Centre universitaire de santé McGill
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McGill University Health Centre
Research Institute

Summer Student Research Day

August 12, 2022

Cruess Amphitheatre and Online

Centre universitaire de santé McGill
Institut de recherche

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Dear summer students and RI-MUHC community,

We are happy to welcome you back to our annual RI-MUHC Summer Student Research Day. Summer students have been hard at work in the lab this summer and this is their opportunity to share their research findings with their peers and the RI community. With 16 oral presentations in the morning and 72 poster presentations in the afternoon, our goal is to create an inclusive and stimulating learning environment in which new ideas can be exchanged and new connections can be made. So don't be shy! Ask your questions, provide encouragement, and help create interesting conversations!

We hope you enjoy the presentations and look forward to welcoming you.

The Organizing committee

Emily Bell, Ariel de Roo, Marie-Claude Gingras, Inga Murawski and Lindsay Naef

Agenda

8:30 Registration

9:00 Welcome remarks from Dr. Rhian Touyz, Executive Director and Chief Scientific Officer

9:00 Oral presentations: Session 1

10:30 Health & Coffee Break

10:45 Oral presentations: Session 2

12:15 Poster instructions and Closing remarks

12:15 Lunch break

13:30 Poster Session

ORAL PRESENTATIONS

Join Zoom Meeting: HERE

Meeting ID: 885 8020 7410 - Passcode: 930432

Session 1 (9:00-10:30)

1- Zhao Wan Er Jin	Dr. Donald Sheppard (IDIGH)
2- David Derish	Dr. Renzo Cecere (CHAL)
3- Armen Erzingatzian	Dr. Julia Valdemarin Burnie(CRP)
4- Joyce Li	Dr. Noemi Dahan-Oliel (IRR)
5- Ziad Nadori Lamlili El Mazoui	Dr. Alexandre Reynaud (BRAIN)
6- Liam Roberts	Dr. Nada Jabado (CHHD)
7- Cyril Kazan	Dr. Tomoko Takano (MeDiC)
8- Evelyn Chan	Dr. Dr. Marta Kaminska (RESP)

Session 2 (10:45-12:15)

9- Celeste Laporte	Dr. Carolyn J. Baglole (RESP)
10- Muhammad Shahzad	Drs. Spicer & Cools-Lartigue (CRP)
11- Catherine Zhu	Dr. Moshe Ben-Shoshan (IDIGH)
12- Nicholas Lee	Dr. Stephane Laporte (MeDiC)
13- Farah Zahoua Alem	Dr. Jesper Sjöström (BRAIN)
14- Julia Visch	Dr. Matthias Friedrich (CHAL)
15- Zeynep Yalcin	Dr. Rima Slim (CHHD)
16- Danielle Cutler	Dr. Julio Flavio Fiore Junior (IRR)

ORAL #1 - Correlation of exopolysaccharide expression in isolates of Pseudomonas aeruginosa and response to eradication therapy in patients with Cystic Fibrosis

Zhao Wan Er Jin[1], François Le Mauff[1], Donald Sheppard[1]

[1]McGill University

Background: By adulthood the majority of patients with Cystic Fibrosis (CF) are infected by the Gram-negative bacterium *Pseudomonas aeruginosa*. Despite the availability of antibiotic treatments to cure acute infections with this organism, treatment of CF patients remains challenging as failure to eradicate bacterial colonization occurs in up to 40% of the cases. While reason for treatment failure remains poorly understood, antibiotic resistance has been shown to be enhanced by the ability of the bacteria to form biofilms. In *P. aeruginosa*, two exopolysaccharides, Pel and Psl, play an important structural and functional role in the formation of these biofilms.

Hypothesis: We hypothesize that failure to eradicate *P. aeruginosa* infections in CF patients is linked to the differential secretion of biofilm exopolysaccharides.

Methods: A set of 14 clinical isolates were obtained from a clinical trial of tobramycin eradication therapy in CF patients. Seven strains were collected from patients in which eradication therapy was successful while 7 others were isolated from patients in whom eradication therapy was unsuccessful. The capacity of strains to secrete the exopolysaccharide Pel was measured by a newly developed enzyme-linked immunoassay (EIA) and the localisation of Pel and Psl within the biofilm was assessed by confocal microscopy.

Results: Previous work from our group revealed that there were no differences in the quantity of secreted Psl between persistent and eradicated strains of *P. aeruginosa*. Consistent with these findings, studies using our newly developed Pel EIA revealed no significant difference in the quantity of secreted Pel between the two sets of strains. Localization of Pel and Psl was therefore performed by confocal immunofluorescent microscopy. In contrast to the findings with secreted exopolysaccharide quantification, microscopy studies to visualize these two polysaccharides within the biofilms revealed significant strain to strain variability in exopolysaccharide expression.

Conclusion: This study demonstrates that despite producing the same amounts of secreted exopolysaccharides, each clinical isolates of *P. aeruginosa* exhibited significant differences in biofilm-associated exopolysaccharide expression. Ongoing studies will seek to determine if there is a correlation between biofilm exopolysaccharide composition and resistance to tobramycin eradication therapy. The outcome of this study could provide a better understanding of the biofilm related resistance mechanisms in *P. aeruginosa* as well as an optimized avenue for novel therapies development.

Oral #2 - Investigating the Cellular Powerhouse through a Patient-Specific Lens: A Study of Mitochondrial Function and Apoptosis in Hypoxia-Treated iPSC-Derived Cardiomyocytes

David Derish[1], Patrick Young[2], Ida Derish[3], Élise Rody[4], Bin Yu[5, 6], Michelle van Holten[3], Janice To[7], Jeremy Zwaig[3], Renzo Cecere[3, 6]

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Background: During a myocardial infarction, restricted oxygen leads to hypoxia-mediated cardiac damage, triggering apoptotic pathways within cardiomyocytes (CMs). Critically, CM injury is irreversible: cardiac tissue cannot regenerate, leaving survivors with permanently damaged heart regions, reduced quality of life, and disproportionately elevated cardiac-related mortality. Pathways of cardiac injury are not yet fully understood, but current literature on dilated cardiomyopathy (DCM) points to mitochondrial dysfunction as a possible culprit in the process of heart failure. Indeed, due to the mitochondria's pivotal role in triggering apoptosis, mitochondrial dysregulation may be an underlying cause of the fragility of cardiac tissue. Since 2006, researchers have been generating unlimited numbers of induced pluripotent stem cells (iPSCs) from blood; these cells can be differentiated into cardiomyocytes (iPSC-CMs) while retaining patient-specific genetic features. By utilising patient-derived iPSC-CMs for in vitro cardiac injury modelling, our project examines apoptotic and mitochondrial responses to hypoxia, thereby elucidating how cardiomyopathies can impact cell survival during infarction events.
Our research asks: when comparing iPSC-CMs from DCM patients (n=4), and healthy donors (n=2), after exposure to hypoxic conditions, are there differences in i) cellular function; ii) expression of pro-apoptotic markers; and iii) mitochondrial activity? We hypothesized that iPSC-CMs derived from DCM patients may be predisposed to more significant injury, correlating with an underlying mitochondrial dysfunction as well as the cells' inability to respond adequately to stress and injury as part of their pathophysiology.
Methods: We isolated peripheral blood mononuclear cells, proceeding with transfection of episomal vectors using Epi5™ Episomal iPSC Reprogramming Kit (ThermoFisher), to obtain patient-specific iPSCs. Pluripotency was confirmed via immunofluorescence, prior to the differentiation of iPSCs into cardiomyocytes using the STEMdiff™ Cardiomyocyte Differentiation Kit (STEMCELL Technologies).
Once spontaneous beating occurred, we recorded daily videos (n=4 per day) to assess baseline contractility over time. The iPSC-CMs were stained for cardiac markers (GATA4, TNNT2, SERCA2a, CX34) to confirm successful differentiation; RT-PCR was performed with additional cardiac and maturation markers. Furthermore, we exposed iPSC-CMs to 0% oxygen for 24 hours. Crystal Violet and Alamar Blue were then used to assess differences in viability and cell metabolism, respectively.
Results: We saw disparities between DCM- and control-derived iPSC-CMs, in cardiac marker mRNA levels prior to hypoxic injury. These patient-specific differences were reflected on a functional level: contractility over time varied largely between lines, pointing to overall quicker and irregular rates in DCM-derived iPSC-CMs. After hypoxia, western blotting revealed differences in protein expression as well as factors involved in heart disease mechanisms. This was corroborated by a significant reduction of post-hypoxic metabolic function in iPSC-CMs from DCM patients, as compared to controls. Next, we aim to quantify mRNA levels of apoptotic markers (Bax, BCL2, CASP3, FAS, HIF-1α) as well as mitochondrial localization via staining of the mitochondrial membrane (MitoTracker™ ThermoFisher) after hypoxia.
Conclusion: Mitochondria-mediated apoptosis is an inexorable feature of cardiac cell death during heart attacks. By furthering our understanding of DCM pathophysiology, our research hopes to

analyse potential prognostic markers of heart failure, inform early screening of DCM, and propose future therapeutic targets.

Oral #3 - Comparative study of doxorubicin encapsulation into nanoparticles using three different chaotic advection-based micromixers

Armen Erzingatzian[1, 2, 3], Ruben R. Lopez[2], Chaymaa Zouggari[2, 3], Julia V. Burnier[2, 4, 5], Vahe Nerguizian[6]

[1]Department of Biology, McGill University, [2]Cancer Research Program, Research Institute of the McGill University Health Centre, [3]École de Technologie Supérieure, [4]Experimental Pathology Unit, Department of Pathology, McGill University, [5]Gerald Bronfman Department of Oncology, McGill University, [6]Department of Electrical Engineering, Ecole de Technologie Supérieure

Background

Extracellular vesicles (EVs) released by cells carry cargo enclosed by a lipid bilayer giving them an important role in cell-cell communication, especially in cancer growth and metastasis. Liposomes are effective nanocarriers for different molecules similar in composition and size to naturally occurring EVs. Micromixers are devices that allow to produce liposomes consistently while controlling their characteristics, size, size distribution (PDI), and zeta potential. Variables such as the total flow rate (TFR), flow rate ratio (FRR) and lipid formulation shape the liposomes' characteristics. Designs such as the PDM, SHM and ring micromixer have microchannels that increase the mixing rate and nanoprecipitation. The objective of this study is to compare, between the 3 different designs, the encapsulation efficiency of doxorubicin (DOX, a fluorescent chemotherapeutic molecule) and the physicochemical properties of the liposomes.

Methods

The devices were designed on SOLIDWORKS 2022 and then 3D-printed with the Pr110-385 3D printer using clear microfluidic photopolymer resin. They were cleaned, flushed with isopropanol and cured under UV light for 30 minutes. Lipids diluted in ethanol (DSPC, CHOL, PEG-PE) were injected in one inlet, while PBS 1x with diluted DOX was injected in the other. Flow conditions were set using computer-controlled syringe pumps. Liposomes were collected at the outlet and dialyzed overnight. To calculate the encapsulation efficiency, liposomes were lysed using 1% Triton X-100 in PBS and fluorescence before and after lysis was assessed using the Qubit 4 Fluorometer. Liposome's hydrodynamic diameter, PDI and zeta potential were determined by dynamic light scattering, and the concentration and size were determined by nanoparticle tracking analysis.

Results

The PDM and ring-micromixer devices were successfully printed at scale 1x with final channel widths of 450um and 280um respectively, but the SHM design's very small features (down to 30um features) proved to be a limitation when using the 3D printing method. The print was successful after the design was enlarged by a scale factor of 3, for a final width of 600um. The addition of nitrile rubber O-rings to the Luer connectors at the inlets and outlets of the device was more effective in creating a seal than the use of barb connections. Liposomes were successfully produced with controllable characteristics using the PDM. Results pertaining to the other micromixers and the encapsulation efficiency are still to come.

Conclusions

Micromixers were successfully printed for the PDM and the ring micromixer, where the SHM needed to be scaled to be 3D printed. It was found that 3D-printed devices such as the PDM produce liposomes of controllable size. In the future, we will include encapsulation efficiency results and LNP-cell interactions.

Oral #4 - Coding Data Elements for Arthrogryposis Multiplex Congenita to the Human Phenotype Ontology (HPO): Challenges and Lessons Learned

Joyce Li[1, 2], Daniel Blanshay-Goldberg[1, 2], Carlos Arthur Sobreira[2, 3], Shahrzad Nematollahi[2, 4], Noemi Dahan-Oliel[2, 4]

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Background

Arthrogryposis multiplex congenita (AMC) is a group of rare congenital musculoskeletal conditions characterized by joint contractures in two or more body parts. The disease occurs in approximately 1/3000 births. AMC research currently lacks a standardized and unified data collection framework. Human Phenotype Ontology (HPO) is a comprehensive online database comprising a standardized vocabulary for phenotypic abnormalities in human diseases. Using the results of a parent project to identify consensus-based data elements for an international registry on AMC, this project aims to map AMC phenotypes to HPO coded to create a standardized terminology for data collection and exchange.

Methods

We started with 2245 variables extracted from 482 data elements. As a team of four medical and microbiology students, our study took place on June- July 2022 at the Shriners Hospitals for Children-Canada. Mapping algorithm was developed by the AMC advisory committee and its reliability was tested on a random set of variables, yielding a satisfactory agreement of 75% (ICC=0.75, 95%CI:0.50, 0.91). The mapping algorithm yielded three mutually exclusive groups: Presence of the exact HPO code (Group A); Presence of multiple, imprecise HPO codes (Group B); or Absence of exact or similar HPO codes (Group C). Required information for each HPO code included definitions, common synonyms, and cross-reference codes to external databases, such as SNOMED, UMLS, MeSH, OMIM, and Orphanet. Regular AMC expert panels were conducted to monitor the progress and address face or content discrepancies.

Results

The proportion of variables in each Groups B or C was 14% (314 and 308 variables; respectively). The sections on joint contracture and multiple pregnancy had the highest proportion of variables in Group C. We have collaborated with the HPO developer's team to update the ontology in order to address variables in Groups B &C. For instance, extensive suggestions have been made to add joint contracture variables to the ontology, and new hierarchical links were proposed to facilitate navigation. An example of the suggestions is presented for hand contractures (Graph 2). We have also searched medical terminology databases, such as SNOMED, MeSH and ICD v.10 &11 to find scientifically valid definitions for these newly proposed terms as well as existing undefined terms. Required modifications to the AMC data elements are also being undertaken following feedback from the AMC expert panels.

