results reveal that in step 1, age and sex did not significantly account for variance in QoL. Adding anxious and avoidant attachment in step 2 significantly explained an additional 21% of the variance in QoL, $F(2,49) = 6.66$, $p = .003$. Avoidant attachment ($pr = -.389$, $p = .005$), but not anxious attachment ($pr = -.085$, $p = .552$), was a significant unique predictor in the model. Including health self-efficacy in step 3 significantly explained an additional 24% of the variation in QoL, $\Delta R^2 = .238$, $F(1,48) = 21.25$, $p < .001$, for a total of 46.3% of variance explained, $F(5,48) = 8.27$, $p < .001$, $R^2 = .46$. In this model, avoidant attachment ($pr = -.479$, $p < .001$) and health self-efficacy ($pr = .554$, $p < .001$), but not anxious attachment ($pr = -.038$, $p = .796$) were significant unique predictors of QoL. A similar pattern of results emerged with PD as the outcome variable.

Conclusions: High avoidant attachment was related to poorer QoL and more PD. After accounting for age, sex, and anxious and avoidant attachment, higher health self-efficacy was related to better QoL and less PD. Findings contribute to our understanding of adaptation to CID, and may help further tailor rehabilitation interventions.

Determining the Mechanism of IFN-? Suppression in Surgically Stressed NK Cells

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Background:
Surgery is a necessary intervention for patients with solid cancers, however surgery may increase rates of metastasis and death. Evidence supports a role for the suppression of the cellular immune response, including Natural Killer (NK) cells. We have shown a profound decrease in extracellular NK cell-derived IFN-γ in post-operative colorectal cancer patients following stimulation with a cocktail of activating cytokines containing interleukin (IL)-2 and IL-12. The intracellular mechanism(s) responsible for defective IFN-γ production by surgically stressed NK cells are unknown; however, we hypothesize that *the inability of surgically stressed NK cells to produce IFN-γ is the result of dysfunctional stimulatory cytokine signalling pathways*.
Objectives:
The objectives were toanalyze intracellular and extracellular IFN-γ by flow cytometry and ELISA in NK cells from cancer surgery patients using multiple stimulatory conditions *in vitro*.

Methods:
Blood was collected from healthy donors and cancer surgery patients at Baseline and on POD1 and peripheral blood mononuclear cells (PBMCs) were isolated by Ficoll density centrifugation. PBMCs were incubated in the presence or absence of activating stimuli (PMA-ionomycin and IL-2/12) before undergoing intracellular staining to analyze the expression of IFN-γ in $CD56^{+}CD3^{-}$cells by flow cytometry. Cell supernatants were collected from these samples and extracellular IFN-γ was quantified by ELISA.

Results:
IFN- γ production has been assessed in thirteen patients with various cancer types. Analysis of intracellular IFN-γ by flow cytometry revealed a reduction in both the percentage and median fluorescence intensity of IFN-γ+ NK cells following surgery in 11/13 patients when stimulated with IL-2/12. Interestingly, the production of IFN-γ in response to PMA-ionomycin was impaired in only 5/13 patients, suggesting variability in the ability to overcome this post-operative defect. A similar defect in extracellular IFN-γ was observed on POD1. It is clear from flow cytometric analysis that reduced IFN-γ measured by ELISA is the result of a defect in production in response to IL-2/12, and not in its release.

Conclusions:
Our optimized assay showed a consistent inability of surgically stressed cells to produce intra- and extra-cellular IFN-γ in response to IL-2/12, as opposed to PMA-ionomycin. This supports our hypothesis that the defect responsible for reduced IFN-γ lies in the signaling pathways downstream of IL-2 and IL-12. Knowledge of the mechanism(s) behind decreased IFN-γ will pave the way for novel perioperative therapeutics to reverse NK cell dysfunction and decrease metastatic recurrence in surgical oncology patients.

Hypogammaglobulinemia and rheumatic diseases.

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Background

Combined variable immunodeficiency (CVID), the most common primary immune deficiency disease. Patients with CVID can, in 20-25% of cases, be diagnosed with a concurrent autoimmune disease. Furthermore, there are patients with autoimmune diseases who may have undiagnosed CVID and that are predisposed to an increased risk of infections with routine antirheumatic treatment. The association between autoimmune diseases and CVID suggests screening should de done in these patients.

Objective

The purpose of our research project is to explore the Ottawa hospital's (TOH) electronic health records of patients with hypogammaglobulinemia and document screening patterns for rheumatic disease occurring within this patient populations. Ultimately, we aimed to explore the screening practices and rheumatologic diagnoses in CVID patients.

Methods

We collected and analyzed 671 patient charts from the Ottawa hospital (TOH) laboratory information system to identify individuals with hypogammaglobulinemia between January 2015 and January 2018. Our inclusion criteria entailed: 1) Patients older than 18 2) Testing for hypogammaglobulinemia performed at TOH 3) Treating physician present at TOH with available documentation 4) IgG levels of 3.0 g/L or lower 5) Evidence of impaired B cell function if available. Patients with hematologic cancers or B cell depleting therapies were excluded. We extracted data pertaining to: indication for antibody testing, ordering physician specialty, antibodies tested, clinical manifestation of patient, and final diagnosis.

Results

31 patients with hypogammaglobulinemia were included in our chart review. Recurrent infections was the most common clinical presentation (45%). The specialty most involved was infectious disease (29%). The most common panel of tests ordered together was RF, ANA, and ANCA (19%). The most common indication for antibody testing was excluding the presence of a primary inflammatory process (52%). Furthermore, in only 10% cases was the indication for antibody testing to rule out the presence of a concurrent autoimmune disease in a patient with CVID.

Conclusions

There is a lack of uniformity regarding the screening for autoimmune diseases in patients with hypogammaglobulinemia. Further study is required to determine the best practice in assessing for rheumatic disease and inversely to screen for immunodeficiency in rheumatic patients.

The association of kidney function and albuminuria with the risk and outcomes of syncope: A population-based cohort study.

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Background

The risks and subsequent outcomes of syncope among seniors with chronic kidney disease (CKD) are unclear.

Objective

The aim of this study was to examine the association of kidney function (as measured by eGFR and ACR level) with incident syncope and to examine the association of syncope with adverse outcomes in patients with CKD.

Methods

We conducted a population-based retrospective cohort study of 272,146 patients ≥66 years old in Ontario, Canada from April 1st2006 to March 31st2016. Using administrative healthcare databases, we examined the association of estimated glomerular filtration rate (eGFR) and urine albumin to creatinine ratio (ACR) with incident syncope, and the association of incident syncope with the composite outcome of myocardial infarction, stroke, death by levels of eGFR/ACR through Cox proportional hazards models with adjustment for confounders.

Results

A total of 15,074 incident syncopal events occurred during the study period. The adjusted risk for syncope was higher with a lower eGFR and higher ACR in a step-wise manner [eGFR 60-<90: HR 1.17 (1.09-1.26) vs. eGFR <30: HR 1.67 (1.50-1.87) with eGFR ≥90 referent; ACR >30: HR 1.15 (1.07-1.24) with ACR <3 referent]. Among the 12,710 patients with a first syncope event and 1 year of follow up, the adjusted risk for the composite outcome was higher with a lower eGFR and higher ACR in a step-wise manner [eGFR 60-<90: HR 1.05 (0.90-1.22) vs. eGFR <30: HR 1.62 (1.34-1.96) with eGFR ≥90 referent; ACR >30: HR 1.77 (1.60-1.96), ACR <3 referent].

Conclusions

In older adults, a lower eGFR and higher ACR are associated with a higher risk of a hospital encounter for syncope, and of complications after a hospital encounter with syncope.

Bifluorescent analysis of alpha-synuclein aggregation

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Background

Parkinson's disease (PD) is an incurable neurodegenerative synucleinopathy. Patients often have an extensive prodromal phase that features gastrointestinal (GI) deficits (e.g., constipation). The Braak hypothesis postulates a prion-like spread of alpha-synuclein aggregates from the enteric to the central nervous system, wherein the vagus nerve acts as the propagation highway.

Objective

Research is directed toward analyzing the initiation site and mechanism of alpha-synuclein aggregation.

Methods

Our novel model utilizes bifluorescent complementation, wherein the aggregation of two alpha-synuclein monomers stimulates the assembly of a functional Venus fluorescent protein. This quantifiable fluorescent signal will enable assessment of the critical initiation mechanisms of PD, which could not previously be studied. We call this our BiSyn/UBC-Cre-ERT2 model (i.e., Bifluorescent complementation of alpha-Synuclein aggregates using tamoxifen-induced Cre recombinase under the ubiquitin promoter).

Results

In vivo – Although our transgene is ubiquitous, recent experiments have shown that the associated protein expression is mosaic. This inconsistent pattern of expression begins in early mouse development and persists into adulthood.

In vitro –Like our mice, the MEFs also vary in transgene expression. In contrast, with this system cells are able to undergo selection using geneticin such that those expressing the transgene are protected from protein synthesis inhibition by the neomycin resistance gene. This selection process allows us to develop an entire line of transgene-expressing cells. We are then able to activate transgene recombination using tamoxifen, as we would *in vivo*, and subsequently stimulate aggregation of synuclein using either rotenone or preformed fibrils.

Conclusions

In vivo – We do not expect that the difference in protein expression between individuals will affect our *in vivo* studies because we are considering the propagation of aggregates to have a prion-like mechanism. Therefore, we hypothesize that our fluorescent signal will initiate in BiSyn-expressing cells, and will propagate to other cells via connective pathways.

In vitro – Our mouse embryonic fibroblast (MEF) culture system has been an important tool in understanding our model. We hypothesize that synuclein aggregation will assemble the Venus protein, emitting quantifiable levels of fluorescence.

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Evaluation of the FIGO classification in predicting anemia and transfusions in women with abnormal uterine bleeding

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Background:
Abnormal uterine bleeding (AUB) is one of the most common complaints for which women present in gynecologist's office or primary-care setting and often require medical or surgical management. Despite numerous efforts to treat heavy bleeding, many of these women have anemia as a result of their acute or chronic loss of blood volume leading to increased morbidity and mortality in this population. Since its release in 2011, the FIGO classification system has been used to classify women with AUB. Because it can be applied preoperatively, it can serve as a valuable tool for identifying women who are at higher risk of anemia and requiring transfusion prior to surgery, thus not only reducing morbidity and mortality but also increasing the quality of life of women with AUB.

Objective:
To describe the risk of anemia or transfusion based on selected FIGO classifications for abnormal uterine bleeding.

Methods:
A retrospective cohort study was conducted to assess the prevalence of anemia and transfusion in women with abnormal uterine bleeding who underwent a gynecological surgery for non-malignant indication at the Ottawa Hospital between April 1st, 2016 and March 31st 2017. Using a standardized data collection form, a chart review was conducted to collect FIGO classification and relevant outcomes. Data analysis using SPSS was conducted to compare rates of anemia or need for transfusion by FIGO classification in women with AUB.

Results:
320 patients were identified as having gynecological surgery for abnormal uterine bleeding. Average age of patients was 43.93(±7.95), and most patients were undergoing hysterectomy (88.1%). The mean preoperative hemoglobin was 124, 23.1% of the cohort had pre-operative anemia and 10.6% had transfusions. Most patients had leiomyomata (74.7 %), adenomyosis (43.8%) or both (84.7%). FIGO classification categories found to be associated with significantly less preoperative anemia were malignancy/hyperplasia as well as non-submucosal leiomyomata ($p<0.05$). None of the FIGO classification categories found to be associated with need for transfusion.

Conclusion
This cohort study identified using administrative health data, provides an overview of pre-operative anemia and transfusions in women with AUB. While lower risk of pre-operative anemia was associated with non-submucosal leiomyomata and malignancy/hyperplasia, other categories were not. These findings can be used by health care professional when planning medical management and can contribute to enhancing quality of life and reducing morbidity in women with AUB.

The effects of opioids administration on the severity of Obstructive Sleep Apnea: A systematic review

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Dr. Gaspard Montandon. Scientist, Keenan Research Centre for Biomedical Science, Assistant Professor, Division of Respirology, Department of Medicine, University of Toronto.