Conclusion

Throughout this project, we have gained valuable experience in multidisciplinary collaborations by working to integrate the opinions of experts in bioinformatics, obstetrics, orthopedics, occupational therapy, physical therapy and other clinicians. Unified databases are increasingly appealing for a holistic clinical view of individual with rare diseases such as AMC. These databases can improve data sharing and knowledge transfer, and allow application of medical technologies such as machine learning models to integrate patient data for research and clinical management. By using HPO, we have created a unique and comprehensive data collection framework for AMC, which can be applied readily in other rare musculoskeletal conditions.

ORAL #5 - Effect of Amblyopia on Motion Prediction Tasks

Ziad Lamlili El Mazoui Nadori[1], Alexandre Reynaud[2]

[1]Undergraduate Student-BRaIN program, [2]Assistant Professor, Ophthalmology & Visual Sciences

Amblyopia is a condition that leads to impaired vision in one eye. Research showed that amblyopia patients have a deficit in motion tracking tasks. They also showed a deficit in gaze control and global motion in the amblyopic eye. Another study, focusing on the fellow eye of amblyope, found a deficit in motion perception even in the fellow eye. Motion prediction consists in predicting the position of a moving object based on previous frames. According to previous studies, speed, size, and occlusion time affect the prediction motion tasks. Furthermore, a study conducted on subjects with normal vision showed that eye movement influences how well the subject does the task. The ones that followed the moving object had better accuracy in estimating its position than subjects who didn't follow the object. Based on these previous studies, we wanted to test motion trajectory estimation in amblyopia. Specifically, whether amblyopes can judge when a hidden moving target will reach its goal.

To design our experiment, twenty participants were assigned to two groups (control and amblyopia patients). The control group had normal vision. The other group had amblyopia. Both groups were presented with the same stimulus on a CRT screen at 56 cm from the screen. Its resolution was 1280x1024 at a refresh rate of 100 hz. The stimuli was a rectangle (5 deg x 1 deg) moving at a constant speed (2.5; 5; 10; 15 deg/s) during a viewing distance (3; 6; 9 deg) before being occluded (1, 2, 4, 8 deg). At the end of the occluded portion, a black rectangle is presented, and the subjects need to stop the movement of the white rectangle when they think that both rectangles overlap.

Preliminary results conducted on control patients showed a normal distribution around an average of 0-degree value for the difference between the perceived distance and the real distance. This result is expected since control patients shouldn't have a problem with prediction motion tasks. We expect to see a different average and higher variability in the amblyopic group.

ORAL #6 - Expression of EZHIP in Osteosarcoma and its Effect on Mesenchymal Differentiation

Liam Roberts[1, 2], Wajih Jawhar[1, 2], Geoffroy Danieu[1], Alva Annett[1, 3], Anna Castillo Orozco[1, 3], Sungmi Jung[4], Robert Turcotte[5], Nada Jabado[1, 3], Livia Garzia[1, 6]

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Background: Osteosarcoma (OS) is the most common bone cancer and accounts for 20% of all bone tumours; malignant and benign. OS most commonly occurs during teenage growth spurts around the epiphyseal growth plate which house mesenchymal stromal cells and different osteoprogenitor populations. The genomic landscape of osteosarcoma has been comprehensively profiled by different groups, yet very little is known about the role of epigenetics and chromatin remodelling in these malignancies. We hypothesized that histone mutations and aberrant expression of the onco-histone mimic, EZHIP, play a role in osteosarcoma pathogenesis.

Methods: The expression of oncohistones (H3K27M and H3G34W) and the oncohistone-mimic EZHIP was evaluated by immunohistochemistry in a cohort of pediatric and adult osteosarcomas. We correlated their expression with histone methylation status (H3K27me3) and overexpressed these mutations in a panel of mesenchymal cell lines to study their effects on differentiation. Finally, we assessed whether EZHIP confers resistance to chemotherapy using in-vitro drug assays.

Results: EZHIP is highly expressed in a subset of osteosarcomas and its expression is correlated with the relative loss of the heterochromatin mark H3K37me3. EZHIP overexpression in the osteosarcoma cell of origin (human mesenchymal stromal cells) alters its differentiation propensity and leads to the upregulation of oncogenic pathways and chemosensitivity to epigenetic drugs.

Conclusion: In addition to the previously reported oncogenic mutations of histone proteins, we describe a potential role for EZHIP, an oncohistone mimic, in a subset of Osteosarcomas. We conclude that altered levels of epigenetic players such as EZHIP may constitute a novel mechanism of OS pathogenesis and strategies targeting such mechanisms should be considered in the future as treatment options for osteosarcoma patients.

ORAL #7 - Study of Rho-GTPase regulators in podocytes

Cyril Kazan[1], Tomoko Takano[1], Sajida Ibrahim[1]

[1]McGill University

In healthy kidneys, the glomerular filtration barrier prevents plasma proteins from the blood to enter into the urine. Glomerulopathies, such as focal segmental glomerulosclerosis (FSGS), cause the disruption of this barrier and the leakage of proteins into the urine. FSGS is a cause of Nephrotic Syndrome, which is characterized by proteinuria, hypoalbuminemia, and edema.

Podocytes are highly specialized cells that play a key role in the glomerular filtration barrier. Their interdigitated foot processes constitute the functional structure of this filter. In FSGS, podocytes undergo detachment and their foot processes become effaced altering the filtration barrier and leading to proteinuria.

Studying the disruption of the podocyte cytoskeleton is important in understanding podocyte abnormalities. Rho-GTPases are known to play a role in cytoskeletal modulation, and a dysregulation of these GTPases could be behind cytoskeletal disruption and podocyte damage. Studies have showed that Rho GTPases activity is either increased or decreased in FSGS models.

Rho GTPases are molecular switches that are tightly regulated by guanine nucleotide exchange factor (GEFs), GTPase-activating proteins (GAPs) and guanine nucleotide dissociation inhibitors (GDIs).

Our lab has identified by BioID pull-down assay the panel of GAPs and GEFs that interact with the three prototypical Rho-GTPases in podocytes (Cdc42, Rac1 and RhoA). Knocking out these GAPs and GEFs in cultured human podocytes using CRISPR-Cas9 gene editing could provide great insight into the role each of these proteins is playing in the regulation of Rho-GTPase activity. This could lead to a better understanding of the pathophysiology of proteinuria, and to the discovery of potential treatment targets.

Oral #8 - Analysis of Predictors for Continuous Long-Term Positive Airway Pressure (CPAP) Adherence in Patients with Parkinson's Disease, Related to Obstructive Sleep Apnea, and Associated Syndromes

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Rationale: Patients with Parkinson's disease (PD) are often affected by obstructive sleep apnea (OSA). Continuous positive airway pressure (CPAP) is the mainstay of therapy, but adherence may be difficult in PD. Our aim was to characterize CPAP adherence and predictors of adherence in a randomized controlled trial in PD patients over a six-month period.

Methods: Patients with PD, OSA and mild cognitive impairment were randomised to CPAP or placebo and reassessed at one, three months, and six-months. Demographic, clinical and sleep characteristics were assessed, including the Montreal Cognitive Assessment (MoCA), PD Sleep Scale (PDSS), Epworth Sleepiness Scale (ESS), Beck Depression Inventory (BDI), PD disease severity and non-=motor symptoms (MDS-UPDRS), and presence of REM Sleep Behavior Disorder (RBD) Independent t-tests and Fisher's exact tests were used to compare variables of interest between consistent CPAP users and non-users. Linear regression was used to assess predictors of average daily CPAP use in patients with available data at the 6-month follow-up.

Results: Of the 48 patients who were randomized to CPAP (mean age 68.9 (SD 10.6), PD duration 9.5 years (SD 5.2), 77.1% male), after a six-month period, 35 had regular CPAP usage with a mean of 3h06 (standard deviation 1h57) per day and use on a mean of 66.5% (SD 26.8) of days. There were 13 CPAP non-users: 4 were followed but did not use CPAP and 9 dropped out of the study. Between CPAP non-users and regular CPAP users, no significant differences were observed in baseline demographic or questionnaire data, except for a trend to a lower MoCA score in non-users (20.5 in non-users, vs. 23.5 in users, p=0.07). Moreover, none of the baseline variables examined were significant predictors of the average daily CPAP use at 6 months.

Conclusion: CPAP use remains challenging in PD patients with OSA and reduced cognition. Though lower cognitive function may be a factor in poorer CPAP adherence, we have not identified any clear predictors

ORAL #9 - The role of the aryl hydrocarbon receptor (AhR) in lung fibroblast differentiation: implications for pulmonary fibrosis

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Background: Pulmonary fibrosis (PF) is a chronic lung disease characterized by the progressive and irreversible scarring of alveolar tissue resulting in impaired lung function. In Canada, the incidence of PF is 10 cases per 100 000 people each year; however, the median life expectancy following diagnosis is only 3-5 years. Risk factors include air pollutants such as cigarette smoke and toxic dust, which damage lung tissue and may trigger aberrant repair responses resulting in fibrosis. This repair process is driven by the release of transforming growth factor-beta (TGF- β) - a cytokine that plays a key role in the development of fibrosis by inducing extracellular matrix (ECM) deposition. Mechanisms controlling the development of fibrosis are poorly understood but could involve the aryl hydrocarbon receptor (AhR), a ubiquitously expressed transcription factor that is activated by toxicants found in air pollutants, and whose activation has been linked to TGF-β pathways.

Hypothesis & Aims: The precise role of AhR in the context of pulmonary fibrosis is poorly understood; therefore, we set out to determine whether the AhR controls the deposition of ECM proteins in mouse lung fibroblasts (MLFs) under pro-fibrotic conditions (*i.e.,* TGF-β stimulation). We predict that in response to TGF-β, the AhR will be implicated in fibroblast differentiation and promote ECM production.

Methods: MLFs cultivated from AhR heterozygous (*Ahr*$^{+/-}$) and knock-out (*Ahr*$^{-/-}$) mice were used for experiments. *Ahr*$^{+/-}$ and *Ahr*$^{-/-}$ MLFs were either untreated or treated with 5 ng/ml of mouse rTGF-β and incubated for 24, 48, and 72h before total protein extraction. Levels of the ECM markers fibronectin and collagen-1, and myofibroblast differentiation marker alpha-smooth muscle actin (α-SMA) were measured by Western Blot. Additionally, mass spectrometry analysis was performed on the lysates from the 48h time point.

Results: Our results showed that stimulation with TGF-β induced α-SMA expression in MLFs of both genotypes, but α-SMA was significantly higher in the *AhR*$^{-/-}$ MLFs. In addition, an increase in collagen-1 protein levels was seen in both genotypes following TGF-β treatment, but more significantly in the *Ahr*$^{+/-}$ cells.

Conclusion: The AhR may play an important role in fibrosis, in part, by regulating ECM production. Further investigation could be aimed at determining the mechanism through which the AhR controls lung fibroblast differentiation.

ORAL #10 - Investigating Clipped Histone H3 as a Novel Prognostic Biomarker for Non-Small Cell Lung Cancer

Muhammad Shahzad[1], Meghan De Meo[1], Roni Rayes[1], Lorenzo Ferri[1], Jonathan Cools-Lartigue[1], Jonathan Spicer[1]

[1]Department of Surgery, Division of Thoracic Surgery, McGill University, Montreal, QC, Canada

Background: The role of the tumor microenvironment (TME) in cancer pathology is far-reaching and complex. Within the TME, tumor-associated neutrophils (TANs) drive cancer progression through various mechanisms. An important such mechanism is NETosis, during which TANs elaborate protein-decorated DNA webs called neutrophil extracellular traps (NETs). The pivotal role of NETs in cancer has prompted interest surrounding their use as a clinical biomarker. Such use of NETs as biomarkers would require specific, sensitive, and quantifiable molecular signatures to distinguish NETs from other circulating DNA. Recently, a novel post-translational modification event underpinning NETosis was described, involving serine protease-mediated cleavage of histone H3 N-terminal tails. This H3 Clipping (hereafter referred to as H3Clip) has been suggested to be a signature of NETs. We therefore sought to assess H3Clip as a marker of NETs and evaluate its potential clinical relevance as a prognostic biomarker for non-small cell lung cancer (NSCLC).

Methods: A monoclonal antibody specific to H3Clip (3D9 mAb) was assessed as a marker of NETs and compared to existing signatures. High resolution immunofluorescence images were taken of neutrophils undergoing NETosis and other forms of cell death to investigate H3Clip as a marker of NETs. To elaborate potential clinical relevance, H3Clip was then assessed as an NSCLC prognostic biomarker. Pre-treatment plasma was available from 29 NSCLC patients. Circulating H3Clip expression was thus measured using enzyme-linked immunosorbent assay and 3D9 mAb. Circulating H3Clip was assessed as a predictor of overall survival (OS).

Results: In this study, we demonstrate that 3D9 mAb for H3Clip is a superior marker of NETs compared to the existing H3Cit/MPO co-stain signature because our immunofluorescence results show that 3D9 is unique to neutrophils actively undergoing NETosis and can detect NETosis earlier than the conventional MPO/H3Cit co-stain. The specificity of 3D9 as a marker of neutrophils undergoing NETosis was validated since PMA stimulated neutrophils had a higher H3Clip signal, as measured both through fluorescence and the H3Clip ELISA, compared to unstimulated neutrophils, neutrophils treated with NETosis inhibitors, and (un)stimulated cancer cells. Given this validation, we assessed H3Clip expression in our MUHC NSCLC cohort, finding expression was significantly elevated in patients with more advanced-stage disease (stages II+) as compared to those with stage I disease ($p<0.05$). Additionally, high H3Clip predicted significantly reduced OS ($p<0.05$).

Conclusion: In this study we validate H3Clip as a novel signature of NETs that is superior to conventional markers. These findings are clinically relevant since circulating H3Clip was predictive of worsened OS in NSCLC, supporting further investigation of its use as a prognostic biomarker. Our results therefore support continued exploration of H3Clip and NETs as cancer biomarkers.