Dr. Moussa Meteb. Research Assistant, Ottawa Hospital Research Institute.

Aseel Ahmad. Research Assistant, The Ottawa Hospital Research Institute, and Honours Bachelor in Health Sciences (BHSc), University of Ottawa.

Randa Ahmad. Research Assistant, The Ottawa Hospital Research Institute, and Honours Bachelor in Health Sciences (BHSc), University of Ottawa.

Background:

Obstructive sleep apnea (OSA) is a common condition characterized by repetitive nocturnal complete collapses (apneas) or partial collapses (hypopneas) of the upper airway during sleep. These events are associated with oxygen desaturation and/or arousal from sleep.

Many patients with diagnosed and undiagnosed OSA use opiates/opioids to treat conditions like acute and chronic pain. There is a potential that administration of these drugs to people with co-existing OSA may worsen OSA.

OSAS has been associated with elevated cardiovascular risk and increased morbidity and mortality. Observational studies have shown that adequate treatment of OSA with CPAP can reduce the incidence of cardiovascular events in patients with any severity of symptomatic OSA.

Objectives:

1. To investigate the effects of opioids administration on the severity of Obstructive Sleep Apnea (OSA) (as measured by the apnea-hypopnea index (AHI), oxygen desaturation index (ODI) or respiratory disturbance index (RDI), or degree of nocturnal oxygen desaturation), and sleep architecture, and sleep quality in individuals with OSA.
3. What are the short and long-term consequences for the use of opioids in adults (≥18) males and females with known OSA, compared to those with OSA not treated with opioids?
   In adults (≥18) with known obstructive sleep apnea and on opioids, what is the effect of positive airway pressure treatment on the severity of OSA (as measured by the apnea-hypopnea index (AHI), oxygen desaturation index (ODI) or respiratory disturbance index (RDI), or degree of nocturnal oxygen desaturation), short and long-term consequences compared to individuals with OSA and on opioids who are not treated with PAP?

Methods:

We searched MEDLINE, EMBASE, the Cumulative Index to Nursing and Allied Health Literature (CINAHL), PsycINFO, Cochrane Databases and bibliographies of identified articles and reviews, will be considered eligible.

Results:

Conclusions:

The Role of SAP102 as a Modulator of Dopamine D1 Receptor Function

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Background:
Dopamine (DA) is a catecholamine neurotransmitter that regulates diverse brain functions including motivation and reward, motor control, cognition and affect. The five subtypes of DA receptors are grouped into D1-class receptors (D1R and D5R) and D2-class receptors (D2R, D3R and D4R) based largely on their canonical signaling pathway corresponding to facilitation of increased (D1-class receptors) or decreased (D2-class receptors) cAMP production through activation of their associated G proteins. D1-class receptors have a high degree of amino acid sequence identity overall, but certain regions, including the third intracellular loop (IL3) and c-terminal tail (CT), have much lower sequence identity which suggests a role of these regions in the differential regulation of D1R and D5R functional properties through post-translational modifications and interactions with different signaling partners. Our lab used yeast two-hybrid screens with IL3 and CT regions of D1R and D5R as bait to identify potential proteins in D1-class receptor signaling complexes. We found a protein called synapse associated protein 102 (SAP102) that interacts with the IL3 of D1R but not D5R, and confirmed the interaction in HEK293 cells and mouse striatum and hippocampus using co-immunoprecipitation. SAP102 is a member of the PSD-95 subfamily of membrane-associated guanylate kinases (MAGUKs) which primarily function to bind to and stabilize proteins at synapses, and are involved in the regulation of glutamate receptor expression and trafficking. Interestingly, altered SAP102 levels and function have been observed in conditions with dopaminergic dysfunction, including models of Parkinson's disease and L-DOPA-induced dyskinesia.
Objective:
We aim to investigate the role that SAP102 may play in the assembly of the D1R signaling complex and modulation of D1R signaling pathways and trafficking.
Methods:
ELISA, immunoblotting, whole-cell cAMP assays and radioligand binding studies were performed using HEK293 cells.
Results:
SAP102 was found to modulate D1R-mediated cAMP production and activation of ERK signaling pathway. Additionally, we found that SAP102 may enhance D1R expression and facilitate greater agonist-induced internalization.
Conclusions:
SAP102 appears to be important for D1R function, thus devoloping an understanding of the mechanisms underlying the effects of SAP102 on D1R function could have relevance for the development of improved therapeutic options for condtions with dopaminergic dysfunction.

Meaningful and Active Patient Engagement in Research: Our Institution's Journey

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3. The Ottawa Hospital

Background: The spectrum of patient engagement in research ranges from information sharing to full collaboration and partnership. Academic hospital patient engagement occurs across multiple domains, including point-of-care and research. Our success in integrating research engagement within the hosptial level, began by harnessing existing resources across domains and working collaboratively. Shifting the paradigm of research to a patient-and-family-centred model requires a concerted effort, support, and strong leadership. We will describe our journey, successes to date, and the barriers encountered. We will outline the resources that were co-designed with patients and reveal our sustainability plan.

Objective: For patients to become partners in research, a cultural shift is needed within the traditional model where the patient experience is not treated as expertise. This poster describes the approach and process taken by the Ottawa Methods Centre (OMC) and the Ottawa Hospital Patient and Family Advisory Program (TOH-PFAP) in creating and establishing an infrastructure for patient engagement.

Methods: By embedding the Strategy for Patient-Oriented Research (SPOR) guiding principles into the TOH-PFAP infrastructure and framework, we aligned the goals of both programs. Our partnership supports high-quality patient-centred research across TOH and the Ottawa Hospital Research Institute (OHRI). We have created an employee facing SharePoint site to act as the ultimate resource hub for TOH and OHRI staff looking to engage patients in their work. The SharePoint site houses the tools and resources that we have created including a checklist for getting started with patient engagement. The entire process has involved patient partners.

Results: Co-design is imperative. Following the re-design of the TOH-PFAP framework, the program was re-built through collaboration with patients. Many resources for research, including checklists and recruitment forms, were created. The barriers we encountered include a lack of guidance and the unpopularity of research among patient advisors. In investigating the latter, we found that our patient demographic is concerned with the time commitment required for many research projects. The time commitment is strict and patients are looking for flexibility. To address this, training is planned for both researchers and patients to help elucidate the many roles for patients and the types of engagement that exist (Evidence: https://www.ncbi.nlm.nih.gov/pubmed/29392529).

Conclusions: Patient engagement in research can be established, promoted, supported, and sustained within existing hospital-wide PFAP activities. Replication is possible wherever PFAP has been established.

The efficacy and safety of MSC therapy in preclinical animal models of myocardial infarction: A systematic review and meta-analysis

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Background: Post-surgical myocardial infarction (PMI) is one of the most common causes of perioperative morbidity and mortality. It is a unique form of myocardial infarction, due to significant inflammation associated with the disease caused by surgical stress. Small clinical studies have assessed mesenchymal stromal cells (MSCs) for non-operative acute MI, however, they have not been evaluated in patients with PMI. Due to the anti-inflammatory properties of MSCs, and the heightened state of inflammation associated with the post-surgical period, it is possible that MSC therapy can be of even greater benefit in patients with post-surgical MI. Prior to considering a clinical trial of MSC therapy for PMI, we performed a systematic review and meta-analysis of available preclinical data.

Objective: To evaluate the efficacy and safety of MSC therapy for acute MI in preclinical animal models.

Methods: MEDLINE, Embase, and BIOSIS were searched from inception until January 18th, 2018. Controlled comparative (MSC vs vehicle) *in vivo* studies of MI or cardiac ischemia-reperfusion injury were included. Only studies that were perioperative (i.e. anesthetic provided before or concurrent to acute MI) were included. Our primary outcome of interest was the change in left ventricular ejection fraction (LVEF), and our secondary outcome was mortality. Random effects meta-analysis was performed with LVEF expressed as standardized mean difference (SMD) ± 95% confidence intervals (95% CI) and mortality as risk ratio ± 95% CI.

Results: A total of 4,076 citations were reviewed and 284 studies were included. The risk of bias of included studies was unclear across most domains. The majority of studies were performed in rodents, and administered bone-marrow derived MSCs intra-myocardially less than 24 hours post-MI induction. The administration of MSC therapy was associated with a significant increase in LVEF (n=3738 animals) (SMD 1.92; 95% CI 1.75-2.09, I^2=82.4%), as well as a significant reduction in the risk of death (n=3150 animals) (RR 0.77; 95% CI 0.65-0.92, I^2=0%). Although significant methodological (study design, species, dose, cell source, etc) heterogeneity existed between studies, the effect of MSC therapy was consistent across multiple subgroups.

Conclusion: The present study provides the most comprehensive preclinical systematic review and meta-analysis to date of MSC therapy for MI. Our results demonstrated the ability of MSC therapy to increase LVEF and decrease mortality, compared to vehicle control. Our results suggest MSC therapy for PMI might be considered for future clinical translation.

Investigating Roles of Circulating MicroRNAs and Immune Cells in Sepsis

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Introduction and Aim: Sepsis is a complex syndrome that can be initiated by the host body's own overwhelming response to infection, potentially progressing to a prolonged immunosuppressive state in patients and long-lasting debilitated immune response in survivors. Each year, sepsis and sepsis-induced multiple organ dysfunction account for millions of deaths worldwide. There are currently no specific treatments for sepsis other than supportive care. MicroRNAs (miRNAs) are a class of small non-coding RNAs that are key regulators of gene expression, including a wide spectrum of targets related to inflammation and infection. Sepsis is associated with alterations in circulating miRNAs that may reflect an underlying response to infection and/or inflammation. Our hypothesis is that circulating miRNAs may be released from cells or tissues in response to organ injuries occurred during sepsis, and the modulation of these miRNAs in animal models of sepsis may result in therapeutic benefits. Here, we sought to identify miRNAs with potential therapeutic effects for sepsis.

Methods and Results: Using high-throughput PCR arrays, we previously identified several miRNAs that were concordantly increased in plasma of septic shock patients and mice with experimentally-induced sepsis compared to their respective healthy controls (n=6-8 per group). One miRNA candidate of interest, miR-146a, has been implicated in the modulation of inflammation and was selected for further study. We conducted gain- and loss-of-function experiments by transfecting macrophages cell line Raw264.7, with synthetic miRNA mimics and related inhibitors of miR-146a (Life Technologies). Elevated and decreased levels of miR-146a were confirmed in cells transfected with the miRNA mimickers and inhibitors, respectively, by RT-qPCR. Macrophages exposed to proinflammatory stimuli (i.e., lipopolysaccharide and interferon-gamma) showed increased levels of induced nitric oxide synthase (iNOS) activity, but this was selectively attenuated in cells transfected with miR-146a mimic, compared to cells that received a negative control mimic or miR-146a inhibitor.

Conclusions: Supplementation of miR-146a levels in macrophages attenuates inflammation-induced iNOS activity, suggesting elevated levels of miR-146a found in the plasma of septic shock patients may represent a compensatory mechanism to offset the harmful vasodilation and low blood pressure in these patients. However, the impact of miR-146a on hemodynamics and organ disfunction in sepsis require further research using preclinical animal models.

Molecular control of genome architecture

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BACKGROUND: The nuclear lamina regulates higher order genome organization. There are two key proteins required for heterochromatin tethering, Lamin A and the Lamin B receptor (LBR). Uniquely, adult mouse rod photoreceptors express neither of these proteins. As a result, they have an unique nuclear architecture. Heterochromatin occupies the centre of the "inverted nucleus", while euchromatin surrounds the nuclear periphery. This feature of adult mouse rods makes them a convenient model for visualizing and studying the molecular determinants of higher order genome organization. Importantly, in several mouse models of retinal degeneration, chromatin inversion is lost, raising the question of how higher order nuclear organization contributes to the degenerative process.

OBJECTIVE: Although the laminar tethering of heterochromatin is a well-described phenomenon, the molecular mechanisms regulating this process remain poorly understood. We propose to use mouse rod photoreceptors as an assay system for tethering sufficiency. We hypothesize that the C-terminus of Lamin A binds heterochromatin directly to facilitate genome partitioning. Our goals are to perform a structure-function analysis of lamin A, in order to identify the critical domain responsible for heterochromatin tethering, and to elucidate the genomic and transcriptomic consequences of heterochromatin tethering.

METHODS: To determine how heterochromatin tethering proteins such as Lamin A and LBR affect genome organization, we ectopically express them in mouse rods. We inject plasmid DNA into the eye subretinally in 1 day old CD1 mice and transfect the DNA by in vivo electroporation. After 6 weeks, when rod nuclei are mature, we prepare the eyes for analysis. ATAC-seq and RNA-seq experiments will help to determine the genomic and transcriptomic changes of the observed phenotype changes of Lamin A.

RESULTS: Lamin A causes profound reorganization of the rod nucleus and is sufficient for heterochromatin tethering. On the other hand, GFP-tagged Lamin A doesn't affect the rod genome. A recombinant lamin comprised of the Lamin B1 N-terminus and Lamin A C-terminus causes partial tethering of heterochromatin in mouse rods.

CONCLUSIONS: The Lamin A C-terminus might be the key for heterochromatin tethering, but N-terminal occlusion also appears to be function-blocking, suggesting that there are multiple domains containing affinities for different types of heterochromatin. In the future, we will develop new molecular tools for identifying and studying genome/lamina interactions. We will use them to better understand the role of higher order nuclear organization on genome control and cellular function, and also how these pathways break down during retinal degeneration.

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The impact of frailty on outcomes in adult trauma patients: a systematic review and meta-analysis

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Background: Many geriatric trauma patients have frailty, a multidimensional syndrome relate to accumulation of age- and disease-related deficits, which contributes to poor outcomes. Frailty tools have been systematically reviewed in trauma, however the association of frailty with outcomes after a traumatic injury has not been synthesized.

Objective: To measure the association between frailty and outcomes after multisystem trauma.

Methods: A systematic search was developed and underwent the PRESS checklist. The search was applied to MEDLINE, EMBASE and CINAHL from inception to May 2018. Epubs ahead of print, in-process and other non-indexed citations were included. Keywords for frailty were combined with trauma specific keywords. No language limitations were applied. Studies where the majority of patients were not trauma admissions or had isolated fragility fractures, isolated burns or isolated head trauma were excluded. A protocol was registered. The primary outcome was mortality. Studies included in the primary analysis had to adjust for injury severity and mechanism of injury to decrease risk of confounding bias. Secondary outcomes included health, resource use, and patient experience outcomes.

Results: We identified 1 181 titles; 56 underwent full text review; 14 were included for final analysis. Included studies represented 3 704 total participants (range from 63-879). Ten included studies were prospective and 4 were retrospective. Studies used 8 different frailty instruments, including muscle cross-sectional area (n=2), Vulnerable Elders Survey (n=2), comorbidity-polypharmacy score (n=1), Clinical Frailty Scale (n=1), psoas: lumbar vertebral index (n=1), upper-extremity function (n=1), frailty index (n=3), and Trauma-Specific Frailty Index (n =3). Of 772 people without frailty, 37 died (5%). Of 1 189 people with frailty, 145 died (12%). On an unadjusted basis, frailty was associated with mortality (odds ratio 2.76, 95%CI 1.90-4.01, P<0.01). Full results of the meta-analysis will be presented at the conference.

Conclusions: A growing literature describes the association between frailty and outcomes in adult trauma patients. Findings of our study will inform prognostication for patients, families and clinicians and will identify important knowledge gaps for future studies.

Surgical outcomes for transgender men undergoing mastectomy and hysterectomy

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BACKGROUND: The term transgender refers to an individual whose gender identity is opposite to their assigned sex at birth. Approximately 0.6% of people in the United States identify as transgender, and the majority pursue the expression of their gender identity through transition. Transitioning is largely a medical and surgical process, and gender-affirmation surgery is a pertinent step in relieving gender dysphoria for transgender individuals. Gender affirmation surgery can be classified as "top surgery" (at the chest) and "bottom surgery" (below the waist). About 93% of transgender men have had or desire mastectomy and about 79% have had or desire hysterectomy as part of gender affirmation. Society has become much more progressive, and LGBTQ+ issues have been garnering increasing attention and acceptance. This social shift has allowed transgender individuals to express their identities much earlier than in the past. An increasing number of mastectomies and hysterectomies are being performed for transgender men each year, therefore surgical outcomes warrant consideration. At present, there is little research concerning surgical outcomes for transgender patients undergoing gender affirmation surgery, however the existing research seems to demonstrate low post-operative complication rates.
OBJECTIVE: To determine rates of surgical complications in transgender men seeking gender-affirmation surgery, including mastectomy and hysterectomy.

METHODS: A descriptive study using the American College of Surgeons National Surgical Quality Improvement Program (NSQIP) Participant User Files (2015 – 2016) was conducted. Transgender patients were identified based on International Classification of Diseases (ICD10) diagnostic codes (Z87.89, F64). Surgical cases of mastectomy and hysterectomy were identified using Current Procedural Terminology procedure codes (19301, 19303, 19304, 19318, 58150, 58262, 58542, 58550, 58552, 58570, 58571).

RESULTS: We identified 104 cases of mastectomy and 252 cases of hysterectomy for transgender men. Transgender men appear to seek top and bottom surgery on average around 29 to 30 years of age. Over 90% of top and bottom surgeries were performed in an outpatient rather than inpatient setting. Over 90% of hysterectomies were performed laparoscopically for transgender men. Surgical site infection was the most common complication for both top (1.9%) and bottom (1.6%) surgery. Transgender men demonstrate low operative complications for both mastectomy and hysterectomy.

CONCLUSION: Transgender men seeking gender affirmation surgery do not appear to be at elevated risk of surgical complications. Our findings support transgender men as suitable candidates for mastectomy and hysterectomy with appropriate surgical outcomes.

WITHDRAWN

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WITHDRAWN

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Viral Sensitizers Potentiate Infection of Cancer Cells via NFB

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Background: Genetically engineered oncolytic viruses (OVs) can be effective anti-cancer agents. However, the heterogeneity of tumours and attenuation of OVs can limit their efficacy. Our group has previously shown that small molecules called Viral Sensitizers (VSes) used in combination with OVs can potentiate the infection of cancer cells up to over 1000-fold resulting in tumour specific killing both *in vitro* and in mouse tumour models.

Objective: In this study, we investigated the effects of VSe1, and analogs of VSe1 with more favorable physicochemical properties, on innate cellular antiviral signalling to further refine the mechanism of action of this class of compounds.

Methods: Oncolytic VSV-resistant renal carcinoma cells were treated with VSe1 or analogs, or vehicle, followed by infection with VSV. The expression of various cytokines, activation and nuclear translocation of transcription factors involved in the antiviral response were analyzed using real-time PCR, ELISA and Western immunoblotting. In parallel, affinity capture experiments revealed that these small molecules interact with glutathione-s-transferase-π (GSTP1-1). GST inhibition assays and knockdown experiments were used to assess the impact of VSe1 and analogs on glutathione metabolism.

Results: Our results show that VSe1 and analogs inhibit OV-induced nuclear translocation of NFκB and expression of antiviral cytokines such as IFNβ, TNFα, and IL-6. While VSe1 and analogs induce oxidative stress and deplete cellular glutathione, siRNA knockdown of GSTP1-1 does not alter viral sensitivity of cancer cells.

Conclusions: More work is necessary to investigate whether these viral sensitizers are acting through a direct or indirect mechanism. Future work including the use of click-chemistry will aim to determine whether VSe1 and analogs act through direct binding or modification of redox-sensitive residues of NFκB.

Identifying small-molecule treatments for the rare lung cancer Lymphangioleiomyomatosis

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BACKGROUND

Lymphangioleiomyomatosis (LAM) is a rare monogenetic lung cancer characterized by immature smooth muscle-like cells invading the lung parenchyma and axial lymphatics. The genetic basis of LAM is a loss-of-function mutation in either the TSC1 or TSC2 gene, leading to hyperactivation of the mechanistic target of rapamycin (mTOR) signalling. An mTOR inhibitor, sirolimus, is the only treatment option for LAM patients, yet the treatment demonstrates limited efficacy. Thus, there exists a critical need for more effective therapy options.

Our lab has established a variety of patient-relevant LAM cell models (LCMs), including the isolation of smooth-muscle cells from TSC2-/- teratoma explants (ESC and iPSC-derived). By testing an 800-drug library of Health Canada-approved cancer therapeutics, we have identified a short-list of small molecules which demonstrate differential toxicity between LCMs and unedited controls.

OBJECTIVE

The goal of this project is to further investigate our selected therapeutics and build pre-clinical evidence for candidate translation.

METHODS

First, we will conduct dose-toxicity assays in LCMs and controls. We will then employ our small molecules in a lung-mimetic 3D culture system to elucidate differential inhibitory effects on invasiveness. The top drug candidates will be assessed in a mouse model of LAM. To robustly simulate LAM in vivo, we will perform peripheral-tail vein injections of our LCMs in immunodeficient mice, whereby injected cells migrate to the lung and recapitulate LAM pathology. Daily oral treatments of our top candidates will be administered, and LCM growth will by monitored by bioluminescence imaging and pathological analysis at the experimental end-point.

SLK Deletion Restores Myoblast Differentiation downstream of TGF-beta in a p38 Dependent Manner

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Duchenne Muscular Dystrophy is a fatal X-linked disorder affecting 1 out of every 3500 male births. The underlying cause of Duchenne is mutations within the dystrophin gene resulting in loss of protein expression, which leads to myofiber instability and damage. The constant damage of skeletal muscle causes sustained immune infiltration, marked by increased levels of cytokines, such as TGF. Interestingly, TGFcan decrease the myogenic potential of satellite cells, thus preventing muscle regeneration. Previously, our lab has shown that knockdown of the Ste20-Like Kinase, SLK, in normal mammary epithelial cells was sufficient to delay TGF induced epithelial to mesenchymal transition. Therefore, we speculated that decreasing SLK levels would be sufficient to decrease the anti-myogenic effects of TGF both in cultured myoblasts and in a mouse model of muscular dystrophy. In the first section of this study, we explored the effect of muscle specific deletion of SLK on muscle development and regeneration. Skeletal muscle specific deletion of SLK did not impair muscle development, but caused a myopathy in older mice. Additionally, muscle regeneration was delayed, but not inhibited by SLK deletion. These findings indicated that SLK has beneficial roles in skeletal muscle, but was not absolutely required for optimal muscle development and regeneration. In the second section, we investigated the potential for SLK knockdown to mitigate the anti-myogenic effects of TGFin vitro. Decreasing levels of SLK restored myoblast differentiation in the presence of TGF in a p38 dependent manner. In the final section, we determined that SLK levels are elevated in dystrophic muscle and that subsequent deletion of SLK in the mdx mouse enhances terminal differentiation of myoblasts without further exacerbating the pathology of the disease. Collectively, this work demonstrates that SLK inhibition can provide a protective effect against the anti-myogenic effects of TGF via upregulation of p38 activity.

Ontario Tumour Bank Initiative at The Ottawa Hospital

Harman Sekhon[1], Angel Arnaout[1], Sebastien Gilbert[1], **Nikita Rayne[2]**, Kerin Hudson[2], John Bartlett[3], Monique Albert[3]

The Ottawa Hospital[1], The Ottawa Hospital Research Institute[2], Ontario Institute for Cancer Research[3]

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 samples from over

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 February 2018 issue Oncogene by revealing an adhesion molecule as a new potential target of cancer therapy.

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 Kerin Hudson.

Pre-exposure Prophylaxis Provides More than Simply HIV Risk Reduction

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Background: HIV pre-exposure prophylaxis (PrEP) was first approved in Canada in 2016. Since then, the number of gay and other men who have sex with men taking PrEP has grown steadily.While there are sound clinical data on the efficacy of PrEP, how PrEP is used as well as its risks and benefits in the real world remain largely undescribed.