ORAL #11 - Safety of COVID-19 mRNA vaccination in children with chronic urticaria

Catherine Zhu[1], Alex Nguyen[1], Connor Prosty[1], Vera Laboccetta[2], Pasquale Mulé[3], Elena Netchiporouk[4], Michelle Le[4], Xun Zhang[2], Sharon Baum[5], Reman Hakroush[6], Shoshana Greenberger[6], Moshe Ben-Shoshan[3]

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Background: Chronic urticaria (CU) is a mast cell-driven disease characterized by the development of cutaneous wheals, angioedema, or both for at least 6 weeks. It affects 1% of children and has a substantial impact on the quality of life. Given the sparse data on the safety of Coronavirus disease 2019 (COVID-19) vaccines in children with CU and the potential risk of urticaria flare post-vaccination, we aimed to assess the safety of the Pfizer-BioNTech BNT162b2 mRNA COVID-19 (BNT162b2) vaccine and the characteristics of COVID-19 infection among CU children.

Method: This study recruited children with CU (aged 5-18 years old) from Montreal Children's Hospital, The Children's Clinic (Canada), and Sheba Medical Center (Israel). Participants were administered standardized questionnaires querying on the first and second dose BNT162b2 vaccine, post-vaccination side effects including allergic reaction (urticaria/angioedema, current CU flares/need for updosing CU medications, respiratory symptoms, anaphylaxis), flu-like symptoms (chills, fatigue, myalgia, low-grade fever), injection site reaction (pain, redness, swelling), and high fever (>39C). Data were also collected on participants infected by COVID-19. Data analysis was performed using R v.4.2.0.

Results: A total of 101 children with CU responded to the questionnaire. Their median age was 13.0 years (IQR=9.3-15.0) and 50 (49.5%) were male. In our cohort, 74 children (73.3%) received the first dose and 56 (55.4%) also received the second dose. No patients reported any allergic symptoms following vaccination. Of the patients who received the first dose, 6 (8.1%) reported flu-like symptoms, 20 (27.0%) injection site reaction, and 1 (1.4%) high fever. For the second dose, 10 children (17.9%) reported flu-like symptoms, 18 (32.1%) injection site reaction, and 1 (1.8%) high fever. Using univariate and multivariate logistic regression, variables such as parental education, sex, and urticaria score over seven days (UAS7) were found to not be significantly associated with COVID-19 vaccination. The main reasons for vaccine hesitancy in the parents of CU children were fears about the general safety of the BNT162b2 vaccine (16.0%) and waiting to consult with their pediatrician to discuss vaccination (16.0%). Among all children in our cohort, 17 (16.8%) reported COVID-19 infection, and 9 (52.9%) of them were unvaccinated at the time of infection. Most infected children (N=10, 58.8%) reported flu-like symptoms, 1 (5.9%) anosmia/ageusia, 1 (5.9%) reported hives flare-up, and 5 (29.4%) were asymptomatic.

Conclusion: Our findings suggest that BNT162b2 COVID-19 vaccination is safe and advisable among children with CU and does not appear to cause adverse events or CU flares. Among our cohort, the majority had mild COVID-19 symptoms and no hives flare-up post-infection. Our study demonstrates the safety of the BNT162b2 vaccine in a significant portion of the population who may be reluctant to be vaccinated. Therefore, our results may increase vaccine uptake and subsequently reduce morbidity, mortality, and the economic burden from COVID-19.

ORAL #12 - Functional Selectivity Regulation of AT1R to Gq and B-arrestin

Nicholas Lee[1] and Stephane Laporte[1,2]

[1]Research Institute of the McGill University Health Centre – MeDiC program; [2] McGill University

Angiotensin II regulates vascular resistance and blood volume via the Angiotensin II type 1 receptor (AT1R). As a G protein-coupled receptor (GPCR), AT1R can signal through G proteins and B-arrestins, which act as adaptors. Specifically, its internalization is mediated by Gαq/11, Gα12/13, Gαi1/2/3 and β-arrestins β-arr1 and 2. Certain ligands may stabilize unique conformations in AT1R, promoting selective signalling known as biased signalling or functional selectivity. Class A GPCRs contain allosteric ligand binding sites extracellularly, intracellularly; ligand binding affects G protein coupling. It was previously found that on the AT1R, the residue at V246 influences Gαq coupling. A particular variant of AT1R with a V246A mutation has been observed to cause favoured activation of Gαq over β-arrestins.

To further elucidate the effect of the V246 residue on functional selectivity in AT1R, we performed site-directed mutagenesis at this position to generate AT1R mutants using two methods. We used AT1R-V246 NNB-F and I245-R primers to generate overlapping strands, which are inserted into a vector at HindIII and XbaI following amplification with dNTPs. Another method was using direct cuts at the origin and using each forward/reverse primer to make a circular product. DMSO competent cells were then transformed using the sequenced products to obtain purified plasmid DNA to be tested via bioluminescence resonance energy transfer (BRET) experiments. Using BRET2 sensors, we investigate the effect of various mutations at the V246 residue on Gαq and β-arr coupling to AT1R.

ORAL #13 - Genetic Classification of Individual Cortical Neurons

Farah Zahoua Alem[1, 2], Hovy Ho-Wai Wong[1, 2], Per Jesper Sjöstrom[1, 2]

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Background

Interneurons (INs) play key roles in signal integration and network stabilization in our brains. However, INs are very diverse, and current profiling methods based on morphology and electrical properties are instrumental but incomplete. Small compartments such as axons are hard to resolve with morphometry and are often severed during dissection, while many cell types have similar electrophysiological properties. Hence, there is a need for an additional complementary technique to ease the identification of cortical INs. This project aims to develop a method that utilizes cell-specific molecular signatures to complement electrophysiology and morphometry data for cell type identification.

Methods

Cell types are genetically identifiable by amplifying specific mRNAs. To ensure feasibility, mRNA extracted from mouse whole-brain tissue was first tested. The method will eventually be refined to single-cell precision with the use of mRNA harvested from whole-cell recording pipettes. The extracted mRNA was reverse transcribed to produce a cDNA library, which was then amplified for specific genetic markers with our custom-designed primers via polymerase chain reaction (PCR). Glial, excitatory, and inhibitory cells were identified from PCR products such as GFAP, VGLUT1, PV, VIP, and SOM.

Results

The PCR products were characterized via DNA agarose gel electrophoresis, which revealed single bands of expected sizes for the PCR products amplified with each of the custom primers. The primer specificity was further confirmed using DNA gel quantification and Sanger sequencing.

Conclusions

The custom-designed primer sets allowed the amplification and detection of the predicted PCR products for the desired markers, thus enabling their future use in a genetic-based cell typing method. To establish this technique, the current protocol will be refined to single-cell precision. The goal is to ultimately allow the amplification and detection of genetic markers from mRNAs that passively diffuse into the pipettes when patching is performed. This will preserve the neuronal morphology, enabling a three-pronged cell typing approach combining electrophysiology, morphometry, and genetic data.

ORAL #14 - Angina Burden and Coronary Vascular Function in Women with Ischemia and No Obstructive Coronary Arteries—Role of Oxygenation-Sensitive Cardiovascular Magnetic Resonance Imaging

Julia Visch[1, 2], Judy M Luu[1, 3], Elizabeth Hillier[1, 4, 5], Malik Elharram[1, 4], James A White[6, 7], Todd Anderson[6, 7], Matthias G Friedrich[1, 8], Louise Pilote[1, 4]

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Introduction: Patients with ischemia and no obstructive coronary arteries (INOCA) often have coronary microvascular dysfunction (CMD). An association between higher angina burden (lower Seattle Angina Questionnaire [SAQ] scores) and increased severity of CMD has previously been reported. Oxygenation-Sensitive Cardiovascular Magnetic Resonance (OS-CMR) is a novel approach that can potentially evaluate CMD. To date, the relationship between angina burden and OS-CMR biomarkers has not been elucidated.

Methods: We investigated the association between angina burden, quantified by the mean and 5 individual components of the SAQ, with measures of myocardial oxygenation, using OS-CMR. More severe CMD is reflected by a decreased % signal intensity (SI) change after hyperventilation (Breathing-enhanced Myocardial REserve, B-MORE), calculated by [(Hyperventilation – Breath Hold)/Breath Hold] x 100%. Univariable linear regression analysis was used to investigate the associations.

Results: In 44 women with INOCA (mean age 55.1±0.9 years), B-MORE was 5.0±1.4% (Table). Several components of the SAQ were associated with OS-CMR measures of CMD [angina frequency (R^2=0.216, Beta=0.465, p=0.039), disease perception (R^2=0.254, Beta=0.504, p=0.024), and the mean overall SAQ (R^2=0.220, Beta=0.469, p=0.037)] (Panel Figure).

Conclusion: In women with INOCA, angina burden is associated with coronary vascular function, as assessed by OS-CMR. Therefore, this novel approach may provide objective information to detect the presence of CMD.

ORAL #15 - Identifying Pathogenic Variants in the Primary-Ovarian-Insufficiency Genes of Patients Experiencing Recurrent Pregnancy Loss and Infertility

Zeynep Yalcin[1] and Rima Slim[1,2]

[1] Research Institute of the McGill University Health Centre – CHHD Program, [2] McGill University

Primary ovarian insufficiency (POI) develops when normal ovarian function is ceased before the age of 40. It affects roughly 1% of women under 40, and 0.1% of women under 30. POI is characterized by a lack of menstruation (amenorrhea) and deficiency in gonadotropic hormones, leading to suboptimal oocyte quality, miscarriages, and infertility. POI has genetic as well as non-genetic determinants, making it a highly heterogeneous condition. Although considered an idiopathic condition, previous data have implicated a strong genetic component. Thus, genetic screening is crucial in families failing to conceive since the underlying cause might stem from genetic abnormalities.

In this study, we look at various POI genes and their variants in families experiencing recurrent pregnancy loss and infertility. DNA available from the patients was analyzed using exome sequencing, and the sequences were searched for variants in POI genes. In our analysis, we prioritized protein-truncating variants, such as frameshift, stop-gain, and insertion/deletion mutations. Identified variants were confirmed by Sanger sequencing and segregated in available family members. In the upcoming stages of the study, the allele frequencies of confirmed variants in our patients will be compared with the allele frequencies of these variants in the general population. A statistically significant higher frequency of protein-truncating variants in patients will demonstrate their genetic susceptibility to POI.

Here we present various POI genes and the cases of families carrying confirmed variants in these genes. It is crucial to note that our patients have never received a POI diagnosis, however, had miscarriages as well as subfertility. Therefore, finding pathogenic variants in POI genes of these patients will provide them with the appropriate diagnosis, further improving the management of their case and reducing the expenses spent on unsuitable fertility treatments. Results from this study can additionally contribute to developing diagnostic genetic tests and provide better support for the patients.

ORAL #16 - Opioid versus opioid-free post-discharge analgesia after outpatient general surgery: Protocol for a randomized controlled trial

Danielle Cutler[1], Julio Flavio Fiore Junior[1]

[1] Research Institute of the McGill University Health Centre - Injury Repair Recovery Program (IRR); [2] McGill University

Rationale: Canada is facing an epidemic of opioid addiction and overdose. Excessive prescribing has been implicated as an important contributor to this national health crisis. Worldwide, Canada has the second-highest rate of opioid prescription per-capita. Surgery often serves as the initial event for opioid-naïve patients to obtain an opioid prescription and spiral into misuse and addiction. Evidence suggests that non-opioid analgesia is common internationally but not in North America, where opioid tablets are often prescribed instead of or with non-opioid medications. From the perspective of surgeons and other perioperative care clinicians, one answer to the opioid crisis is to avoid post-discharge opioid prescribing altogether using opioid-free analgesia (i.e., pain management using only non-opioid interventions). Prescriber decision-making must be informed by robust randomized controlled trials (RCTs) focused on the comparative effectiveness of analgesia regimens including opioids (opioid analgesia, OA) vs. opioid-free analgesia (OFA).

Research question and hypotheses: The **P**ostoperative **A**nalgesia **I**ntervention with **N**on-opioid **Alt**ernatives (**PAIN-Alt**) trial will address the following research question: Among patients discharged after outpatient abdominal general surgery, to what extent does OFA impact 7-day pain intensity, pain interference, and post-discharge nausea and vomiting (PDNV) in comparison to OA? We hypothesize that the prescription of OFA will be non-inferior to OA by a non-inferiority margin of 1 point (minimally important difference) on the Brief Pain Inventory (BPI) pain intensity and pain interference scales (range 0-10; higher scores = worse outcome).[12] Also, we hypothesize that the prescription of OFA will reduce rates of PDNV in comparison to OA (absolute risk reduction $\geq$ 10%).

Methods: The PAIN-Alt trial will be a parallel, two-group, assessor-blind, open-label RCT conducted at seven tertiary hospitals across four Canadian provinces (Alberta, Ontario, Manitoba, and Quebec). The trial feasibility and design have been supported by a pilot study (n=76). Adult patients (>18 years old) undergoing elective cholecystectomy and hernia repair scheduled for same-day discharge will be considered for inclusion. At postoperative hospital discharge, participants will be allocated 1:1 to receive OA (around-the-clock non-opioids and opioids for breakthrough pain) or OFA (around-the-clock non-opioids and adjustment of non-opioid drugs and/or non-pharmacological interventions for breakthrough pain). Given the pragmatic nature of this trial, the specific OA and OFA regimens will be determined by the patient's primary surgeon, considering the surgical procedure, comorbidities, and patient preferences. The co-primary outcomes of interest will be 7-day pain intensity, pain interference, and rates of PDNV. Secondary outcomes include self-reported physical and mental function, adverse drug events, healthcare reutilization, opioid misuse, chronic pain, prolonged opioid use, and healthcare costs. A sample of 600 participants will be targeted following a priori power calculation. Our primary statistical analyses will be conducted using linear mixed models and logistic regression.

Significance: The overprescription of opioids is one of the driving forces behind the opioid crisis. Patients undergoing outpatient general surgery are frequently prescribed opioids after discharge, but the value of this practice remains uncertain. The PAIN-Alt trial is essential for building a strong body of evidence to mitigate the negative downstream effects of postoperative opioid overprescribing in Canada.