Objective: This study aimed to investigate the efficacy of PrEP, how PrEP is used and its potential risks and benefits.

Method: We conducted a cross-sectional study including 113 men attending the PrEP clinic at the Ottawa Hospital. Mean age was 39.9 years (SD = 10.0).

Results: All participants identified as men and most had sex with only men (92.0%) or mostly men (6.2%). The vast majority had completed postsecondary schooling (86.7%) and 52% had an annual income of $80,000 or more. Essentially all men (98%) took PrEP daily and 74% indicated they rarely if ever forgot to take their pills. Most men disclosed their use of PrEP to both regular and causal sexual partners (87% and 83% respectively). Comparing before to after initiating PrEP, there was no significant change in sexual activity among participants including number of partners or sexual positions. Condom use, however, decreased by half or more. Most striking was the decrease in sexual anxiety. A full 50% of participants reported being stressed frequently or always during sex, and 60% felt stressed frequently or always after sex. Once they started PrEP, only 1.7% reported feeling stressed frequently or always during sex, and no one reported feeling stressed frequently or always after sex. Recreational drug use in the past three months was fairly high, with cocaine (19.5%), MDMA (16.8%) and GHB (15.9%) being the most common.

Conclusion: Our results indicate men taking PrEP tend to be educated and affluent. While taking PrEP did not lead to changes in sexual activity, condom use did decrease as did sexual anxiety.

Restored insulin signaling rescues memory deficits in a preclinical mouse model of Alzheimer's Disease

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Alzheimer's disease (AD) is a primary form of dementia and is characterized by the progressive deterioration of brain tissue, resulting in the early loss of memory in AD patients. One important AD hallmark is the accumulation of amyloid β (Aβ)-containing plaques which may enhance inflammatory processes in the brain. Vascular Aβ deposition is followed by impairment of cerebral blood flow, which also contributes to AD pathology. Type 2 diabetes (T2D) dramatically increases the chance to develop AD, and brain insulin response is impaired in AD patients, indicating a causal link between T2D-related disturbed insulin signaling pathway and AD. Insulin signaling is modulated by the protein-tyrosine phosphatase 1B (PTP1B): High PTP1B activity, which is potentially induced by inflammatory processes, inhibits the insulin signal transduction cascade. Impaired insulin signaling affects synapse formation and memory consolidation in AD patients.

Here, we tested whether blocking PTP1B activity ameliorates AD symptoms in a mouse model that expresses the Swedish and Indiana mutations of the human amyloid precursor protein (hAPP J20 mice).

PTP1B activity was pharmacologically reduced by *in vivo* application of Trodusquemine (6 consecutive doses at 5 mg/kg/5 days) in hAPP J20 mice. In addition, loss of PTP1B activity was achieved by the CaMKII-Cre recombinase driven knockout (KO) of PTP1B. Trodusquemine-treated hAPP J20 mice and PTP1B-KO hAPP J20 mice were used to analyze insulin signaling, amyloid β accumulation, hippocampal neuron death, synaptic dysfunction and memory loss. Despite cognitive function deficits measured by the Morris water maze test, no change in cerebral blood flow determined by laser doppler flowmetry, was detected in 6-month-old hAPP J20 mice; reduced blood flow was reported in these mice at older age, 10 months. Importantly, genetic ablation of PTP1B or treatment with PTP1B inhibitor Trodusquemine prevented cognitive deficits in hAPP J20 mice without affecting cerebral blood flow function, implying that restoring insulin signaling rescues the AD-associated loss of memory via neuronal function.

In conclusion, our study identified a mechanistic relationship between disruption of insulin signaling and the pathological alterations of memory observed in a mouse model of AD.

Significance: Trodusquemine has undergone clinical trials for obesity/diabetes, and could readily be translated to human studies to restore brain insulin signaling to treat AD.

ATAC-Seq reveals differential chromatin landscape in dystrophin-deficient muscle stem cells

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Satellite cells (SCs) exist as a heterogeneous population based on transcription factors Pax7 and Myf5. Induction of Myf5 expression requires Pax7 recruitment of a H3K4 histone methyltransferase complex to enhancer elements upstream of the Myf5 promoter, which recruits chromatin remodeling complexes that favor gene expression. Mdx SCs, used as a model for Duchenne Muscular Dystrophy, show reduced Myf5 expression while mdx myoblasts display reduced H3K4me3 at the Myf5 promoter. Furthermore, electron microscopy studies on SCs revealed a vastly different chromatin state between WT and mdx SCs, whereby mdx SCs displayed more euchromatin compared to WT SCs. Thus, we hypothesize that the chromatin landscape in mdx is perturbed. ATAC-Seq revealed enormous differences between WT and mdx SCs and myoblasts, with only around 30% of peaks being common across all conditions. Interestingly, very few of the differentially accessible regions (DARs) are located at TSS. Most DARs are in distal regions. Performing region-gene association analysis, we identified potential pathways which are affected. Mdx SCs display increased accessibility at regions associated with genes involved in phosphorylation. Among the regions displaying reduced accessibility in mdx SCs are genes involved in SC differentiation, supporting years of research indicating impaired muscle regeneration in mdx mice. Furthermore, many DARs between WT and mdx SCs are located at Pax7 binding sites, as identified by Pax7 ChIP-Seq in primary myoblasts. This supports the role of Pax7 in chromatin remodeling and may imply its function is altered in Duchenne Muscular Dystrophy, corroborating emerging research linking SC defect with the disease.

Production of reactive oxygen species by Sertoli cells is the subfertility inducing mechanism in aging Arsa null mice

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Background: Sulfogalactosylglycerolipid (SGG), the major sulfoglycolipid in mammalian male germ cells, is essential for spermatogenesis (Tanphaichitr et al., Prog Lipid Res 2018, 72:18-41). During normal spermatogenesis ~50% of testicular germ cells (TGCs) become apoptotic and phagocytosed by Sertoli cells (SCs), so that their molecular components can be degraded by SC lysosomal enzymes. In aging mice (≥ 8-month-old) null for *Arsa,* SGG is intracellularly accumulated in SCs especially in lysosomes, leading to lysosomal swelling, typical of lysosomal storage disorder (LSD). Collectively, our results indicate that SC lysosomal ARSA (arylsulfatase A) is responsible for SGG desulfation. Reduced spermatogenesis (50% wild type (WT) rates) and increased numbers of abnormal sperm lead to subfertility in these aging *Arsa*$^{-/-}$ mice. Although it is unclear how SGG accumulation causes impaired SC functions and subfertility, previous observations indicate that mitochondria in LSD cells are dysfunctional due to decreased mitophagy rates. This in turn increases production of reactive oxygen species (ROS) by mitochondria. Herein we hypothesize that SGG accumulation in SC lysosomes in aging *Arsa*$^{-/-}$ mice disrupts mitochondrial function, leading to increased ROS production, thus causing DNA damage to TGCs and sperm with eventual spermatogenesis impairment.

Objective: Determine whether SCs of aging *Arsa*$^{-/-}$ mice produce higher levels of ROS than WT SC counterparts.

Methods: Levels of superoxide in primary cultures of SCs from 8-month-old *Arsa*$^{-/-}$ and WT mice were determined by 2-hydroxyethidium formation following SC incubation with dihydroethidium (DHE). H_2O_2in SC cultures and epididymal fluid of the aging *Arsa*$^{-/-}$ and WT mice were quantified by Amplex Red assay.

Results: DHE assay results revealed no changes of superoxide levels in SCs of aging *Arsa*$^{-/-}$ mice versus WT mice. SCs may have mechanisms to protect themselves from superoxide damage by converting it to H_2O_2, which then is diffusible extracellularly. Released H_2O_2 levels from SCs of 8-month-old *Arsa*$^{-/-}$ mice were indeed higher than those of WT SCs. Furthermore, levels of H_2O_2 in the epididymal fluid presumably reflecting H_2O_2 released by SCs and diffused into the epididymal lumen were higher in 8-month-old *Arsa*$^{-/-}$ mice than the corresponding WT values.

Conclusion/Significance: Our results suggest that the increased production of ROS (especially H_2O_2) by SCs is the primary cause of spermatogenesis impairment in aging *Arsa*$^{-/-}$ mice. H_2O_2 released from SCs would cause DNA damage in TGCs and sperm, which can be assessed by the formation of 8-hydroxydeoxyguanosine. Increased ROS production in SCs would reflect decreased mitochondrial membrane potential (observable by JC-1 dye staining).

Identification of novel pharmacological targets and small molecule inhibitors of adenovirus replication

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Background & Objective: The human adenovirus (Ad) mainly causes minor respiratory illnesses, but can lead to severe disease and death in pediatric, geriatric and immunocompromised patients. No approved antiviral therapy currently exists for the treatment of severe Ad-induced diseases.

Within the first few hours of infection, the Ad DNA enters the host cell nuclei and associates with cellular proteins. This assembly of the viral genome into a protein-associated structure is required for efficient expression of viral genes. Consequently, one approach to treating Ad-induced disease may be to prevent the viral DNA from transitioning to this "active" state. Thus, our objective is to identify novel small molecules inhibiting Ad replication and to investigate the underlying molecular mechanism of the inhibition.

Methods: We have generated a wildtype-like Ad construct encoding the red fluorescent protein (RFP) within the viral genome. RFP from this construct is only expressed following Ad DNA replication, which allows us to effectively monitor virus replication via fluorescence microscopy. Using this construct, we designed an efficient method for screening small molecule libraries to identify novel Ad inhibitors.

Results: We screened over 1300 small molecules to identify compounds that exhibit anti-Ad activity. Several positive hits have been validated to either inhibit or delay Ad replication and reduce virus yield from infected cells. In particular, the histone deacetylase (HDAC) inhibitor vorinostat (an FDA-approved anticancer drug) was found to significantly reduce RFP expression. Follow-up studies revealed that vorinostat delays the onset of virus replication, and decreases virus yield. Vorinostat was also effective against more virulent and clinically relevant Ad serotypes. The drug's inhibitory effects were found to be paritally mediated through the inhibition of HDAC2 activity. Further elucidation of the underlying mechanism and *in vivo* studies are underway.

Conclusion: The costs associated with Ad-induced disease are significant in terms of medical expenses, lost work hours and loss of life in some populations. Identification of novel Ad inhibitors will allow the design and development of more effective antivirals, ultimately leading to decreased disease pathogenesis and higher survival rates. Investigation of the mechanism by which these compounds impact Ad replication will provide insights on virus-cell interactions, and allow us to identify new pharmacological targets for therapeutic intervention.

Role of Astrocytic Dopamine D1 Receptors in Post-Stroke Motor Recovery

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Background: Stroke recovery represents a growing challenge in the field of neuroscience. There is no specific therapy available that can fully improve the motor skills of stroke patients. Previous studies which have implemented pharmacological therapy for stroke recovery using amphetamines and L-DOPA drugs have yielded inconclusive results. These drugs activate wide range of proteins on the surface of the cell called receptors. This would explain the mixed results between clinical and preclinical research.Our lab evaluates a therapy for stroke recovery that uses a drug called dihydrexidine (DHX in short), dopamine receptor 1 agonist, in combination with exercise training. Astrocyets plays important factor for axonal remodeling and regenration. astrocytes express dopamine reseptor and can respond to dopamine.

Objectives: We purpose that astrocytes are recruited by the DA-D1R pathway to enhance post-stroke recovery. First objective for this Hypothesis, characterizing the spatio-temporal profile of the TH-positive hyper-innervation observed after stroke, and investigate how DA exerts its effect on peri-infarct astrocytes at the molecular level. Second, determining that astrocytic D1R are required in the DHX-mediated enhancement of post-stroke motor recovery.

Methods: The effects of PT on TH-positive hyper-innervation within the peri-infarct region will be assessed using IHC at 4, 10 and 28 post-stroke days. To explore the interplay between the TH-positive hyper-innervation and peri-infarct astrocytes, PT stroke will be performed in Aldh1L1-eGFP mice (pan-astrocyte genetic reporter). IHC will be conducted on brain sections from saline and DHX-treated mice stained with catecholaminergic (TH), dopaminergic (dopamine transporter; DAT), noradrenergic (dopamine β-hydroxylase; DBH) markers and anti-GFP antibody, and immunostaining visualized by confocal microscopy. In addition, at the time points indicated here-above, astrocytes will be isolated from micro-dissected peri-infarct and control cortices using magnetic beads or FACS sorting, and their total RNA will be purified for subsequent RT-qPCR analyses of gene expression.

Results: There is gradual increase in TH- and DAT-positive innervation over time around the infarct site. We expect to see an increase expression of trophic factors in peri-infarct astrocytes.

Conclusion: Astrocytes may contribute to recovery through DHX-induced stimulation of D1R.

Dysregulated androgen-induced exosomal release of mir-379-5p determines granulosa cell fate

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Background: Androgen promotes both follicular growth and atresia stage-dependently. miRNAs repress gene expression by targeting 3' UTR of specific mRNAs and are differentially expressed in DHT-induced PCOS rats. Exosomes are involved in ovarian intercellular communication. However, how exosomal miRNAs determine ovarian cell fate is not understood.

Objective: Our overall hypothesis is androgen regulates exosomal content of miRNAs in a follicular stage-dependent manner, a process which determines granulosa cell (GC) fate. Our specific objectives are to determine if: (1) androgen regulates cellular and extracellular content of miRNAs in GCs, (2) the regulation of these processes is follicular stage-dependent.

Methods: Preantral (PAF; DES-primed rats) and antral follicles (AF; eCG-primed rats) were cultured ± DHT (1 uM; 24 & 36h) and exosomes and microvesicles were isolated from spent media by differential centrifugations and characterized by nanoparticle tracking analysis.

Results: DHT treatment for 36h reduced microvesicle concentration in conditioned media of GCs from both PAF and AF, but reduced GC exosome secretion in AF but not PAF, resulting in a reduction of microvesicles/exosome ratio in PAF-GC. DHT reduced cellular rno-mir-379-5p and increased its exosomal content in PAF GC. rno-mir-379-5p was not detectable in extracellular vesicle-depleted conditioned media, supporting the notion that its secretion is primarily via exosomes. DHT had no influence on the cellular and extracellular content of rno-mir-379-5p in AF. Studies with luciferase assay, and miRNA mimic and inhibitor suggested phosphoinositide-dependent kinase-1 (PDK1) as a downstream target of rno-mir379-5p. The increased of exosomal rno-mir-379-5p in DHT treated-PAF GC is associated with increased cellular content of PDK1, P-PDK1, P-AKT/AKT ratio and MCM2 (a cell proliferation marker) but not in AF GC. Inhibition of exosome release in PAF GC with GW4869 resulted in increased cellular content of rno-mir-379-5p, reduced that of PDK1 and MCM2 in the presence of DHT, indicating that exosomal release of rno-mir-379-5p is a regulatory mechanism for PDK1 cellular content and proliferation. DHT reduced proliferation of AF GC (MCM2). Conversely, the inhibition of cellular rno-mir379-5p using miRNA inhibitor rescued proliferative ability in AF GC treated with DHT.

Conclusions: These findings suggest that increased exosomal release of mir-379-5p from GC in response to androgenic stimulation is a survival mechanism specific for the preantral stage of follicle development.

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Characterization of the anti-tumour immune response induced as a result of MG1-infected cell vaccine pre-immunization in murine leukemia models.

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While blood cancer affects both children and adults, stem cell transplantation (SCT) treatment has different prognosis for the different age categories. Generally children present themselves with acute lymphoblastic leukemia (ALL) and have low relapse rates following SCT. Acute myeloid leukemia (AML) however is more frequent in adults with higher relapse rates after treatment. We think immunotherapy is key in initiating an adaptive immune response against leukemia, preventing its relapse by eliminating the microscopic disease leftover after surgery. Our lab believes infected tumour cells can act as personalized immunogenic vaccines generating an anti-tumour response. When immunizing mice with infected, syngeneic L1210 cells (ALL), 90% of mice survive the challenge with viable L1210 cells. Interestingly, in the murine AML model, immunization with C1498 infected cells extends the survival, but fails to provide long term cures. Our goal is to characterize the immune response induced by these infected leukemia cell vaccines by comparing the activation status of T cells and dendritic cells (DC) post immunization. Preliminary data indicates that C1498 vaccine does not induce CD40 expression on murine DCs after immunization as much as L1210 vaccine does, potentially explaining better memory response and survival rates in the latter. In addition, molecular and flow cytometry data showed that C1498 cells express lower levels of immunogenic markers such as calreticulin when compared to L1210 cells after viral infection, which potentially contributes to the C1498 immune evasion mechanism during vaccination.

Going Paperless in Clinical Research is Feasible, Legal and Desired. Time for Action.

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Background: Despite numerous technological advances, academic clinical research units are often slow to adapt available or emerging technologies. Despite the availability of advanced electronic information systems, paper is still the primary source for data collection, storage and verification in clinical research units.

Objectives: To explain the sources of paper load in clinical research and how to reduce them. Also, to clarify the stance of Regulatory bodies on modernization of clinical research. Finally, to review findings of an International study exploring attitudes of clinical research personnel towards modernization of clinical research operations.

Methods: 1- We measured the total paper count used for clinical research for the Ottawa Stroke Research Group and calculated all associated costs and the environmental impact. 2- We reviewed the current guidance documents by the major regulatory bodies (ICH-GCP, FDA, EMA and HC) on direct data entry, electronic signatures and electronic informed consents. 3- We conducted an international survey to explore attitudes of research personnel towards modernizing clinical research.

Results: 1- Significant costs incurred and significant resources are wasted due to unnecessary overuse of paper. 2- Over the last 5 years there has been significant developments aiming to reduce paper load in the clinical research world. 3- While FDA and EMA are encouraging reduction of paper load, the official stance of HC is not clear. 3- Clinical research personnel overwhelmingly desire modernization of clinical research.

Conclusions: Reduction of paper load is feasible, legal and very much desired by clinical research personnel. We encourage HC to consider updating its current guidelines on clinical research operations.

The relationship between endometriosis phenotype, symptomatology and catastrophizing as a coping response

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Background: Endometriosis is a chronic gynecological disease characterized by growth of endometrial tissue outside of the uterine cavity[1]. The phenotypes of endometriosis are: superficial endometriosis (SE), ovarian endometrioma (OMA) and deep infiltrating disease (DIE)[2]. Women with endometriosis are more likely to catastrophize than the general population due to chronic pain (dysmenorrhea, dyspareunia, non-cyclic pelvic pain, dyschezia and dysuria)[3].

Objectives**:** 1) To examine the relationship between catastrophizing, endometriosis phenotypes and types of pain. 2) Determine relationships between catastrophizing and relevant factors including age, education level, diagnostic delay, and number of pain medication taken for endometriosis.

Methods**:** Patients were recruited at Shirley E. Greenberg Women's Health Centre at The Ottawa Hospital. They completed internationally developed, standardized clinical questionnaires detailing history of pain, medical history and catastrophizing amongst other relevant topics. A chart review was conducted on patients' phenotypes of endometriosis and history of past pelvic surgeries. Forty-nine patients met inclusion criteria and were retained as study participants. Chi-square, one-way ANOVA, Pearson and Spearman tests were conducted to ascertain the relationship between variables. Significance was accepted at the $p<0.05$ level.

Results**:** Thirty one percent of patients had clinically significant total catastrophizing score (≥ 30). Significant associations were found between catastrophizing and number of pain medications ($r= .361$, $p = .012$), age of first endometriosis-related symptoms ($r= -.389$, $p= .010$), period pain affecting daily activities $x^2(1) = 4.920$, $p= .027$, $V = .342$, and not having DIE (i.e. having SE or OMA only): $x^2(1) =4.141$, $p= .291$, $V= .042$. No significant relationships were found between DIE and pain types or between a combination phenotype (DIE + OMA ± SE) and pain types.

Conclusions**:** Regardless of endometriosis phenotype, patients experience different types of pain and can be susceptible to catastrophizing. Pain may be a result of the interaction between endometriosis lesions and nerve fibers rather than a result of the phenotype of endomeriosis[4]. Number of pain medications, younger age at which first symptoms of pain were experienced and period pain are risk factors for catastrophizing. Clinicians should be encouraged to identify and treat patients for catastrophizing in a multidisciplinary setting in order to promote patient satisfaction and positive treatment outcomes.

PAX7 function in muscle stem cells is regulated by acetylation

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Background and Objective: Satellite cells are adult stem cells responsible for muscle regeneration upon injury. The transcription factor PAX7 is a critical regulator of satellite cell survival, self-renewal and proliferation. However, how PAX7 itself is regulated in satellite cells remains unclear. The objective of that study is to identify new regulation mechanisms of PAX7 function in satellite cells.

Methods and Results: Using mass spectrometry, we identified two novel acetylation sites on the PAX7 protein, that regulate its transcriptional activity and chromatin binding. Acetylation does not impact PAX7 protein stability, its nuclear localization or its capacity to mediate protein-protein interactions. To study the function of PAX7 acetylation in vivo, we employed the CRISPR/Cas9 technology to generate mice in which one acetylated residue on PAX7 protein is mutated to an arginine. Abolishing PAX7 acetylation in mice impairs muscle regeneration and leads to progressive satellite cell exhaustion. We also identified molecular regulators of PAX7 acetylation using immunoprecipitation assays in myoblasts. Indeed, we determined that the acetyltransferase MYST1 and the deacetylase SIRT2 both interact with PAX7 and control PAX7 acetylation levels. In addition, decreasing Myst1 and/or Sirt2 levels by RNA interference perturbs the expression of Pax7 target genes. Finally, we cultured muscle fibers treated with either siMyst1 or siSirt2 to follow the fate of satellite cells after their first cell division. We discovered that MYST1 and SIRT2 are important players in regulating the balance between satellite stem cell symmetric *vs*asymmetric division, therefore controlling satellite cell self-renewal and commitment.

Conclusion: Our data support a model in which PAX7 acetylation levels regulate its transcriptional activity, and consequently its function in satellite cells.

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Targeted endothelial cell ablation leads to transient pulmonary arterial hypertension followed by rapid microvasculature repair and resolution in mice

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Introduction: Endothelial cell(EC) apoptosis is increasingly recognized as a central trigger for pulmonary arterial hypertension (PAH), although the mechanisms by which endothelial injury leads to the development of the hemodynamic and pathological features of this disease are not well understood. Therefore, we developed a transgenic model to induce targeted endothelial injury in response to diphtheria toxin(DT) in mice by driving expression of the DT receptor, which is not normally expressed in rodents, using an EC specific promoter. This model was used to test the hypothesis that EC apoptosis is sufficient to induce PAH, and to explore the mechanisms responsible for both the onset and resolution of this phenotype.

M&R: Mice expressing CRE under the VE-Cadherin promoter were crossed with animals harboring the human DT-receptor(DTR) gene flanked by LoxP-sites. Twelve-week-old binary-transgenic (BT) animals received DT via intra-tracheal instillation at doses ranging from 3 to 100ng per animals. Doses 25ng and above led to lethality within 96-hours post administration and necropsy analysis showed extensive pulmonary edema. Administration of 10ng DT led to an increase in RVSP within 72-hours in BT animals (n=18) 34.74 ± 1.26mmHg when compared to saline-treated controls (n=13) 23.78±0.46mmHg ($p<0.0001$) but no change in right ventricle hypertrophy (RVH) at this early. Interestingly, after a single injection of DT, the increase in RVSP was transient and returned to control levels within one-week (24.12±1.43mmHg, n=11). Despite a recovery in RVSP, DT-treated animals displayed a delayed increase in RVH at one-week (0.2656 ± 0.006, n=14) when compared to controls (0.2444±0.004, n=12; $p=0.009$). In addition, older animals (25-weeks)(n=10) that received the DT-treatment had an increased mortality rate (6/10) within the first week when compared to younger animals (12-weeks) (n=17) (1/17). Micro-CT of DT-treated animals showed a 40-percent decrease in total vasculature volume when compared to saline controls. This was associated with a loss in both $CD144^+$ ECs and $AN2^+$ pericytes assessed by flow cytometric analysis, and an increase in $CD11b^+$ macrophage population 72hours-post-treatment.