POSTER PRESENTATIONS

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Meeting ID: 885 8020 7410 - Passcode: 930432

Breakout Room 1:

1-	James Vafiadis	Dr. George Zogopoulos (CRP)
2-	Yeniay Erdem	Dr. Farah ElTurk (CHHD)
3-	Jacob Abdaem	Dr. Abhinav Sharma (CHAL)
4-	Abdulrahman Hamam	Dr. Suhad Ali (CRP)
5-	Allyson Kis	Dr. Louise Pilote (CHAL)
6-	Marie Dumont	Dr. Aimee K Ryan (CHHD)
7-	Jillian Caplan	Dr. Renzo Cecere (CHAL)
8-	Mustafa Fakih	Drs. PA Martineau & R Gawri (IRR)
9-	Sumali Mehta	Dr. Anne-Marie Lauzon (RESP)
10-	Sriya Veerapaneni	Dr. Jesper Sjöström (BRAIN)

Breakout Room 2:

11-	Liliane Marie Fakhouri	Dr. Dr. James Engert (CHAL)
12-	Erin Ward	Dr. James Martin (RESP)
13-	Patrick Young	Dr. Renzo Cecere (CHAL)
14-	Kaiyang Li	Dr. Carolyn Jack (IDIGH)
15-	Leanne Young	Dr. Aparna Suvrathan (BRAIN)
16-	Srishti Ahlawat	Dr. John Kildea (CRP)
17-	Ridhi Mittal	Dr. John Kildea (CRP)
18-	Michelle van Holten	Dr. Renzo Cecere (CHAL)
19-	Olga Tsyruk	Dr. George Thanassoulis (CHAL)
20-	Ketsia Lola	Dr. James G Martin (RESP)

Breakout Room 3:

21-	Hillary Chappus-McCendie	Dr. Stella S. Daskalopoulou (CHAL)
22-	Paolo Medina Iglesias	Dr. Ronald Dandurand (RESP)
23-	Xavier Ng	Dr. Sampath Loganathan
24-	Jessy Chen	Dr. Teruko Taketo (CHHD)
25-	Camilo Sierra	Dr. Andréa Maria Laisner (CRP)
26-	Vanessa Ross	Dr. Maziar Divangahi (RESP)
27-	Chelsea Pozzebon	Dr. Louise Pilote (CHAL)
28-	Massimo Mazza	Dr. Miguel Burnier (CRP)
29-	Alexandra Yacovelli	Dr. Carolyn Jack (IDIGH)
30-	Laure Aubert	Dr. Suhad Ali (CRP)

Breakout Room 4:

31- Olivia Zhou	Dr. Rahul Gawri (IRR)
32- Miranda Zary	Dr. Kevin Schwartzman (RESP)
33- Olivier Renaud-Charest	Dr. Meranda Nakhla (CHHD)
34- Emma Youhnovska	Dr. Miguel Burnier (CRP)
35- Senna Choi	Dr. Jacques Genest (CHAL)
36- Bhumika Sharma	Dr. John Kildea (CRP)
37- Vivian Wu	Dr. Per Jesper Sjöström (BRAIN)
38- Emily Oulousian	Dr. Abhinav Sharma (CHAL)
39- Jacob Nicodemo	Dr. Constantin Polychronakos (CHHD)
40- David El-Adem	Dr. Constantin Polychronakos (CHHD)

Breakout Room 5:

41- Elie Fadel	Dr. Jacques Lapointe (CRP)
42- Leina Kingdom	Dr. Louise Pilote (CHAL)
43- Janice To	Dr. Renzo Cecere (CHAL)
44- Aidan Shoham Amizlev	Dr. Suhad Ali (CRP)
45- Laurence Bélanger	Dr. Dao Nguyen (RESP)
46- Farah Majedi	Dr. Anie Philip (IRR)
47- Zachary Grimard	Dr. Simon Rousseau (RESP)
48- Youssef Hsheimi	Dr. Abhinav Sharma (CHAL)
49- Ariane Leonard	Dr. Alex Gregorieff (CRP)
50- Samy Abu Setah	Dr. Ivan Litvinov (CRP)

Breakout Room 6:

51- Elisa Taine	Dr. Indra Gupta (CHHD)
52- Angele Wen	Dr. John Kildea (CRP)
53- Isabella Gomez	Dr. Per Jesper Sjöström (BRAIN)
54- Xue Er Ding	Dr. Jun Ding (RESP)
55- Mia Su	Dr. Jun-Li Liu (MEDIC)
56- Sarah Moussa	Dr. Elena Netchiporouk (IDIGH)
57- Fadi Touma	Dr. Ivan Litvinov (CRP)
58- Amrit Das	Dr. Stella S. Daskalopoulou (CHAL)
59- Thien Hy Quach	Dr. Aimee K. Ryan (CHHD)
60- Hongda Li	Dr. Nancy Braverman (CHHD)
61- Amina Bouraj	Dr. Suhad Ali (CRP)

Breakout Room 7:

62- Matthew Djan	Dr. Alex Gregorieff (CRP)
63- Andrew Yim	Dr. Joanna Przybyl (CRP)
64- Zachary Rehany	Dr. Dan Poenaru (CHHD)
65- Rubis Lantier-Théberge	Dr. Myriam Srour (CHHD)
66- Connie Guo	Dr. Jesper Sjöström (BRAIN)
67- Emma Rodriguez	Dr. Myriam Srour (CHHD)
68- Maya Bain	Dr. Aimee K. Ryan (CHHD)
69- Kaitlin Olsen	Dr. Irah King (RESP)
70- Tara Gedeon	Dr. Abhinav Sharma (CHAL)
71- Ellie Massey	Dr. Nathalie Lamarche-Vane
72- Pierre Elias	Dr. Gabiel Altit (CHHD)

POSTER #1 - Investigating mechanisms of Cisplatin resistance in BRCA mutated pancreas cancer

James Vafiadis[1], Joan Miguel Romero[1], Yifan Wang[1], Alicia Gomez[1], Geoffroy Danieau[1], George Zogopoulos[1]

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Introduction: Pancreatic cancer is a systemic disease in its earliest stages, since it does not exhibit symptoms until it has metastasized to other organs. Thus, identifying subtypes of patients amenable to current systemic therapies, and mechanisms of resistance to these therapies, is important. Up to 10% of pancreas cancers are homologous recombination deficient (HRD), which can be caused by germline mutations in *BRCA2* and other genes. These cancers are sensitive to platinum-based chemotherapies like cisplatin and poly APD ribose polymerase inhibitors (PARPi). Identifying mechanisms of resistance to these treatments is crucial for these patients.

Methods: Six SCID-Beige mice were implanted with patient-derived tumours in the following scheme: three mice had both flanks implanted with tumours derived from a cisplatin-resistant tumour, and three mice were implanted in the same manner with a cisplatin-sensitive tumour as control. Both tumours came from patient-derived xenografts treated with cisplatin to generate resistance, originating from a patient carrying a *BRCA* mutation, as previously described (Wang, 2020). The cisplatin-resistant tumours were administered intraperitoneal cisplatin injections at 4mg/kg biweekly once a single tumour had reached a given size threshold of 5x5 mm^3 in order to assure resistance was maintained. The cisplatin-sensitive mice were administered control. Three additional mice were implanted with a PARPi-resistant tumour, as described above.

Results: Three mice in the cisplatin-resistant arm had tumour growth, while only one mouse in the cisplatin-sensitive group had tumor growth. The tumours are still being grown in the mice until endpoint is reached. At endpoint, tumours will be excised and will undergo WGS and single-cell RNA sequencing (scRNAseq) and ATAC-seq, followed by multi-dimensional single-cell analysis for identification of subpopulations present that confer resistance.

Conclusion: Once sequenced, we expect to see mutations within the resistant tumours in comparison to the treatment sensitive tumours. Further, subpopulation of cells uniquely expanded in the cisplatin-resistant tumours are expected to be identified by scRNAseq, and will be analyzed in hopes of improving understanding of how chemotherapy resistance works in HRD patients.

POSTER #2 - Development of an extraction liquid chromatography-tandem mass spectrometry technique to quantitate the level of dihydroceramide species in plasma of MPS III patients

Yeniay Erdem [1] and Farah ElTurk[1,2]

[1] Research Institute of the McGill University Health Centre – CHHD Program; [2] McGill University

MPS type III, also known as Sanfilippo syndrome is a lysosomal storage disorder and is one of the seven types of mucopolysaccharidoses. MPS III is caused by a deficit in one of the enzymes heparan-N-sulfatase, α-N-acetylglucosaminidase, α-glucosaminidase acetyltransferase or N-acetylglucosamin-6-sulfatase, required for the metabolism of a family of glycosaminoglycans, the heparan sulfate (HS*)*. The storage of HS in cells and biological fluids inflicts detriments to one's health. Neurodegeneration and other neurological symptoms (e.g., epilepsy) have been the main symptoms observed in youth diagnosed with this syndrome. Up to date, there is no cure for this disorder, but ongoing research is conducted to better understand the pathophysiology and introduce treatment options. In our laboratory, we try to look for new biomarkers that can be used for early diagnosis and monitoring of disease severity and treatments. Several studies have suggested a specific group of molecules called sphingolipids as potential biomarkers for inflammation and neurodegenerative diseases and neuropsychiatric disorders.

Sphingolipids belong to a class of biomolecules called lipids. They play important metabolic and structural roles in maintaining cellular homeostasis. Previous studies have also shown the implication of these molecules in several diseases. For example, sphingosine-1-phosphate (S1P d18:1) has been found to be involved in cancer development and immune response; ceramides, another family of molecules within the group of sphingolipids have been found to play a significant role in skin disease and cancer. Other sphingolipids have also been found to be implicated in diabetes type 2, Alzheimer's disease, and cognitive impairment.

Dihydroceramide is a subgroup of sphingolipids used as a precursor of the *de novo* ceramide synthesis. Technological advances in lipidomic research have allowed the discovery of their implication in disease, contrary to the past belief of dihydroceramide being biologically inactive in the cell. These species have been shown to play an essential role especially in cancer and metabolic conditions. It was suggested that dihydroceramide species could be used as a potential biomarker for various diseases.

Sensitive, accurate and precise methods of identification and quantification of sphingolipids are therefore crucial elements for evaluating their possible implications in disorders that could make them potential biomarkers. Studies have shown the liquid chromatography (LC) combined with tandem mass spectrometry (MS/MS) method is an efficient way to measure concentrations of sphingolipids in patient blood samples.

In this study, a link between dihydroceramide concentration in MPS III patients' blood samples is being evaluated by developing a robust and reproducible LC-MS/MS method. This will allow to determine the possibility of using the dihydroceramide species as a biomarker for MPS III disease.

Poster #3 - Optimization Of Guideline-directed Medical Therapy In Patients With Heart Failure With Reduced Ejection Fraction Following Coronary Care Unit Admission

Jacob Abdaem[1], Amir Razaghizad[1], Julian Guida[1], Guang Zhang[1], Nadia Giannetti[1], Abhinav Sharma[1]

[1]McGill University Health Centre

Background: Multiple guideline-directed medical therapies (GDMTs) have been shown to decrease cardiovascular mortality and heart failure hospitalization in patients with heart failure with reduced ejection fraction (HFrEF). However, the use of these therapies in current practise remains unclear.

Objective: Evaluate the use of GDMTs in patients with HFrEF during and after admission to the Coronary Care Unit (CCU).

Methods: This was a single-center retrospective cohort study. Inclusion criteria were an index hospitalization to the CCU of the Royal Victoria Hospital between May and November 2021 and a left ventricular ejection fraction (LVEF) < 40%. Patients were eligible for inclusion regardless of clinical presentation. Baseline characteristics were described with medians, interquartile ranges, and proportions. Proportion of GDMT use was evaluated at CCU admission and discharge. Proportion of GDMT addition within 60 days post-discharge was also assessed.

Results: Overall, 70 patients were included, of whom 59 survived until discharge and 58 survived until 60 days post-discharge. The median age, LVEF and eGFR were 66 years, 27.5% and 74.0ml/min/1.73m2 respectively. Among all patients (n=70), the proportions of use of GDMTs at admission were 41.4% for ACE inhibitors/ARBs, 52.9% for beta-blockers, 14.3% for MRAs, 12.9% for sacubitril-valsartan and 8.6% for SGLT2 inhibitors. Among surviving patients at discharge (n=59), the proportions of use were 54.2%, 93.2%, 40.6%, 16.9% and 18.6% for each GDMT respectively. And then, among patients who survived until 60 days post-discharge (n=58), an additional 8.6%, 1.7%, 8.6%, 12.1% and 0.0% were prescribed each GDMT respectively.

Conclusion: The proportions of GDMT use in HFrEF patients before-and-after CCU admission were low for most agents. There is a need for alternative strategies to better optimize rates of GDMTs among HFrEF patients.

Poster #4 - Characterization of novel PRL/PRLR-signaling network in breast cancer

Abdulrahman Hamam[1], Dana Hamam[1], Suhad Ali[1]

[1]McGill

Prolactin (PRL) is a key hormone essential for mammary lobuloalveolar development and differentiation allowing successful lactation. Accumulating evidence implicates a critical anti-tumorigenic role for PRL and its receptor (PRLR) in breast cancer. Further investigations are required to identify the pathways in which PRLR is mediating its anti-tumerging role in breast cancer. In support of this, we have performed a large-scale unbiased proteomics analysis of PRLR/interacting proteins in the luminal A MCF7 cells. This analysis revealed a significant interaction between PRLR and several proteins that are associated with different physiological processes and pathways that may play a role in supressing tumour formation (such as KFC, SPICE1 among others). In this study, we will validate the interaction of these proteins with PRLR upon stimulation of MCF7 cells with hPRL by preforming immunoprecipitation assay (IP) and western blot. Furthermore, the expression of these proteins will be characterized in different breast cancer cell lines (MCF7, MDA231, T47D, SKBR3). Additionally, bioinformatic tools will be used to evaluate the prognostic values of these genes in breast cancer patients and study their correlation with PRLR. Characterizing the interaction of these proteins with PRLR will allow us to understand more about the mechanistic way of which PRL/PRLR signalling pathway is involved in and eventually allows to develop more prognostic and diagnostic tools for treating breast cancer patients. Our results will reveal new possible players in the PRL/PRL signalling pathway in suppression of breast tumorigenesis, providing further insight into the anti-tumorigenic role of PRL in BC.

POSTER #5 - Implementation of Point-of-Care Ultrasound in a university affiliated medical center in Northern Ethiopia

Allyson Kis[1], Jonathan Houle[1], Ning-Zi Sun[2], Workagegnehu Hailu[3], Louise Pilote[4]

[1]McGill University, [2]MUHC, [3]University of Gondar, [4]RI-MUHC

Background: Good quality ultrasound machines have become more affordable and portable with evolving technology. Their use in low-resource settings may improve care notably by allowing for faster and more accurate diagnoses, better resource allocation and safer procedures. Many studies reported successful Point-of-Care Ultrasound (POCUS) implementation in low-and middle-income countries (LMICs), although, to our knowledge, none have conducted a comprehensive needs assessment prior to its implementation nor have used validated evaluation models to assess respective curricula through learner performance and patient related outcomes. We therefore aimed to develop, implement and evaluate a remote POCUS curriculum tailored to the needs of a university-affiliated hospital in Northern Ethiopia.