Conclusion: We have demonstrated that direct EC injury and apoptosis is sufficient to induce a transient PAH phenotype in BT-DTR mice. This model will be used to define the role of EC loss and microvascular degeneration in the development of PAH, as well the mechanisms responsible for rapid vascular repair. This work provides novel insights into mechanisms and potential therapeutic targets for the treatment of this disease

miR-486-5p protects against ischemia-reperfusion acute kidney injury in mice

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Background

Acute kidney injury (AKI) is a highly prevalent clinical disorder with significant mortality and no current treatment. We previously showed that endothelial colony forming cells (ECFCs) release exosomes highly enriched in the pro-survival miR-486-5p. In our murine model of AKI, intravenous (i.v.) injection of ECFCs or their exosomes protects against kidney ischemic injury, associated with reduction in PTEN, a target of miR-486-5p. Biodistribution studies reveal that exosomes selectively target the ischemic kidneys, and increase kidney levels of miR-486-5p. Whether exosomal miR-486-5p is critical to prevention of ischemic injury is unclear.

Objective

The aims of the present study were i) to investigate the effect of direct administration of miR-486-5p to mice with kidney ischemic injury, and ii) to compare levels of miR-486-5p within different organs after treatment with miR-486-5p mimic or ECFC exosomes.

Methods

ECFC-derived exosomes were isolated by differential centrifugation and characterized using nanoparticle tracking analysis and immunoblot. Kidney ischemic injury was induced in FVB mice by bilateral renal vascular clamping (30 min), followed by 24 hrs of reperfusion. Exosomes (20 µg) or Invivofectamine-mimic complex containing miR-486-5p (1mg/kg) were injected at the start of reperfusion via tail vein. Organs and blood were removed 24 hrs after reperfusion for further analyses.

Results

Infusion of exosomes or miR-486-5p mimic significantly improved kidney function compared to mice with ischemia-reperfusion alone, reflected by decreases in plasma creatinine and blood urea nitrogen (BUN) ($P<0.05$, $n=5$). Exosomes selectively increased miR-486-5p levels in kidneys at 24 hrs ($P<0.05$, $n=5$). Mice injected with miR-486-5p mimic showed significant increases in miR-486-5p levels in kidneys, but also in liver and spleen ($P<0.01$, $n=5$). Administration of miR-486-5p mimic did not affect miR-486-5p levels in brain, lung or heart.

Conclusions

Systemic administration of miR-486-5p mimic prevents kidney ischemic injury in mice, associated with increased miR-486-5p levels in kidney; increases are also seen in the liver and spleen. These results suggest that the protective effects of ECFCs or their exosomes in ischemic AKI may be largely mediated by pro-survival miR-486-5p.

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Evaluating a Personalized Approach for the Treatment of Mental Health Disorders in First Responders

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Introduction: Among the general Canadian population, 8.0-9.2% report lifetime prevalence rates of Post-Traumatic Stress Disorder (PTSD) [1]. Lifetime prevalence rates among Canadian first responders are far greater, with estimates falling between 8%[2]-32%[3] for police officers; 26% for paramedics [5]; and 17% for firefighters [4]. This vulnerability is not limited to PTSD, first responders also report elevated rates of depression [6,7], anxiety [7], alcohol abuse and dependence [6,8] and suicidality [9]. Further, there are unique clinical challenges with providing mental health care to this population in routine health care settings, such as emergency departments, which are places that first responders are known in their professional capacity. Treatment and employer resources for first responders are variable, and there are no standard evidence-based workplace guidelines for organizations to follow.

Methodology and Results: A three-phase research plan was designed in collaboration with highly diversified representatives from a large number of first responder organizations:

Phase 1 includes the completion of a needs assessment of the City of Ottawa's Tri-Services (Police, Fire and Paramedics) to identify preferences for mental health treatment and access to care. Structured qualitative interviews will also be conducted with participants representing particular sub-groups within the first responder community, in order to identify barriers to care and implement effective strategies during planning and set-up of a First Responder Operational Stress Injury (OSI) Clinic. The results of Phase 1 will guide the design of Phase 2, a pilot randomized controlled trial of the First Responder OSI Clinic. Phase 2 aims to assess the feasibility and acceptability of: 1) setting up a First Responder OSI Clinic using a First Responder Mental Health Team (FRMHT); and 2) the design and implementation of a preventative pre-screening method using two different intake assessments. N=40 participants will be referred to one of three treatment groups based on clinical assessment: 1) lifestyle recommendations; 2) Employee Assistance Program; or 3) FRMHT. The primary outcome is number of days taken off of work in a year. Additionally, information regarding the economic impact of mental health disorders in the workplace will be examined during both Phases 1 and 2, which will inform an economic analysis in Phase 3.

Conclusion: Results of Phases 1 and 2 will inform the design and implementation of a full-scale RCT (Phase 3), which will evaluate the effectiveness of the First Responder OSI Clinic, the Preventative Pre-Screening Method and the FRMHT.

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). Methodologically rigorous multicenter preclinical studies may address some of these issues. In order to address feasibility of the multicenter preclinical approach, we performed a pilot study using a mouse model of acute lung injury (ALI).

Methods: The protocol was approved by Animal Care and Veterinary Services at uOttawa, McGill, and St. Michael's Hospital. Given the complexity of ALI models, for the pilot 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'). Following anesthetic induction (ketamine/xylazine) and intubation (22 G cathlon) mice were randomized to receive either a) 50 micrograms of LPS in 50 microL of PBS (n=6/lab) or b) PBS alone (n=4/lab) by intratracheal instillation. The intervention was blinded 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 were collected to assess secondary outcomes including inflammation (cytokines) and barrier disruption (protein concentration in BAL).

Results: We successfully: a) harmonized protocols for disease induction between centers, b) gained multi-institutional animal care approval, c) coordinated mice shipment from a single colony to each center, d) acclimatized mice for one week at each center and e) synchronized conduct of experiments. Minimal protocol violations were noted. All 40 mice were successfully randomized and administered either PBS or LPS intratracheally. Three mice (7.5%) died periprocedurally or prior to sacrifice; this was within the expected 5-10% expected dropout rate. Despite between center variation, LPS treated mice demonstrated significant changes in cytokines and barrier function (Figure).

Conclusions and Future Directions: The PreMATIC pilot is the first multicenter preclinical study in Canada and demonstrates a feasibility to harmonize protocols and experiments across different research centres. Prior to testing an interventional therapy in an appropriately powered study, further pilot studies will assess additional outcomes and address identified protocol issues to reduce violations. By using this approach, we hope to conduct the world's first multicenter preclinical trial of a stem cell therapy which may provide more robust evidence on potential for clinical translation.

Retinitis Pigmentosa: Exploring Treatment with WN1316 and XIAP

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Background: Retinitis pigmentosa (RP) is a progressive eye disease that occurs in approximately 1 in 5000 people, globally. The disease targets rod photoreceptors causing a tunnelling effect on vision until all retinal activity is lost and complete blindness occurs. The most common form of this disease results from a proline to histine change at position 23 (P23H) on the rhodopsin gene, causing apoptotic death of rod cells. Despite the mutation being localized to rods, cone photoreceptors also die in the late stages of RP. The mechanism for cone loss is unknown but it is theorized to be a result of increased oxidative and metabolic stress due to the loss of rods.

Objective: This study examines a therapeutic strategy that targets both oxidative stress and apoptosis. Treatment with WN1316 (2-[mesityl(methyl)amino]-N-[4-(pyridine-2-yl)-1H-imidazol-2-yl] acetamide trihydrochloride), which upregulates an endogenous antioxidant pathway regulated by the Nrf2 gene, will be used to target oxidative stress. Gene therapy with the X-linked inhibitor of apoptosis (XIAP) will be used to target apoptotic pathways.

Methods: WN1316 will be tested in combination with XIAP to see if there is an additive effect on RP disease prognosis in comparison to either treatment alone. Using electroretinography and histology, the function and structural integrity of the photoreceptor layer will be tracked in all test subjects for 5 months to measure declines in electrical activity, retinal function, and vision loss.

Sex-related changes of cerebrovascular function in two mouse models of ischemic stroke

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Cerebral function relies on a steady supply of oxygen and nutrients from the blood stream, thus relying on healthy blood vessels and cerebral blood flow (CBF). As such, the brain is particularly vulnerable to vascular failure. Aging, poor diet and other risk factors affect vascular health and promote the onset and/or progression of neurological disorders including ischemic stroke (IS), the most prevalent form of human stroke. IS represents a major cause of infirmity and death worldwide, with more than 400,000 people living with disabling consequences in Canada. While neuronal plasticity represents a central focus in stroke research, vascular responses to IS remain poorly understood. This study aims at better understanding the spatio-temporal profile of hemodynamic responses to IS in female and male mice. Indeed, as IS differentially affects females and males, it is crucial to investigate sex differences by building in experimental groups that better represent the reality of a human population. Our multidisciplinary approach combines sophisticated imaging modalities (laser-Doppler flowmetry and photoacoustic imaging), as well as anatomical and molecular methods to investigate cerebrovascular responses following photothrombotic (PT) or endothelin-1 (ET-1)-mediated IS in the somatosensory cortex of adult outbred (Swiss Webster) mice. Immediately (15 min) after PT stroke induction, we measured a significant decrease in baseline CBF in females (-32% ± 6.5; n=5), while no change was observed in males (+1.6% ± 12.1; n=5) within the direct vicinity of the injury. However, 48 hours post-stroke, a similar decrease in baseline CBF was found in both females and males (-55% and -45%) but not 3 weeks post-stroke. Following ET-1-induced IS, we found comparable reductions in baseline CBF in both females and males, as well as similar reperfusions 48 hours post-stroke. Intriguingly, 3 weeks after ET-1 stroke, the diminution in baseline CBF was maintained only in females. Our data thus suggest sex-related changes in the CBF responses to IS in two mouse models, suggesting a sex-dependent CBF regulation and resistance to IS. This work is complemented by anatomical and molecular readouts to assess the underlying mechanisms of these changes observed after IS. For instance, in the cerebral cortex of both females and males, microvascular branching and density, as well as changes in the expression of gene candidates related to angiogenesis and CBF, are being quantified. This original study will provide a novel understanding in cerebrovascular remodeling following IS and its associated sex-difference in mice.

Neuropeptide Y regulates proliferation and apoptosis in ovarian granulosa cells during follicular development

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Background

Mechanisms of follicular selection and development are not completed understood. Reproductive function of Neuropeptide Y (NPY) in the brain is known but that in the ovary is unclear.

Objective

The objective of this study was to investigate the role of NPY in folliculogenesis and pathogenesis of polycystic ovarian syndrome (PCOS).

Methods

To understand NPY expression pattern in granulosa cells (GCs) at different stages of follicular development, immunohistochemistry, real-time polymerase chain reaction and Western blot were performed on preantral, early antral and late antral follicles (PA, EA and LAF respectively), in 21-days old Sprague Dawley rats injected with eCG (10 IU; ip). For PCOS model rats, rats (21-day of age) were implanted with dihydrotestosterone capsule (83 μg/day) and euthanized at 1 month thereafter. To examine NPY function on proliferation and apoptosis in GCs, Ki67 immunocytochemistry and TUNEL assay were performed.

Results

NPY protein expression was in a follicular stage- and cell type- dependent. NPY protein and Npy mRNA were present at the highest level in EA-GCs. NPY receptors protein in GCs were follicular stage-dependent. GCs from PCOS model rats contained less Npy mRNA and more NPY receptor type 2 and 5 protein. Addition of recombinant NPY (0.1 nM) to EA-GCs cultures increased Ki67-positive but decreased TUNEL-positive cells. In contrast, in LAF-GCs, NPY reduced Ki67-positive and increased TUNEL-positive cells. Such effects of NPY were not observed in PA-GCs. CGP71683 (NPY receptor type 5 antagonist) and PD98059 (MAP kinase inhibitor) inhibited NPY induced Ki67-positivity in EA-GCs.

Conclusions

These results indicate that NPY regulates GCs proliferation and apoptosis in a follicular stage-dependent and autocrine manner. NPY may be involved in pathogenesis of PCOS.

The development of astrocyte interactions with the cerebrovasculature

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Background

In the central nervous system, neurons and blood vessels are linked together in a multicellular complex called the neurovascular unit (NVU). The coupling between neuronal activity and hemodynamics (neurovascular coupling) allows blood vessels to respond to elevated neural activity by increasing blood flow. A key component of the NVU is the astrocyte. Its processes encompass neural synapses and express neurotransmitter receptors, allowing these glial cells to detect synaptic activity. They also surround blood vessels with their endfeet and play a role in controlling vasodilation and vasoconstriction. The mechanisms that control these interactions are currently an area of active research. However, very little work has been done to explore the role of astrocytes in the development of the NVU.

Objective

It is known that the NVU is not functional at birth but matures a few weeks after. We explored how this maturation occurs and what role astrocytes play in vascular network expansion (i.e. angiogenesis).

Methods

We used a mouse model (in which astrocyte are genetically labeled with GFP; Aldh1L1-eGFP) to explore what role astrocytes play in proper cerebrovascular development and how they mature and incorporate into the NVU. To initiate this investigation, we used immunohistochemistry to visualize astrocytes (eGFP), endothelial cells and neurons. The stepwise recruitment of astrocytes around blood vessels was systematically charted throughout postnatal development (from birth to adulthood), allowing for a novel and precise morphological description of NVU formation.

Results

We show that, 7 days after birth (P7), astrocyte density is high in the cerebral cortex, but these glial cells do not contact blood vessels yet. At P14, while parenchymal astrocyte density progressively decreases, we began to detect astrocyte endfeet around blood vessels. Endothelial coverage of endothelial cells by astrocyte endfeet increased significantly from P14 to P50. Interestingly, while all “fibrous” (i.e. white matter) astrocytes expressed GFAP as expected, protoplasmic cortical astrocytes (eGFP+) also expressed GFAP when close to or in contact with blood vessels.

Conclusion

These novel observations allow for a more precise morphological description of NVU formation. They suggest that the NVU in mice forms between P7 and P14, that most astrocytic proliferation occurs between P0 and P7, and that astrocytes diffuse across the growing brain between P7 and P14.

Getting Ahead of the Curve: A Demonstration of Early Economic Evaluation

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Background: Early Health Economic Evaluation (EHEE) is a form of cost-effectiveness analysis about a specific disease or population, but is built prior to any completed clinical investigations of an intervention. It is a means of determining whether an intervention is likely to be deemed cost-effective before the researcher or producer invests significant and scarce resources into an intervention that may ultimately never reach the patient. It is becoming possible to build these evaluations thanks to advances in decision modeling methods and greater access to large administrative databases with robust information on patient cohorts.

Objective: This presentation will introduce the functions and methodologies of EHEEs, using three example studies in which the author built the economic evaluation models. Primary attention is given to how major stakeholders, including clinicians, researchers, and decision makers can utilize EHEEs in unique ways.

Methods: Three disease profiles of interest are modeled: (1) severe sepsis in the elderly, (2) bronchopulmonary dysplasia in preterm infants, and (3) adult acute lymphoblastic leukemia. The intervention in all three is an innovative stem cell therapy currently in pre-clinical development. EHEE models were built to characterize the healthcare trajectory under current standards of care and comprehensive patient outcomes, accounting for various patient characteristics. Several points of intervention are coded into the model to allow testing of how changes in select clinical outcomes thanks to a hypothetical intervention will affect long term patient quality of life, life expectancy, and cost to the health system.

Results: The resulting model describes the current clinical trajectory of patients and overall burden of care associated with the disease. Without knowing the precise clinical impact of a yet-to-be-tested intervention, the EHEE analysis calculates the 'therapeutic headroom' to determine what level and combination of improvements will be necessary to be confident the intervention will be deemed cost-effective and adopted by the health system.

Conclusions: EHEE models, while difficult to build well, provide critical insights to various stakeholders. For clinicians, the standard of care model is, functionally, a clinical decision tool. Decision makers can use the therapeutic headroom calculations to set investment and reimbursement priorities, while researchers can use it to determine the value of information in order to set research priorities for the intervention trials. EHEEs will not replace traditional health economic evaluations, but they can improve the quality and efficiency of health research and innovation.

Assessing the completeness of reporting of study rigour in preclinical oncolytic virus immunotherapy studies

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Background: Oncolytic virus (OV) immunotherapies are promising treatments being considered for 'bench-to-bedside' translation. Recent research has shown encouraging results in animal models and the potential translation to humans is gathering significant interest and funding. However, irreproducibility of basic scientific results represents a significant barrier to the successful movement of preclinical research to the clinical setting. To address this issue and improve the possibility for successful clinical implementation, the National Institutes of Health has developed a set of preclinical reporting guidelines to help improve the rigour of research and its reporting. To date, the quality of reporting in the preclinical OV immunotherapy literature has yet to be evaluated.
Objectives: The primary aim of this review is to evaluate how completely preclinical *in vivo* OV immunotherapy studies adhere to guidelines for transparency in preclinical reporting to enhance the rigour of research conduct. Our secondary objective is to develop an evidence map identifying the current trends in OV immunotherapy research (e.g. common virus, cancer, and animal models).
Methods: Each domain from the guidelines was operationalized into a set of 'yes' or 'no' questions, with each question addressing a single item from the recommendations. A systematic strategy was developed to search MEDLINE and Embase to identify all articles published from January 2016 to June 2017 relevant to preclinical *in vivo* OV cancer research. Articles were screened and data extracted independently and in duplicate. Studies that included *in vivo*animal experiments investigating OV immunotherapy were included. Studies with only *in vitro* and/or *ex vivo* experiments were excluded.
Results: The literature search returned 1554 articles. After title, abstract, and full-text screening 236 articles met our predefined eligibility criteria. In the included studies adenovirus (43%) was the most often used viral platform. Frequently investigated cancer models included colorectal (14%), skin (12%), breast (11%), and liver (10%). A xenograft implant (61%) in immunodeficient mice (85%) was the most common animal model. Across the studies that were considered in our review, we found the recommendations from all domains of rigour were poorly reported: use of reporting standards (0.4%), distinction between biological and technical replicates (1%), statistics (49%), randomization (1%), blinding (2%), sample size estimation (0%), and inclusion/exclusion criteria (0%).
Conclusions*:* Completeness of reporting in the preclinical *in vivo* OV immunotherapy literature is poor suggesting poor research rigour. This may hinder efforts to replicate work and translate findings from 'bench-to-bedside.'

The role of extracellular vesicles derived from adipose tissue macrophages in polycystic ovarian syndrome

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Introduction: Polycystic ovarian syndrome (PCOS) is a heterogeneous disorder characterized by ovulatory dysfunction, hyperandrogenemia, obesity and insulin resistance. Macrophages play key role in inflammation and the balance between M1 (cytotoxic and pro-inflammatory) and M2 (anti-inflammatory) macrophage has an important role in follicular growth, atresia and ovulation. Chronically androgenized rats, implanted with 5α-dihydrotestosterone (DHT), are used as animal model to mimic ovarian changes of PCOS women, including antral follicle growth arrest and anovulation. However, what is the exact etiology of the PCOS and how androgen excess induces PCOS-related ovulatory dysfunction is not fully understood. We hypothesized that adipose tissue-derived macrophages play an important role in the pathogenesis of PCOS by secreting vesicles to alter granulosa and theca cell functions. Our objective was to investigate the role of extracellular vesicles derived from adipose tissue macrophages in PCOS and how extracellular vesicles are involved in ovarian cell-macrophage communication.

Methods: Female Sprague Dawley (SD) rats at the age of 21 days were randomly assigned into DHT and CTR groups. DHT (7.5 mg) or Sham capsules were implanted to rats for 7, 15, 30 days. M1 (CD68+CD163-) and M2 (CD68+CD163+) macrophages in adipose tissues attached to ovary were identified and quantitated by immunofluorescence. In addition, macrophages were isolated from the adipose tissues attached to the ovary and identified by flow cytometry (+CD68).

Results: Both M1 and M2 macrophages in ovarian adipose tissue increased with age of the test animals. 15 and 30 days of DHT treatment significantly increased M1/ M2 ratio in adipose tissues attached to the ovary, but not total number of M1 and M2 macrophages compared to CTR group. DHT treatment for 15 and 30 days significantly promoted M1 macrophage polarization but inhibited M2 macrophage polarization in 30 days comparing to CTR group. In addition, the percentage of macrophages in total cells isolated from adipose tissues of untreated rats was up to 80%.

Conclusions: Androgen excess induces pro-inflammatory condition (higher M1/ M2 ratio) in ovarian adipose tissues in vivo, which supports the notion that pro-inflammatory may participate in the PCOS pathogenesis.

Deciphering the functional redundancy of USP4 and USP15

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Ubiquitin-specific proteases (USPs) are a class of deubiquitinating enzymes that catalyze the removal of ubiquitin, a polypeptide post-translational modification that is essential in many cellular processes, from various proteins. Previous work done in the lab established that the USP paralogs USP4 and USP15 emerged from an ancestral USP about 500 million years ago from a whole genome duplication, and the majority of known vertebrate genomes contain a functional copy of both. These paralog USPs are known to regulate processes including cell growth, innate immunity, and embryonic development, and are also involved in disease states such as cancer. Genetic crosses of mice with knockout mutations performed in the lab have determined it is essential that at least one allele of the paralogs be functional, as when both USP4 and USP15 are knocked out no viable pups are born. At embryonic day 12.5, double-null embryos are physically smaller compared to embryos that are heterozygous at both genes. These smaller embryos have severely underdeveloped livers with evidence of fibrosis and decreased hematopoiesis. Finally, embryos which have at least one functional copy of USP4, but are null in USP15 appear to have an intermediate phenotype. Mouse embryo fibroblasts have been derived from embryos of all genotypes. Levels of USP4 and USP15 substrates will be assayed in these cells to explore the extent of functional redundancy of the USPs and the effects their deficiencies have on critical signalling pathways. These findings will have implications for potential targeted therapies.

Chronic fluoxetine induces serotonin axonal plasticity in recovery from post stroke depression

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Background: Fluoxetine (FLX), a serotonin-selective reuptake inhibitor that increases synaptic 5-HT, can improve recovery from stroke, including from post-stroke depression (PSD). But as FLX is not always effective, we developed a mouse model of persistent PSD to address and enhance FLX's mechanism of action. Microinjection of endothelin-1 into the left medial prefrontal cortex (mPFC) of male C57BL6 mice induced a focal stroke that resulted in chronic anxiety, depression and spatial learning-memory deficits. Chronic FLX treatment induced recovery from PSD, along with a re-balancing of the activity of ipsi/contralesional side (Vahid-Ansari F, Albert PR; NeuroRx 2018). FLX increases synaptic 5-HT levels by blocking 5-HT transporters (SERT). It remains to be elucidated how FLX-induced 5-HT activity results in behavioral recovery.

Objectives: Since 5-HT axons can regrow months after injury, we used anti-SERT staining to look for FLX-induced changes in 5-HT fibers, which could mediate PSD recovery.

Methods: Using an optimized methodology, 5-HT fibers (SERT+) were quantified in Z-stack images (z slice:0.24 µm thickness) at 63X using the ZEN software. Next, deconvolution was done using AutoQuant X3.1 to reduce background while increasing image resolution. The density of 5-HT axons was quantified in the left vs right mPFC (CG, PI), nucleus accumbens, septum, hippocampus (CA1, CA2/CA3, DG), amygdala and dorsal raphe. The average axonal projections and the number of varicosities across the Z-stack images were extracted using Fiji's Z-project function. Using Imaris x64 9.1.2, SERT+ fiber length density and the number of SERT+ varicosities were calculated in brain tissues of WT, PSD, and PSD with running wheel or treated with FLX.