Methods: We used the Context, Input, Process and Product (CIPP) model to structure the curriculum and its evaluation process. Decisions regarding our target population, curriculum structure, form and content were based on a previously conducted comprehensive needs assessment. Our learners are a selected group of motivated residents and physicians at the University of Gondar Hospital. The curriculum will be delivered remotely over a duration of 12 months and comprises of high-yield scans targeted towards internists working in the emergency department and inpatient settings as well as relevant POCUS guided procedures. Learners will have access to a series of recorded lectures accompanied by documents for reference as well as an online platform where they can upload scans, ask questions and receive feedback from experienced POCUS users in Canada. Three local staff physicians were identified to receive in-person training and become local experts to then lead small group sessions and Objective Structured Clinical Examinations (OSCEs) as well as participate in periodic meetings to ensure progression and development of learners. Curriculum evaluation will be done through assessments of learner performance and perceptions as well as through POCUS use related data collection. Learner performance will be monitored through periodical examinations: 4 written and 2 OSCEs. POCUS related data will be collected through questionnaires filled out by learners for each scan performed where they can comment on the use of POCUS on management, diagnosis and patient outcomes. The impact of the curriculum on lengths of stay and mortality will be evaluated through a chart review and comparison with historical controls.

Expected results: We expect to see improvements in written and practical assessment results, increased comfort, and increased usage of POCUS by residents and faculty members. We also expect improvements in management and patient related outcomes such as increased safety and success of procedures, modified diagnoses, improvements in management, and decreased length of stay. Furthermore, we hope that implementing POCUS in a university setting will facilitate the sustainability of the curriculum and diffusion of POCUS related knowledge and skills to incoming residents and faculty members.

POSTER #6 - Claudins have a unique position pattern in the cell during neural fold fusion in chick

Marie Dumont[1] and Aimee K Ryan[1,2]

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Tight junctions are multiprotein complexes that seals between epithelial cells and are created during the first stages of embryonic development. The claudin protein family is essential for the formation and function of tight junctions. Over 20 claudin family members have been found in vertebrates and each one has its own expression pattern. Neural tube development can be divided in four steps: neural plate formation, elongation, elevation, and subsequent fusion of the neural folds, to form the closed neural tube that is vital for vertebrates. Data from our lab showed that depleting Claudin-3, -4 and -8 prevents the first two steps from occurring properly, causing neural tube defects. Depleting only Claudin-3 inhibits the last step of neural tube closure, neural fold fusion. My project is focused on characterizing claudin expression patterns during neural fold fusion. I hypothesize that I can specifically locate claudins in transverse sections of embryos during neural fold fusion. To answer this, I performed immunofluorescence on each claudin and a tight junction marker in chick embryo sections. This study is focused on Claudin-1(Cldn1), -3, -4, -8, -10 and -14 because they are known to be involved in neural tube development and/or they are expressed in the non-neural ectoderm during neural fold fusion. For now, I showed that Cldn3 is in the apical domain of non-neural ectoderm and that Cldn4 is in the apical domain of both non-neural and neural ectoderm. I am currently doing immunofluorescence on Cldn1, 8, 10 and 14. For the next step, I will focus on characterizing claudins at the apical region cells that are making contact at the midline. This research will bring a better understanding of claudins' function during neural fold fusion and reveal the exact position of claudins during the first stages of development.

POSTER #7 - Induced Pluripotent Stem Cell-Derived Cardiomyocyte Transplantation in Animal Models of Heart Failure: a Meta-Analysis of In Vivo Studies

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When a heart is deprived of oxygen for minutes to hours, the cardiac cells die. These cardiac cells, termed cardiomyocytes (CM) do not regenerate, which ultimately leads to heart failure. Technological advances have allowed us to create cardiac stem cells that have the potential to heal heart tissue. One type of stem cell called an induced pluripotent stem cell (IPSC) has shown promise for cardiac regeneration in an artificial environment. IPSC cells can be transformed into cardiomyocytes through various techniques, a process referred to as cell differentiation. Recently, researchers have begun testing these IPSC derived cardiomyocytes (IPSC-CMs) in animals. However, the advantages and limitations of in-vivo IPSC-CMs for cardiac regeneration are varied and have yet to be synthesized. Through this systematic review and meta-analysis, we will provide insight into the advantages and limitations of iPSC-CMs for cardiac regeneration in animal models of heart failure.

Information sources will be searched according to a predetermined and peer reviewed search strategy (see figure). Collaboration between reviewers will be blinded. The primary outcomes will be: 1) left ventricular ejection fraction and 2) fractional shortening, while the secondary outcomes will be: 1) left ventricular diameter during systole and diastole, 2) end systolic volume, 3) end diastolic volume, and 4) infarct size. Means and standard deviations will be extracted for meta-analytic review using the software RevMan Web. Effect measures will be calculated for all outcomes using standardized mean differences and outcome measures will be analyzed as a pooled analysis. Between and within-study heterogeneity and sensitivity analyses will be conducted. Data will be combined across studies for all IPSC-CMs in animal models, compared to controls. Additional subgroup analyses will be performed, including a meta-regression for each subgroup. Assessment of the strength of evidence will be conducted using Grading of Recommendations, Assessment, Development and Evaluations (GRADE) and publication bias will be assessed by visual inspection and quantification methods.

Evaluating the state of the literature in animal models brings us one step closer to applying IPSC-CMs to the treatment of heart failure in humans.

Poster #8 - Novel tenocyte culturing technique through cellarator-mediated surface stretching

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Patients with ligament tears often undergo operative ligament reconstruction using various grafts to regain original function. Harvested tendons are often used as allografts for reconstruction; however, less invasive and less sacrificial alternatives have been explored in bioengineering settings, such as seeding artificial scaffolds with tendon cells providing optimal mechanical properties with physiological compatibility. Tendon tissue extracted from donors post-operatively are beneficial for research, therapeutic and tissue engineering applications. Primary tenocytes (tendon-derived cells) are difficult to culture primarily due to their relatively short lifespan, and the lack of an established growth compartment incorporating mechanical stresses comparable to those seen physiologically. Expanding and splitting tenocytes through passaging causes them to lose phenotypic expression of important tenogenic markers such as collagen I & III, scleraxis, tenascin-C and decarin. This reduction in the level of markers makes usage of passaged tenocytes in experiments inappropriate as they are no longer representative of tenocytes *in vivo*. Therefore, a new technique is needed, where primary tenocytes are allowed to expand to high confluency, while maintaining their tenogenic expression of various markers. This project aims to propose a novel technique in tenocyte cell culturing, where a constant and gradual uniaxial loading of a cell culture surface can provide the mechanical stimulation and the space needed to culture primary tenocytes to high populations, while maintaining appropriate levels of tenogenic markers. Tenocytes will be harvested from donors undergoing surgery and isolated into primary cells and then cultured for 10 days on 2 sets of activated surfaces: an expanding silicone plate and petri dishes. The petri dishes will serve as a control, where they will be passaged from smaller dishes to larger ones – which is the current method for expanding tenocytes. Quantitative PCR will be used to compare levels of expression of collagen I & III, scleraxis, tenascin-C, and decarin. It is hypothesised that tenocytes undergoing uniaxial expansion at the culture surface will not have a significant difference in marker expression between days 0 and 10, while the passaged tenocytes on the petri dishes will have a significant difference in marker expression between days 0 and 10. If the results support the proposed hypothesis, the novel tenocyte culture technique can be used to better mimic their physiological gene expression, allowing for more applicable experimentation, and improved tissue engineering applications.

Poster #9 - Optimizing the Isolation of Contractile Smooth Muscle Cells from Cryopreserved Tissue

Sumali Mehta[1], Matheus Schultz[1], Pranjal Seth[1], Linda Kashmar[1], Gijs Ijpma[1], Anne-Marie Lauzon[1]

[1]MUHC

The functional properties of smooth muscle have been extensively investigated by measuring the contractility of smooth muscle tissue strips, lending valuable insights towards our understanding of the pathophysiology of diseases such as asthma and hypertension. To increase the throughput of smooth muscle studies, working at the cellular level would be ideal. Furthermore, cellular studies eliminate diffusion barriers and vestigial effects from epithelial layers or unexpected paracrine substances. However smooth muscle cells (SMCs) are known to dedifferentiate and lose their contractile phenotype in culture, complicating mechanical studies at the cellular level. The maintenance of the freshly isolated SMCs fusiform morphology and contractility remains a major challenge to single-cell mechanics studies. While previous studies have confirmed the maintenance of these features in freshly isolated SMCs, the number of contractile cells and the reproducibility of these preparations have been variable and challenging. In addition, the current protocols utilize a relatively small piece of smooth muscle tissue from euthanized animals, making the isolation of SMCs from fresh tissue relatively wasteful. Here, we discuss the optimization of a new protocol for the isolation of fusiform contractile SMCs from cryopreserved smooth muscle tissue. Smooth muscle was obtained from equine tracheal sections and cryopreserved with some epithelium and connective tissue following previously reported protocols. The cryopreserved tissue was then acclimated in DMEM +2% FBS before isolation of SMCs with two consecutive enzymatic digestions. Inspection of isolated cells was performed through fluorescence microscopy with the viability dye calcein-AM, and contractility of SMCs was assessed by addition of 10^{-5}M methacholine. The optimization process yielded variable results with some preparations showing viable, fusiform, SMCs with a contractility comparable to cells isolated from fresh tissue. However, other preparations yielded few poorly contractile cells. Further work will be needed to determine the key steps necessary for reproducibility of SMC isolations from cryopreserved tissue.

Poster #10 - Presynaptic NMDARs in neurotransmission

Sabine Rannio[1], Sriya Veerapaneni[1, 2, 3], Aurore Thomazeau[1], Jesper Sjöstrom[1, 2]

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Background

Synaptic plasticity is the ability of neurons to modify their connectivity over time. It is a key mechanism involved in a variety of brain processes such as development, homeostasis, and learning and memory, and is often impaired in pathological conditions such as Alzheimer's disease, schizophrenia, and epilepsy. N-methyl-D-aspartate receptors (NMDARs) play a crucial role in synaptic plasticity and thus are of major interest. Classically, NMDARs have been thought to be exclusively expressed as postsynaptic (postNMDARs) ionotropic receptors, allowing sodium and calcium ions to pass into the cell through a channel pore to trigger intracellular signalling cascades. But there has been increasing evidence that some NDMARs may signal metabotropically, that is, without ion flux, and NMDARs have also been detected at the presynaptic site (preNMDARs). Thus, our understanding of NMDARs in synaptic plasticity is incomplete and requires further investigation. However, pharmacological tools to explore the distinct roles of pre- vs. postNMDARs are limited. Therefore, in our project, we are taking a genetic deletion approach to study the contribution of different NMDAR pools in neurotransmission and synaptic plasticity in the neocortex.

Methods and Findings

We have generated a global and sparse deletion model to remove NMDARs from a subset of primary visual cortex (V1) layer 5 pyramidal cells (PCs) identified by a fluorescent tag. Using NMDA uncaging, we showed that NMDARs were sufficiently deleted in tagged cells in the global (0.02 ± 0.5 pA, n = 31 vs. control -48 ± 6 pA, n=19; $p < 0.001$) and the sparse deletion model (0 ± 0 pA, n = 9 vs. control -31 ± 8 pA, n = 14; $p < 0.001$). We then investigated spontaneous release by studying miniature excitatory postsynaptic currents (mEPSCs) in the global deletion model and found that mEPSC frequency was not affected by wash-in of an NMDAR antagonist, AP5, unlike control (92% ± 4%, n = 18 vs control 60% ± 12%, n = 5; $p < 0.05$). Now we investigate changes in mEPSC frequency in response to AP5 in the sparse deletion model to conclusively attribute regulation of spontaneous release to preNMDARs as suggested by pharmacological studies.

Conclusion and Future plans

As genetic deletion removes both ionotropic and metabotropic NMDAR signalling, it will consolidate the contribution of preNMDARs to the regulation of neurotransmitter release. We will then use the sparse deletion approach to distinguish the specific roles of pre- vs postNMDARs in short- and long-term synaptic plasticity. This will provide further insight into synaptic plasticity mechanisms in brain development, learning and memory, but also brain pathologies, and potentially open new avenues for therapeutic intervention.

POSTER #11 - Discovering Novel Risk Factors for Aortic Stenosis

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[1]RI-MUHC

Valvular aortic stenosis (AS), a condition in which the aortic valve becomes dysfunctional due to calcification or fibrosis of the valve's leaflets, causes an obstruction of blood flow out of the heart and into the aorta. Valvular aortic stenosis is a cause of heart failure and premature death in patients that become symptomatic. While progression of the disease is slow (~20 years to become symptomatic), once this condition develops, there is no pharmacological treatment. The only option for severely symptomatic patients is to undergo a valve replacement therapy, which is both costly and invasive.

While many factors influencing the development of AS are known, many are still undiscovered. The goal of this study is to determine new risk factors in valvular aortic stenosis, thus improving our understanding of AS development and potentially develop a predictive model, which could improve prevention in at-risk patients.

This project aims to identify biomarkers related to aortic stenosis. We will also investigate their added value to the standard AS susceptibility prediction model, which accounts for known risk factors (such as age and sex). Afterwards, we will perform additional analyses and research to understand how these newly identified biomarkers might be involved in the etiology of AS.

To do so, we began by organizing data in the UK Biobank and selecting the variables that we'll be analyzing to configure the dataset that will be used in the study. This dataset consists in a sample of 274,794 participants stratified into two groups: those who have AS (3,821) and those who don't have AS (270,973). We will establish the prevalence of some classic risk factors for aortic stenosis such as age, male sex, cigarette smoking, elevated blood pressure, dyslipidemia, adiposity and kidney dysfunction/metabolism, but also the prevalence of some emerging risk factors, notably Lipoprotein(a).

Then, we'll examine a variety of models and search for novel biomarkers that might contribute to aortic stenosis. The final step will consist in data interpretation and additional research to provide context to our findings.

POSTER #12 - TH2 cytokine-primed NHBEs show increased expression of inflammatory mediators upon challenge with synthetic double-stranded RNA

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Background: It is known that T_H2 cells and their effector molecules, IL-4 and IL-13, mediate the characteristic inflammatory response seen in the airways in allergic asthma. Viral infection, which typically results in the body mounting a T_H1 immune response, is a major cause of asthma exacerbation. Although T_H1 and T_H2 responses are known to counter-regulate each other, interactions between the two immunopathologies are not fully understood in the case of virus-related asthma exacerbations. We wished to explore these interactions using an *in vitro* normal human bronchial epithelial (NHBE) cell culture primed with T_H2 cytokines to simulate asthmatic bronchial epithelium and we hypothesized that bronchial epithelium primed with T_H2 cytokines would secrete more pro-inflammatory mediators in response to viral triggers.

Objectives: To simulate a viral exacerbation in asthma *in vitro* in NHBE cell culture using polyinosinic:polycytidylic acid (P(I:C)), synthetic double-stranded RNA that is commonly used to model viral infection. To assess the levels of inflammatory mediators, including IL-8 and tumor necrosis factor in response to stimulation with P(I:C), IL-4, and IL-13.

Methods: NHBE cells were seeded in submerged culture on day 0 in PneumaCult™ Ex-plus medium. The cells were treated with 1ng/mL recombinant human IL-4 or IL-13 or with vehicle control on day 1 for 48 hours. On day 3, the cells were challenged with 10µg/mL P(I:C); after 5 hours or 24 hours, RNA was extracted using the Trizol method, and the expression of relevant inflammatory mediators was assessed using quantitative real-time PCR (qRT-PCR). Culture supernatant was removed for analysis of protein secretion using ELISA according to the manufacturer's instructions.

Results: qRT-PCR data showed a synergistic induction of the inflammatory mediators TNF and IL-8 by T_H2 cytokines and P(I:C); the expression of IL-8 and TNF by the NHBE cells primed with IL-4 or IL-13 in response to 5hour P(I:C) challenge was significantly greater than the expression of these pro-inflammatory cytokines when challenged with P(I:C) alone. Although T_H2 cytokines increased IL-8 protein secretion when compared to the untreated control, there was no statistically significant synergistic induction of IL-8 secretion after 5 hours of P(I:C) challenge. The P(I:C) and T_H2 priming interaction was further explored by testing different concentrations of P(I:C) up to 100µg/mL for 24 hours; however, a synergistic induction of IL-8 was still not seen at the level of protein secretion, and a maximum dose response from P(I:C) did not appear to be achieved.

Conclusions: In this *in vitro* model of viral exacerbation of asthmatic bronchial epithelium, the treatment of T_H2-primed NHBE cell culture with P(I:C) resulted in the synergistic induction of inflammatory mediators at a transcriptional level, but this synergy was not detected at the level of protein secretion. It is possible that 24 hours was not long enough to see this change; this can be tested with a 48hour P(I:C) challenge and the use of a transfection reagent to help the P(I:C) reach its intracellular receptors.

POSTER #13 - Characterization of doxorubicin induced cardiotoxic injury in patient-specific induced pluripotent stem cell-derived cardiomyocytes

Patrick Young[1], David Derish[2], Ida Derish[3], Élise Rody[4], Bin Yu[5, 6], Michelle van Holten[3], Janice To[7], Jeremy Zwaig[3], Renzo Cecere[3, 5]

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Cardiovascular disease (CVD) is the leading cause of death globally with approximately 18.6 million deaths annually as of 2019. Predictive models indicate, despite current medical interventions, the rate of CVD is expected to increase in the future, coupled with worsening morbidity, and an increasing economic burden on the healthcare system. Although modern treatments seek to reduce the effects of CVD, the non-regenerative nature of cardiomyocytes determines that ischemic episodes result in permanent cardiac function loss. There are no current curative interventions for CVD besides invasive and largely inaccessible transplant surgery. In order to develop novel therapies for patients, a personalized and more accurate cellular model is necessary to understand the mechanisms which may lead to cardiomyopathy.

Doxorubicin (Dox) is a widely-used chemotherapy treatment for solid and hematological tumors. It is well established however that any administration of Dox can result in Dox-induced cardiotoxicity (DIC) and dox-induced congestive heart failure (DiCHF). The mechanisms of injury from Dox exposure are not understood, nor is the reason that some patients are more prone to its cardiotoxic effects. Patient sensitivity to DIC ranges from immediate and acute, to dormant and chronic, to no detectable effects. For this reason a patient-specific method of investigation, such as the use of induced pluripotent stem cell-derived cardiomyocytes (IPSC-CMs), is integral to understanding the mechanisms of Dox injury. This will allow physicians to prescribe targeted chemotherapeutics and may also pave the way to developing drugs to counteract DIC. This study thereby seeks to generate and characterize IPSC-CMs, determine the most representative *in vitro* concentrations of Dox to model *in vivo* Dox exposure, and to assess patient-specific reactions to Dox.

We are investigating the variable patient responses to Dox, keeping in mind the cumulative nature of DIC and its genome-wide modifications. After Dox exposure, the IPSC-CMs are expected to show signs of DIC as measured by assays evaluating viability, metabolic activity, calcium flux, and hypertrophy. IPSC-CMs were generated from DiCHF (n=1) and healthy control (n=1) patients. Peripheral blood mononuclear cells were isolated and reprogrammed using the Epi5™ Episomal iPSC Reprogramming Kit (ThermoFisher). Cardiomyocyte differentiation was performed using the STEMdiff™ Cardiomyocyte Differentiation Kit (STEMCELL Technologies) and characterized via immunofluorescence and RT-PCR with respective markers. The cells were prepared for Dox-injury with doses ranging from 0μM-2.5μM to mirror *in vivo* plasma concentrations. After a 24-hour incubation with Dox, the cells were assessed through Crystal Violet (CV), Alamar Blue (AB), Fluo-4AM, and microscopy-based imaging-analysis to determine viability, metabolic activity, calcium flux, and hypertrophy respectively.

Preliminary data demonstrated significant dysregulation of calcium flux in control and DIC patients in response to Dox. Pre-injury, DIC patients showed lower viability in comparison to controls. Surprisingly, post-Dox viability did not change for DIC patients but was significantly lowered in controls. This hints that previous genetic modifications via Dox may have been maintained for DIC patients but were newly introduced to controls. This study emphasizes patient-specific and cumulative

cardiotoxic effects of Dox, expanding our understanding of *in vitro* modeling of plasma Dox concentrations.

POSTER #14 - McGill Adult Atopic Dermatitis Digital Outcomes (MAADDO) - Iterative Process

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Atopic dermatitis (AD), also known as eczema, is the most common skin disease worldwide, and affects up to 10% of adults and 20% of children in developed countries. AD is costly to both individuals and the healthcare system. In the United States alone, AD is estimated to cost from $364 million to $3.8 billion per year. The disease manifests primarily as intense itching, excoriation (repeatedly picking at one's own skin), lichenification (thick and leathery skin), and skin discoloration. Debilitating itch, raw, bleeding skin, oozing sores, and superinfections are common and cause great discomfort, loss of sleep, and interruption of daily life, with resulting psychosocial comorbidities, including anxiety, depression, and feelings of isolation. AD is a complex disease to manage for both patients and clinicians due to its recurrent, relapsing, and temporal nature. The current standard-of-care (SoC) for AD management is complex and time-consuming; further limited by the lack of resources at points of care, the SoC leads to repeated treatment failures. As a result, patients are self-managing their disease by turning to online resources, oftentimes unvalidated. To address this gap, the lab of Dr. Carolyn Jack, located in Montreal, Canada, developed a mobile health (mHealth) app, EczemaQ.

This pilot tool, which is currently in a beta phase, was designed to provide remote access to care and personalized medicine to adult patients living with atopic dermatitis, empowering this population through shared decision-making and self-management of their disease. During Phase 1 of the MAADDO study, EczemaQ will be optimized through an interactive, explanatory, and sequential mixed methods process. Both patient and clinician feedback on the usability, relevance, perceived benefits and barriers of the application will be collected. Quantitative feedback will be collected through the Technology Acceptance Model 2 (TAM2) questionnaire. Then, in-depth qualitative feedback will be collected through focus group sessions. Together, both quantitative and qualitative feedback will be analyzed and communicated to the app developers to ensure EczemaQ is tailored to the needs and requests of its end users. In the subsequent phase, EczemaQ will be investigated in a randomized controlled trial (RCT) as an intervention in AD management; changes in AD disease outcomes and the level of patient engagement will be measured. We hypothesize that this award-winning pilot will be effective versus SoC only in improving AD disease outcomes and patient engagement by delivering validated educational content to adult patients living with atopic dermatitis and facilitating remote self-management.

Currently, we are in Phase 1 of the MAADDO study, the iterative process of the EczemaQ app. We are actively recruiting adult patients with AD to test drive the app and provide their feedback. The next three weeks are critical for initial data collection and analysis; by early August, we will have preliminary results to present. We anticipate having both quantitative data through the TAM2 questionnaires and qualitative data through thematic analysis of the transcripts from the focus groups.

POSTER #15 - Precise measurement of motor learning in a mouse model of Fragile X Syndrome

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Fragile X Syndrome (FXS) is a leading genetic cause of intellectual disability frequently co-diagnosed with autism that currently has no cure; the disorder causes severe intellectual disability and behavioral and learning challenges such as motor learning deficits. The cerebellum is known to be involved in the coordination of motor learning, and a hallmark of cerebellar damage is dysmetria, a unique decomposition of reaching movements resulting in a deficit in reach endpoint precision. Previous analysis of cerebellum-dependent motor learning in rodents have not measured this learning precisely, often being limited to measures of success or failure of a task determined by human observation. Such results based on success and failure rates only show that FXS individuals do not perform as well as wildtype (WT) ones, but it is not sufficient in helping us understand how or why. In order to elucidate the role of the cerebellum in motor learning, more detailed analysis of exactly how and when different parameters of a movement improve during learning are required; recent advances in computer vision fueled by deep learning have made fast, robust measurements of animal behavior possible, revolutionizing behavioral neuroscience. Powerful deep-learning-based tools such as DeepLabCut (DLC) specifically designed to aid the measurement of behavior have been achieved within the last few years, opening a plethora of avenues for new research that would not have been possible before. To investigate these precise behavioral differences, we implemented two different methods of quantifying behavior to analyze a mouse model of FXS while learning a single-pellet paw reach task, a skilled movement that requires precise and coordinated motor movements in the forelimb where mice learn to reach through a small slit to retrieve a food reward: the current gold standard method of marker-based motion capture (OptiTrack) and a marker-less deep learning pose estimation algorithm (DLC). Our project aims to precisely quantify behavior throughout learning a skilled forelimb reaching task, using deep learning and motion capture to evaluate the trajectories and kinematics of reaching in a non-invasive way. This precise measurement will allow us to link the precise behaviors to its underlying molecular mechanisms in the cerebellum, helping us to elucidate how synaptic and cellular plasticity in the cerebellum support learning and how this process goes awry in disease. This insight will ultimately enable the creation of more effective and targeted treatments for individuals with FXS and potentially other similar disorders.

POSTER #16 - Head Neck Cancer

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Background: The head and neck region, including tumorous volumes within it, is not anatomically rigid, but is very radiosensitive. This means that its 3D shape is in constant flux during radiotherapy, and is impacted by tumour shrinkage, inflammation, weight loss, and changes that affect the patient's muscle density, fat distribution, edema, and fluid accumulation. Indeed, HNC patients who undergo radiotherapy often experience treatment-related toxicities that result in anatomical changes. These anatomical changes in turn can render the treatment plan invalid, requiring replanning, which if performed ad hoc, as is currently the case, can be disruptive for the treatment planning team. Therefore, in this research project we are investigating if we can predict if and when a head and neck cancer patient requires replanning using an AI algorithm.

Methods: In previous work, our team recognized that systematic anatomical changes in the patient result in a change in the minimum distance between the planning target volume (PTV) and the patient's skin. This minimum distance can be used to predict whether the patient will require replanning or not. The faster this distance decreases the more likely and the sooner the patient will require replanning. In this summer research project, we are analysing the weight loss of the patient, and using the x_min, which is the minimum distance between the PTV and the skin of the patient, to predict whether the patient needs to be replanned or not and when. This work is being done using supervised machine learning algorithms. First classification algorithms are being used to predict whether a patient will require replanning or not and later, regression will be used to predict when the patient would require replanning. Various parameters will be considered like the patients age, gender, minimum distance between the tumour and the patient's skin in every fraction. All these will contribute to predicting when the patient would require replanning and will hence help in optimising the use of hospital resources.

Results: To date, we have accumulated data on 59 head and neck cancer patients, of which 31 required replanning. We are presently preparing the data for classification algorithms.

POSTER #17 - OncoBuddy AI Matching Algorithm

Ridhi Mittal[1,2] and John Kildea [2,3]

[1]Laurie J. Hendren scholar working in the Opal Health Informatics Group; [2] Research Institute of the McGill University Health Centre – Cancer Research Program; [3] McGill University

Background: Cancer patient peer support is a beneficial tool for current and previous patients who can share lived experiences and provide a greater sense of empathy and moral support to one another. In fact, matching patients according to similar experiences, both medically and personally, increases the efficacy of support. But, current peer support is inefficient as it is conducted manually and relies on a coordinator to match patients based on a few known factors. Therefore, this research study will examine ways to develop AI-powered matching algorithms that will more efficiently and effectively match cancer patients according to a wider and more complex set of factors than can be done manually.

Methods: In this summer research project, we are designing and developing an AI-matching algorithm for the OncoBuddy/OncoConseil project and evaluating its effectiveness to ensure recommended matches will result in appropriate peer support. We are comparing multiple existing AI models, (i.e. the Deferred Acceptance Algorithm and the Genetic Algorithm) and testing the models on synthetic patient data that we have generated with statistical inferences from the existing Opal database and StatsCanada. A fitness function derived from previous research will determine the efficacy of the matching algorithms.

Results: Currently, we have generated a synthetic dataset of 1770 patients to be used for training and testing purposes. Initial design and development of the AI algorithms is underway, and will soon be compared using the methods described prior.

POSTER #18 - On a path of discovery towards a patient specific induced pluripotent stem cell-derived cardiac endothelial cell phenotype: a preliminary characterization.