Results: Chronic FLX treatment significantly increased SERT+ fiber length density at the lesion site in the left mPFC (L vs R CG), and left amygdala but not other areas compared to untreated or running wheel-treated PSD brains. FLX induced-increases in SERT+ fibers were associated with higher numbers of SERT+ varicosities in the left mPFC and amygdala, compared to PSD brains.

Conclusion: Using our PSD mouse model, in which chronic FLX but not running wheel reverses anxiety, depression and cognitive phenotypes, FLX also induced a novel recovery of 5-HT innervation in mPFC and amygdala. Ongoing studies to quantify and map synaptic contacts will address the functions of 5-HT neuroplasticity at the target sites.

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Combatting Post-Stroke Depression via Optogenetic Stimulation of the Serotonin System in a Transgenic Mouse Model

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Background: Post-stroke depression (PSD) is a condition that afflicts one in three stroke survivors. PSD significantly hampers post-stroke recovery and is a risk factor for further complications. Serotonin (5HT) is a neurotransmitter found in mammals, and reduced 5HT activity in the brain has been linked to depression. The most common clinical treatment for PSD are the selective 5HT reuptake inhibitors (SSRIs), but there exists a 3-6 week latency period in many patients before the onset of antidepressant action, and remission is only 50%. Increasing 5HT levels is a known mechanism of action for many antidepressants, but the precise role 5HT plays in depression is not known.

Objective: We hypothesized that by directly stimulating the 5HT system an acute and robust antidepressant effect can be produced. We also hypothesized that an increased stimulation output would result in an increase in antidepressant effect.

Methods: We previously developed a mouse model of PSD that lacks motor deficits via two small ischemic lesions in the left medial prefrontal cortex. These PSD mice were shown to improve with chronic SSRI treatment. To directly stimulate 5HT neurons, we used PetChR2 mice that express the blue light-activated channelrhodopsin2 (ChR2) selectively in 5HT neurons. The PSD lesion was done in the PetChR2 mice, and they were implanted with a 200μm light fibre over the dorsal raphe region. During behaviour testing, constant stimulation at 465 nm was applied for one third of the total time.

Results: The dual surgery resulted in minimal mortality. In unstimulated mice, the ischemic lesion in PetChR2 mice replicated the anxiety and depression phenotype seen in PSD model during behaviour testing. While cohort numbers are still small, encouraging trends were seen in stimulated mice in depression tests. When stimulated under low output, a significant antidepressant effect was seen in PetChR2 mice carrying two copies of the ChR2 transgene during forced swim test. When stimulated under high output, PetChR2 mice displayed an antidepressant response. However, a persistent anxiogenic and antidepressant phenotype also emerged over several weeks of high output stimulation in the PetChR2 mice.

Conclusions: The persistent PSD phenotype was replicated in ChR2 transgenic mice. The depression phenotype appears responsive to acute 5HT stimulation under both output conditions. This provides a useful model to target 5HT release in specific brain regions to understand the effects of 5HT on anxiety and depression, and to enhance recovery.

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Serine, a component of one-carbon metabolism, is transiently transported during meiotic maturation in oocytes

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Background: Products of one-carbon metabolism play a variety of important roles in cellular systems, including neutralizing reactive oxygen species(ROS), purines and thymidylate synthesis for DNA, and providing methyl groups for epigenetic methylation. Folate is actively taken up during oocyte growth, and it is known that the folate cycle is a key component of one-carbon metabolism, for which serine is the main methyl donor. While the transport of many amino acids has been studied in oocytes and preimplantation embryos, serine has largely been overlooked despite its potential role in many important processes.

Objective: We aim to profile the transport of serine in oocytes and identify the responsible transporter(s).

Methods: We used radiolabeled [3H]serine (1uM) and competitive inhibition with an excess (5mM) of substrates that would distinguish between candidate transporters for identifying the major serine transporter in mouse oocytes. Serine uptake was also measured with and without sodium(Na+), since amino acid transport has classically been categorized based on sodium-dependent or -independent activity. By increasing [3H]serine concentrations, the kinetics of the transporter was established as well. Additionally, we maintained meiotic arrest of oocytes in the germinal vesicle(GV) stage using dbcAMP or natriuretic peptide C(NPPC) to determine whether transporter activity required progression of meiotic maturation.

Results: Throughout meiotic maturation, serine uptake rate is highest (0.04-0.07 fmol/oocyte/min) in the 4 hours following GVBD, after which it is inactivated. In growing oocytes, there is transport of serine at comparable rates to that in fully grown oocytes. We carried out experiments for characterizing the transporter at 4 hours following oocyte isolation from follicles, where transport rate was at its maximum. Substrates of systems B0,+, b0,+, system A, GLYT1, or B0AT2 did not compete for uptake of [3H]serine, and serine transport was eliminated in Na+-free media. Alanine and cysteine in media nearly eliminated [3H]serine uptake into oocytes. Excess leucine and histidine also inhibited serine transport, suggesting SNAT7 as the likely transporter. Measurements of transport kinetics indicated a Km of 203uM and a Vmax of 8.2fmol/oocyte/min. Activity of the transporter is not dependent on resumption of meiosis. Serine transport however increased with NPPC but not dbcAMP.

Conclusions:
Serine transport is active during oocyte growth, and is also transiently activated during meiotic maturation of mouse oocytes. SNAT7 appears to be the most probable transporter of serine in oocytes, as SNAT7 requires sodium to function and is the only transporter that fits the substrate profile we have established. Expression of SNAT7 will be confirmed using RT-PCR.

An evaluation of traumatic spinal cord injuries to assess and improve local prevention strategies

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Background: Over the past twenty years, there has been a shift in the prevalence and etiology of traumatic spinal cord injuries (SCIs), as well as the populations most at risk for this type of injury. Specifically, there has been an increase in age at time of injury and proportion of fall-related injuries. Additionally, literature shows that mechanisms of injury and outcomes vary significantly by region. Therefore, it's important to examine data available in local databases to formulate the most effective traumatic SCI prevention strategies.

Objective: To evaluate the epidemiology and mechanisms of traumatic SCI in local centers (Ottawa) and determine if there is any correlation with prevention programs (i.e. Parachute/ThinkFirst) to improve current SCI prevention strategies. Specific aims include: 1) determine the sociodemographic characteristics, incidence, prevalence, and etiology of traumatic SCI in the Champlain LIHN; 2) compare and contrast the sociodemographic characteristics, incidence, prevalence, and epidemiology of traumatic SCI at the different registry centers to see if there is a difference in injury prevention where there is a Parachute/ThinkFirst chapter.

Methods: A prospective study will be conducted of all patients who have sustained a traumatic SCI between January 2009 and December 2015 and were included in the Rick Hansen SCI Registry (RHSCIR) for the Champlain LHIN. The sociodemographic characteristics, incidence, prevalence, and etiology of traumatic SCI patients at different registry centers where there is a Parachute/ThinkFirst chapter will be described and compared using chi-square test and Mann-Whitney U test for categorical and continuous variables respectively, to see if there is a difference in injury prevention. Natural log transformation will be applied for normalizing distribution of duration of follow-up.

Results: Currently, several SCI prevention strategies exist at the international, federal, provincial, and municipal levels. Although these strategies cover a large range of injury causes (i.e. trauma from cars, sports, and falls), there is a lack of coordination and amalgamation between individual programs. Among these, the largest scale SCI prevention program is administered by Parachute/ThinkFirst, which operates both nationally and locally through various chapters.

Conclusions: The next steps of this project will examine the data from the RHSCIR for the Champlain LHIN and compare patient characteristics at the different registry to see if there is a difference in injury prevention where there is a Parachute/ThinkFirst chapter. Hopefully, the epidemiological findings can be used to guide and improve local traumatic SCI prevention strategies.

Profiling Methylenetetrahydrofolate Reductase (MTHFR) Throughout Mouse Oocyte and Preimplantation Embryo Development

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Background

DNA methylation is a major contributor to the epigenome. Twice throughout gametogenesis and embryogenesis, the methylation pattern is removed and recreated; any disruption to the cellular methyl pool could have detrimental effects on this process. Methylenetetrahydrofolate reductase (MTHFR) is an enzyme in the folate cycle that converts 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate. This product is the substrate for methionine synthase, which is the connection to the methionine cycle. The MTHFR reaction is the determinant for whether methyl groups enter the methionine cycle to eventually produce S-adenosyl methionine, the universal methyl donor.

Objective

We plan to begin to elucidate the role and regulation of MTHFR in early development in a mouse model by observing how it changes during oocyte maturation and preimplantation embryo development regarding its protein expression, mRNA, phosphorylation status and isoforms present.

Methods

We will use western blots to observe protein expression throughout the various stages of development and qPCR to observe relative levels of mRNA over the same stages. Lambda phosphatase treatments will be used in conjunction with Phos-tag gels to detect phosphorylated and unphosphorylated MTHFR.

Results

The preliminary results show that MTHFR protein is expressed throughout all developmental stages studied. Lambda phosphatase treatment affects some stages of development, but not others. Phos-tag gels suggest that there are both phosphorylated and unphosphorylated proteins present, and that this changes over the course of development. Since it has been previously shown that MTHFR activity is regulated by its phosphorylation status, this may provide insight into its regulation in oocytes and early embryos.

Some of our research trainees who hold salary awards

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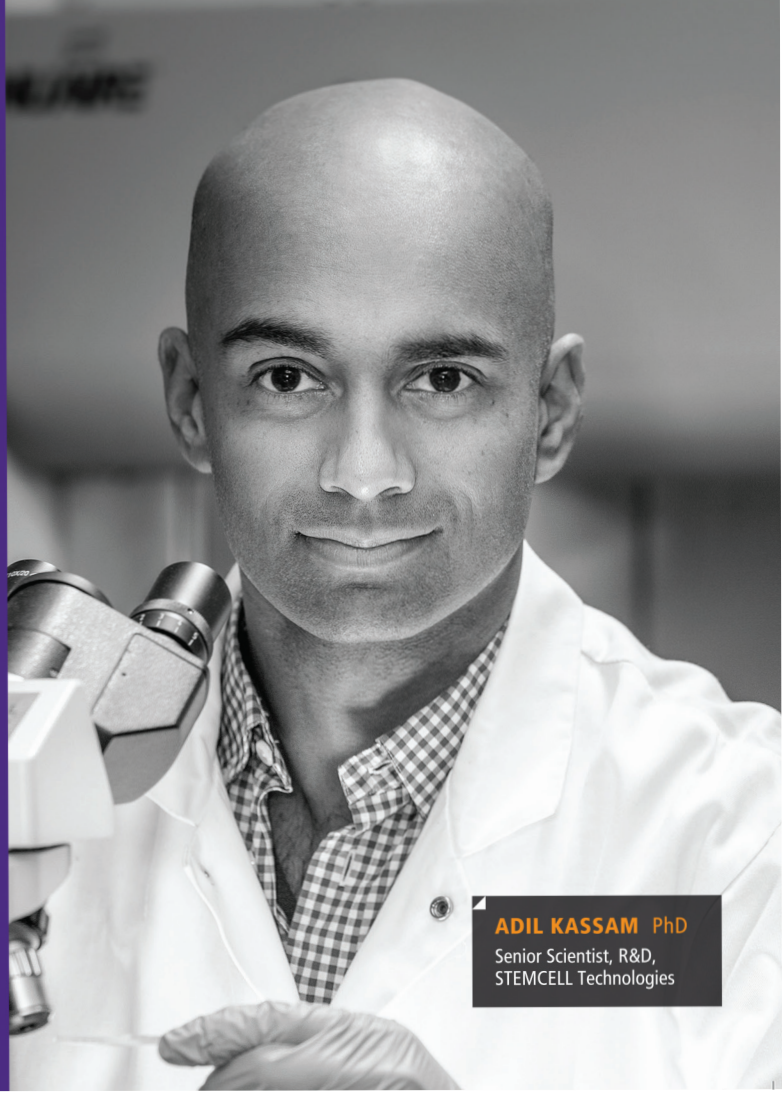

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