Michelle van Holten[1, 2], Ida Derish[1, 2], Elise Rody[3], Bin Yu[2, 4], David Derish*[2, 5], Janice To*[2, 6], Patrick Young*[2, 7], Jeremy Zwaig[1, 2], Renzo Cecere[2, 8], * Equal authorship or contribution.[9]

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Background: The leading cause of mortality worldwide remains cardiovascular disease. Many cardiac disorders are characterized by endothelial cell (EC) pathophysiology. ECs, inter alia, play an important role in producing crucial antithrombotic molecules, modulate blood vessel barrier function and line their luminal surfaces, regulating the transport of oxygen and micronutrients to other cells. Given their involvement in multiple pathologies in cardiovascular, haematological, and immunologic systems, ECs have immense potential in cardiovascular disease research. To study ECs, the reprogramming of human peripheral blood mononuclear cells into induced pluripotent stem cells (iPSCs), and their subsequent differentiation into iPSC-ECs, provides an unlimited cell source, retaining patient-specific genetic backgrounds. Several protocols have been developed accordingly, however minimal research has been done on the phenotypical cardiac specificity of these endothelial-like cells. Currently, co-culturing ECs with cardiomyocytes (CMs) is the optimal method to confer a cardiac phenotype to ECs *in vitro* – a laborious inefficient process. As faithful cardiac *in vitro* models are essential when researching disease mechanisms pertaining to EC injury, our laboratory initiated an in-depth characterization of several iPSC-EC lines. The purpose of this study is to successfully generate, characterise and confer a cardiac phenotype to iPSC-ECs. We hypothesized that iPSC-ECs will display typical EC morphology expressing CD31, CD144 and CD34. Moreover, we expect the iPSC-ECs to lack EC cardiac specific marker CD29. Nonetheless, we hypothesized that iPSC-derived CM secretome treatment of iPSC-ECs will cause CD29 expression, hereby hinting at an induced cardiac phenotype in ECs. CM secretome is an easier accessible method compared to co-culturing with CMs and provides a novel cardiac phenotype induction technique.

Methods and Preliminary Results: We generated iPSC-ECs for characterization, as well as iPSC-CMs for secretome collection (n=1 cardiomyopathic patient, n=1 healthy donor) from peripheral blood, through transfection of reprogramming factors. Initially, pluripotency of iPSCs was confirmed via immunostaining (OCT4, SSEA-4, NANOG and TRA-1-60). After iPSC-EC differentiation (STEMdiff™ Endothelial Differentiation Kit), we confirmed the expression of prominent EC markers (CD31, CD144 and CD34) and cell viability via immunofluorescence and flow cytometry. Flow cytometry analysed single cells via cell marker expression by staining with fluorescently conjugated antibodies, confirming our generated iPSC-EC reliability. Moreover, we collected iPSC-CM secretome, by adding serum-free basal media to iPSC-CMs and incubating them at 37ºc for 24 hours; secretome was then collected, filtered, and stored at -20ºc until further use. Secretome will be administered to iPSC-ECs for 24 hours, and cells will be analysed via flow cytometry, to verify whether cardiac marker CD29 is expressed.

Conclusions: Altogether, this preliminary data suggests our generated iPSC-ECs can be characterized as endothelial-like cells expressing CD31, CD144 and CD34. However, as they do not readily express cardiac-specific marker CD29, we attempted to develop a method via iPSC-CM secretome treatment, to push iPSC-ECs towards a cardiac phenotype, and potentially a more faithful cardiac EC model. This preliminary knowledge will be a steppingstone to develop *in vitro* cardiac 3D organoids. This patient-specific model will aid in researching cardiac physiology, pathology, and the development of novel therapeutics for cardiovascular disorders.

POSTER #19 - Prevalence of Novel and Established Risk Factors in Premature Atherosclerotic Cardiovascular Disease

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Ischemic heart disease (IHD) is the leading cause of death worldwide. Although the literature on IHD is vast, there is limited data on IHD prevalence and risk factors in younger patient populations. Moreover, there has not been much significant improvement in mortality for this young group of patients over the years. Premature cardiovascular disease, specifically an early myocardial infarct (MI) before the age of 60, although rare, has many lasting sequelae including fatal arrhythmias, subsequent heart attacks and sudden death. Moreover, risk factor profiles differ in individuals who have a premature MI compared to their older counterparts. Thus, the objective of this project is to identify risk factors in the UK Biobank cohort of 500,000 patients for individuals ≤60 years of age who have coronary artery disease (CAD). Specifically, we will investigate females ≤ 60 years old and males ≤55 years old because these groups represent approximately 10% of CAD cases for both sexes and allow for more even comparison. We will be working with a sample of 237, 267 participants, 65% of whom are female, with a median age of cases being 52 years old. We will establish the prevalence and relative risks of traditional (such as smoking, diabetes mellitus, hyperlipidemia, hypertension, family history of premature MI and male sex) and emerging risk factors (such as Lp(a), genetic risk score, genetic dyslipidemia) for IHD. The prevalence of genetic dyslipidemias in these individuals will also be determined. The differences in pervasiveness of these risk factors in males and females will also be investigated. Lastly, a model will be developed to predict individual risk of experiencing a premature MI. R statistical software will be utilized for data analysis. Quantifying the risk factors and their overall contribution to predisposing an individual to heart disease has the potential to identify which modifiable risk factors should clinically be focused on. It may also help shape public policy and preventative intervention strategies. Such interventions may help decrease the complications and mortality following a heart attack, which continue to be important public health issues in North America.

POSTER #20 - Quantification of collagen content in mouse lungs using automated image analysis

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Background: Worldwide mortality from idiopathic pulmonary fibrosis continues to rise. Attempts to better understand the pathogenesis of lung fibrosis and test potential therapies have relied heavily on preclinical animal models. Therefore, the methodologies used to assess lung fibrosis are an important consideration. Numerous publications report the quantification of lung fibrosis by means of Ashcroft score (semi quantitative scoring system) or qualitative evaluation of histological stains. However, these approaches are subject to inter and intra-observer variability. Automated image analysis can potentially address the issue of subjective interpretations by providing an objective quantitative assessment.

Aim: To test the performance of an image analysis program to detect collagen using a putative mouse model of lung fibrosis (hypochlorous acid, HOCl).

Methods: Balb/c mice received daily intradermal injections of HOCl for 6-weeks, following published protocols (ATJ Maria et al. Front Immunol. 2018). On day 42, lungs were harvested and subsequently stained with picrosirius red to show collagen deposition. Automated image analysis was then performed on 3 serial sections from each left lung using the Aperio Pixel Count Algorithm. The algorithm detects pixels that match the input parameters which are based on the hue, saturation, and intensity color model. To detect collagen with picrosirius red, the default hue value was used (0.10), a hue width of 0.4 and color saturation of 0.08 were specified. Collagen content (expressed in pixels) was corrected to the area of each lung. To establish the method's reproducibility, one author re-analyzed the mouse lungs two weeks after the initial evaluation (to assess intra-observer variability) and two authors independently analyzed 5 lung sections (in order to assess inter-observer variability).

Results: The algorithm did not detect any differences in the number of picrosirius red pixels between PBS and HOCl- injected mice. The intra-subject coefficient of variation (calculated based on 3 sections for each subject) ranged from 12-24% (mean 17.95%) and 1.57%-36.78% (mean 10.5%), for PBS and HOCl animals respectively. Inter-subject variability between PBS animals was 6.18% and 11.32% between the HOCl animals.

Conclusion: The Positive Pixel Count Algorithm did not show evidence of collagen deposition in HOCl mice which is consistent with the lack of lung function abnormalities. Inter-subject variability was <12% in PBS and HOCl groups. These preliminary findings suggest the algorithm may be a reliable tool for histological collagen quantification. Next, the algorithm will be tested in the bleomycin model of lung fibrosis, and we will evaluate how the analysis results are affected by variation in input parameters.

POSTER #21 - Optimizing the isolation of extracellular vesicles from healthy term placenta

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Background: Preeclampsia (PrE) is a disorder of pregnancy characterized by new-onset or worsening hypertension in combination with end-organ damage after 20 weeks of gestation. PrE is believed to be the result of abnormal placentation which leads to uteroplacental malperfusion and oxidative stress. This results in the release of several factors by the placenta into the maternal systemic circulation, causing endothelial cell dysfunction and vascular impairment which culminate in the PrE symptoms observed later in pregnancy. One group of particles released are the extracellular vesicles (EVs). EVs are membranous cell-derived vesicles that play a role in distant cell communication. Previous studies have reported increased levels of placenta-derived EVs in maternal blood in PrE and that the level of EVs is associated with disease severity. However, very few studies have investigated EVs in the placenta tissue. EV technologies are rapidly evolving and there exists several methods to isolate EVs, including ultracentrifugation, affinity chromatography, and size exclusion chromatography. Ultracentrifugation isolates EVs based on differential sedimentation rates. Affinity chromatography isolates EVs using a membrane that has affinity for and binds EVs. Finally, size-exclusion chromatography isolates EVs using a porous polysaccharide resin. Despite advances in EV isolation technology, it remains unclear which method is most efficient to provide a pure and concentrated EV sample from placenta tissue.

Hypothesis/Objective: We hypothesize that placenta-derived EVs play a role in placenta-mediated endothelial cell dysfunction and may represent a promising early predictive tool for PrE. Our objective is to compare and optimize the methodologies of isolating EVs from term placenta tissue to determine which is the most efficient and yields the purest sample.

Methodology: Healthy term placenta tissue will be obtained from pregnant participants enrolled in our ongoing study at the Royal Victoria hospital, and collected and processed immediately following delivery. The tissue will be homogenized in media, filtered through 100 µL and 70 µL filters, and centrifuged to remove cell debris. The resulting supernatants will be collected for EV isolation, using the three techniques previously described. Briefly, ultracentrifugation will follow differential and ultracentrifugation of supernatants using a protocol previously optimized in our lab. Affinity and size-exclusion chromatography will follow respective kit instructions. The resulting samples from the three techniques will be analyzed by nanoparticle tracking analysis (NTA) to determine the quantity and purity of the EVs.

Anticipated results: We expect all three methods to successfully isolate EVs from our samples. NTA will determine the purity and concentration of EV isolates from each technique. Using this information, we will be able to identify the protocol that provides the highest purity and concentration. This protocol optimization will contribute to a future project in which EVs are isolated from PrE placentas.

POSTER #22 - A Novel Effort-Free Test of Lung Function for the Prediction of COPD Patient Symptoms

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Introduction: Spirometry, the gold standard lung function test, correlates well with patient symptoms but requires cooperation and strenuous effort, and is often impossible in the very young, elderly, cognitively impaired, and neuromuscularly weak. Whole-breath oscillometry (OSC) is an effort independent lung function testing method successfully used in infants and the elderly. Our novel intra-breath OSC (OSC_{IB}), correlates better with symptoms than either OSC or spirometry in adult asthmatics, predicts future respiratory events in healthy infants, distinguishes between children born either preterm or at term and predicts need for surfactant therapy in premature infants. OSC_{IB} detects expiratory flow limitation in patients with chronic obstructive lung disease (COPD) but its ability to predict symptoms is unknown.

Hypothesis: We hypothesize OSC and OSC_{IB} will correlate with symptoms as measured by the COPD Assessment Test (CAT) and Modified MRC Dyspnoea Scale (mMRC) as strongly as spirometry.

Methods: We performed a retrospective chart review using data acquired during routine care. 166 subjects had available patient reported outcomes (PROs) (CAT and mMRC), biometrics, spirometry OSC and OSC_{IB}. Spirometry was performed at a hospital pulmonary function lab respecting ATS criteria and OSC during well office visits. Subjects underwent 3-5 measurements of OSC (tremoFlo C-100, Thorasys, Montreal, Canada) respecting ERS standards. From 3 reproducible tracings resistance and reactance parameters including the reactance-area (AX) were calculated. Subjects underwent a 30-45s OSC_{IB} measurement at 10 Hz yielding 10 Hz reactance from which novel reactance vs flow and volume parameters including the end-inspiratory reactance (XeI) were calculated. Data distributions were tested with Shapiro-Wilk tests and range with boxswarm plots, and outliers rejected. Patients were divided into 3 groups: COPD (symptoms compatible with COPD, ≥10 pack-year smoking history, post-bronchodilator FEV1/FVC<0.70), pre-COPD (same as COPD, but a post-bronchodilator FEV1/FVC≥0.70 and MMEF<65%predicted) and Healthy (disease-free after clinical, physiology, and imaging evaluation). Between group differences for biometrics, PROs, spirometry, OSC and OSC_{IB} parameters were determined using Kruskal-Wallis and post-hoc Dunn tests, and correlation strength between PROs and spirometry, OSC, and OSC_{IB} using Bonferroni-corrected Spearman rho. The study had MUHC-RI IRB approval (14-467-MUHC-T).

Results: After exclusion of 3 subjects with low reproducibility of R and 3 subjects for extreme outlier data, 104 COPD, 31 preCOPD and 25 healthy subjects remained. Groups differed in age, height, and smoking history ($p<0.05$) but not weight ($p=0.72$) and BMI ($p=0.56$). All spirometry and OSC parameters differed between groups ($p<0.01$). Most OSC_{IB} parameters differed between groups. Of spirometry parameters, FEV1 and MMEF correlated best with CAT ($r=-0.49$, $p<0.001$, $r=-0.54$, $p<0.001$, respectively) and mMRC ($r=-0.53$, $p<0.001$; $r=-0.54$, $p<0.001$, respectively). Of OSC parameters, AX correlated best with the CAT ($r=0.45$, $p<0.001$) and MRC ($r=0.49$, $p<0.001$). Of OSC_{IB} parameters, end-inspiratory reactance (XeI) correlated best with CAT ($r=-0.45$, $p<0.001$) and MRC ($r=-0.56$, $p<0.001$).

Conclusion: Spirometry, OSC and OSC_{IB} all demonstrated moderate correlations with subject symptoms measured by CAT and mMRC. Of all parameters, the novel OSC_{IB} parameter XeI showed the strongest correlation with symptoms. Both oscillometry and intra-breath oscillometry may be viable alternatives to spirometry for assessing COPD patients.

POSTER #23 - Ajuba's role in regulation of Notch pathway

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Notch pathway is a highly regulated and conserved cell signaling system in animals. Understanding the Notch pathway regulation in different tissues at the molecular level is imperative to gain insights into the development of an embryo and also malignant transformation in adults. In skin epidermis, Notch signalling is responsible for differentiation and maintenance of the differentiated layers. Ajuba was recently discovered as a novel regulator of Notch pathway. Ajuba directly interacts with Notch1/2 and also a protein called Numb, a negative regulator of Notch signalling. The proposed research project will study how Ajuba interacts with Notch1/2 and its pathway components at the molecular level using genetics and biochemical techniques. Ajuba protein is composed of pre-LIM domain, LIM domain and Nuclear Localization signal (NLS). Our lab has already created Ajuba mutant constructs that either lack 1) pre-LIM domain or 2) LIM domain or 3) NLS signal. In the proposed work, all 3 Ajuba mutant constructs will be expressed in mouse keratinocytes and their interaction with Notch1/2, Numb and other Notch pathway components will be tested using immunoprecipitation techniques. In addition, the localization of Ajuba and its mutants will be explored using fluorescent microscopy techniques. Through the proposed research project, which will be an in vitro component of future animal studies, we aim to get detailed mechanistic insights into Ajuba's role in the regulation of Notch pathway.

POSTER #24 - Aberrant follicular development in the heterozygous Hfm1 null mutant female mouse

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The *HFM1* gene encodes for a DNA helicase involved in meiotic recombination, which is necessary for the proper segregation of homologous chromosomes during meiosis I. Both homozygous and heterozygous *HFM1* mutations have been linked to infertility and premature ovarian insufficiency in humans. *Hfm1*$^{-/-}$ female mice deplete their oocytes by puberty and become sterile. *Hfm1*$^{+/-}$ female mice are fertile, but if and how the HFM1 haplodeficiency affects fertility has not been reported. The objective of our research is to compare follicular development between *Hfm1*$^{+/-}$ and *Hfm1*$^{+/+}$ female mice. We fixed ovaries at 4, 14, 26, and 60 days postpartum (dpp) and processed them for hematoxylin and eosin staining or immunofluorescence staining with anti-MSY2 and -FOXL2 antibodies to identify oocytes and granulosa cells, respectively. In the ovaries at 14 dpp, we categorized the follicles according to the layer of granulosa cells and the presence or absence of antrum. Our results show that in *Hfm1*$^{+/-}$ ovaries (n=8) compared to the *Hfm1*$^{+/+}$ovaries (n=3), (1) no difference was found in the percentages of primordial follicles recruited into the growth phase, (2) more follicles progressed to the most advanced stage, (3) the zona pellucida was poorly formed around the oocytes of multi-layered follicles, and (4) oocytes were more frequently seen detached from granulosa cells. In addition, multi-oocyte follicles were more frequently found in *Hfm1*$^{+/-}$ ovaries at 26 dpp. We conclude that follicular development is aberrant in *Hfm1*$^{+/-}$ ovaries since the young ages, probably leading to subfertility. Our laboratory is currently testing the fecundity of *Hfm1*$^{+/-}$ females up to 10 months old.

POSTER #25 - Exploring the relationship between Family Caregiver Distress and Burden in Psychosocial Oncology

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Background Family members have multiple roles and responsibilities when caring for someone diagnosed with cancer. The distress associated with these roles can result in family members experiencing elevated levels of family caregiver burden, anxiety, and depression. Moreover, caregivers are at increased risk for physical complaints, mental health disturbances and a range of other threats to their overall well-being. The results of previous research yielded a unique screening tool, the Family Member Problem Checklist (FMPC), identifying sources contributing to family members' distress. Due to the COVID-19 pandemic, the demands of caregiving have increased while caregiver community and social support services have been challenged due to the ongoing sanitary measures.

Purpose This study's objectives are to (1) assess the level of distress or burden of caregivers of a person diagnosed with cancer and (2) to identify caregiver needs via the Family Member Problem Checklist (FMPC) to guide the development of future healthcare and community caregiver support strategies.

Methods Recruitment of 84 participants, family members or friends that consider themselves as caregivers of a person with cancer. Study Procedures: once eligibility is determined, self-report questionnaires are given to participants in person, by mail or via an electronic link through Qualtrics with the goal of assessing caregivers' levels of distress and burden. Recruitment is being performed via multiple outreach strategies at the McGill University Health Centre's (MUHC) Cedars Cancer Centre, the West Island Cancer Wellness Centre, through electronic media outreach via the Opal app as well as electronic and physical pamphlets and posters. Study Questionnaires: Distress Thermometer (DT)**,** Family Member Problem Checklist (FMPC), Burden Scale for Family Caregivers (BSFC)**,** Hospital Anxiety and Depression Scale (HADS).

Discussion Caregivers of cancer patients are primarily in the community as opposed to the cancer centres due to restrictions to entry secondary to the COVID-19 pandemic, including wave seven, which makes recruitment into a study such as this more challenging. Therefore, we have been exploring multiple recruitment strategies. Initially, recruitment was possible through the McGill University's Health Centre's (MUHC) Psychosocial Oncology (PSO) program and through in-person contact. Currently, besides the previously mentioned strategies, we are also using community outreach and virtual platforms for participant recruitment.

Keywords Caregiver Distress, Caregiver Burden, Caregiver Needs, Oncology, Mental Health, Well-being

POSTER #26 - β-glucan Administration Route Affects Trafficking and Protection Against Bladder Cancer

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Background: Bladder cancer is the ninth most common cause of cancer death worldwide. Depending on each case, treatments normally involve chemotherapy, surgery and/or administration of Bacille Calmette-Guerin (BCG) intra-bladder (IB). Trained immunity (TI) has been proposed as a molecular mechanism to explain the use of BCG to treat bladder cancer. TI is an innate immune cells' memory, induced following exposure to specific training agents (such as BCG or a fungal cell wall component; β-glucan). For instance, studies have suggested TI as a mechanism of protection against influenza and tuberculosis (TB). In these cases, BCG reprograms monocytes and macrophages epigenetically, which is a characteristic of TI. Moreover, β-glucan has been shown to have antitumor capacity. A recent study demonstrated β-glucan's protective effects against pancreatic ductal adenocarcinoma (PDAC). In fact, the same research found that when administered intraperitoneally (IP) into wild type (WT) mice the majority of β-glucan makes its way to the pancreas, inducing TI. However, whether β-glucan induced TI protects against bladder cancer, and how, remain to be investigated. We have established a murine model to administer β-glucan directly into the bladder. This could allow us to show that β-glucan offers additional protection against bladder cancer when administered IB, as opposed to IP.

Objective and hypothesis: The aim of this study was to investigate the kinetics of β-glucan trafficking and degradation after IB injection, compared to IP administration. We would like to better understand this particle's possible protective qualities against bladder cancer. Considering the antitumor effects of BCG, which is still found in the body 30 days after IB administration, we hypothesized that β-glucan would persist in cells and tissues. This might be a critical component for the long-lasting effects of trained immunity and possible beneficial response against bladder cancer.

Methods: β-glucan was first labeled with fluorescein dichlorotriazine (DTAF) which allows tracking of the β-glucan *in vitro* and *in vivo*. For the *in vivo* experiments, we used our established model of IB injections to deliver β-glucan in the bladder of C57BL/6J female mice. At various days post-treatment, the spleen, draining lymph node, bladder, bone marrow, pancreas were harvested for flow cytometric and microscopic assays to monitor β-glucan degradation/dissemination. In parallel, because macrophages are the only cell type able to digest β-glucan, we derived bone marrow-derived macrophage (BMDM) from C57BL/6J mice *in vitro*. The BMDM were stimulated by different concentrations of labeled β-glucan (BG-DTAF) at day 0 and day 3 and harvested at different time points for analysis.

Conclusion: β-glucan trafficking and degradation have not been studied after IB administration. The results from this study will help to better understand the mechanisms of β-glucan-induced TI against bladder cancer and pave the way to new therapies to treat bladder cancers.

POSTER #27 - Sex differences in the relationship between hypertension, brain structure, and incident dementia: New insights from the UK BioBank

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Background: Hypertension is an established leading risk factor for morbidity and mortality from vascular diseases across the lifespan. It has been more recently linked to incident dementia in older adults. Specifically, the age of diagnosis of hypertension has been identified as a unique contributor to all-cause dementia, independently of blood pressure control. However, it remains unclear whether this relationship differs across the sexes – that is, whether the predictive trajectory of age of hypertension diagnosis on risk of dementia differs between men and women, and if so, how these differences are substantiated in structural brain changes in midlife and in the elderly.

Objective: To investigate whether the association between age of diagnosis of hypertension, structural brain changes, and dementia (all-cause, vascular, Alzheimer) is modulated by sex and, furthermore, whether this relationship remains constant across the adult lifespan.

Method: Baseline data from 502 505 individuals aged 40 to 73 years old were collected from the UK BioBank between 2006 and 2010. Data were retained from participants with a known age of diagnosis of hypertension and who had obtained a brain MRI between 2014 and 2019 (N = 11 399) and from participants with a known age of diagnosis of hypertension who later developed dementia (all-cause, Alzheimer, vascular; N = 124 053). Of the remaining participants, data were stratified by age of diagnosis of hypertension (<35 years, 35-44 years, 45-54 years, 55-64 years, ≥65 years old) and sex. Using propensity score matching, a control participant was randomly selected for each hypertensive participant in each of the stratified age and sex groups. Generalized linear models were used to predict the following: (1) the odds of developing dementia and (2) the magnitude of changes in brain structure as a function of age of hypertension diagnosis and sex, after controlling for major vascular risk factors and demographic covariates.

Anticipated Results: We anticipate that female participants who received a diagnosis of hypertension at an earlier age would be more likely to develop dementia and manifest structural brain changes compared to their older counterparts and matched male participants. That is, the effect of age across the sexes will uniquely index the risk of developing this disease and associated brain changes in later life.

POSTER #28 - Histopathological study of 150 cases of keratoconus

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Background:
Keratoconus is a bilateral and asymmetrical cornea ectasia. In 2008, a study was conducted on 49 cases of keratoconic corneas to document the prevalence of the most common histopathological features (Fernandes et al, 2008). The aim was to document, in an unprecedented manner, the pathophysiology of keratoconus. The purpose of this paper is to provide an update with a sample size that is three-fold and to confirm and add to the previous findings reported. These will help solidify the pathophysiology of keratoconus and to aid physicians in the correct histopathological diagnosis of the disorder early on in development.

Methods:
A total of 150 cases of keratoconic corneas were studied, including the 49 corneas from the Fernandes et al study in 2008, from the MUHC – McGill University Ocular Pathology & Translational Research in Montreal, Quebec, Canada. A retrospective study of the corneas was conducted to document the prevalence of the most common morphological features. The corneas were obtained after penetrating keratoplasty. Histopathological reports were reviewed to obtain data such as age and gender. Specimens were fixed in 10% buffered paraformaldehyde solution, bisected through the centre of the button, and embedded in paraffin. Sections were stained with haematoxylin and eosin and periodic acid-Schiff for light microscopic examination.

Results:
The most common morphological features of the corneas were the following: 87% (N=130) of cases showed signs of epithelial thinning, 91% (N=136) showed breaks in the Bowman's layer, 65% (N=97) showed compaction of the stromal fibres, 63% (N=94) showed folds in Descemet's membrane. Less common features were superficial iron deposition with 27% (N=40) of cases, 30% (N=45) showed deep stromal scarring, 29% (N=44) showed epithelial scarring, 31% (N=47) showed epithelial cell loss, and 18% (N=27) showed breaks in Descemet's membrane. The major differences between the findings are that the prevalence in breaks in Bowman's layer was found to be higher than epithelial thinning in this study. Fernandes et al documented a prevalence of 71% (N=35) for the breaks in Bowman's layer and 82% (N=40) for the epithelial thinning. Another noticeable contrast is the prevalence of endothelial cell loss, which was found to be 22% (N=11) in the previous 2008 study. All cases present either break in Bowman's layer and/or epithelial thinning.

Conclusions:
These new findings confirm the previous reported findings of the 2008 study (Fernandes et al, 2008). Albeit minor differences, this much larger sample size confirms the incidence and prevalence of the histopathological signs of the diagnosis of keratoconus. These new findings also explain the role of the epithelium and the Bowman's layer in the early development of this disease.

POSTER #29 - IL-13 Production by CD45+ and CD45- Cells in Lesional and Non Lesional Skin of Adult Atopic Dermatitis Patients

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Background: IL-13 plays a central role in contributing to the pathogenesis of Atopic Dermatitis (AD), which is a common inflammatory skin disease also known as eczema. This interleukin is a potent mediator of type-2 inflammation, which is the specific type of immune response pattern commonly associated with allergy. Type 2 T cells, natural killer cells, mast cells, basophils, and eosinophils are cells that are known to produce this cytokine. Defining IL-13 secreting cells is key to better understanding atopic dermatitis as they are poorly defined.

Methods: IL-13 and CD45 protein markers were detected using Immunofluorescence (IF) and were imaged using confocal microscopy. Afterwards, cells were counted manually by using the ImageJ software.

Objective: Characterize IL-13 producing cells from adult AD patient skin biopsies by using a CD45, an immune cell marker, and compare it across lesional, non lesional and healthy subjects skin samples.

Results: When looking at lesional skin samples (n=14) from AD patients, a higher number of cells (281 ± 128, mean ± SD) were both IL-13 positive and CD45 positive when compared to non lesional (n=14) skin samples (12.4 ± 19.9, mean ± SD). Regarding $IL\text{-}13^{+}$ and $CD45^{-}$ cells, lesional samples have more cells (34.1 ± 17.8, mean ± SD) than non lesional samples (2.2 ± 6.6, mean ± SD). When looking at the ratio of $IL\text{-}13^{+}$ and $CD45^{-}$ cells with $IL\text{-}13^{+}$ and $CD45^{+}$ cells, we notice that nonimmune cells secreting IL-13 represent 7.8% of the average number of cells in lesional skin. We did not detect $IL\text{-}13^{+}$ cells in healthy subjects (n=2).

Conclusion: There was a higher number of IL-13 producing cells in lesional skin compared to non lesional skin or healthy subjects. Furthermore, most IL-13 producing cells were immune cells in lesional and non lesional skin. It would be interesting to further characterize the different cell populations to better understand the disease.

POSTER #30 - Role Of Prolactin Pathway In Ciliogenesis In Breast Cancer Cells

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Background:
Several studies have highlighted a potential tumor-suppressive role of the primary cilia which would prevent cells from dividing. Primary cilia are immobile organelles, made of microtubules. They are present in most cells including mammary cells. The formation of the primary cilia results from the displacement at the membrane of the mother centriole from the centrosome to form the basal bodies, this phenomenon takes place during the G0 and G1 phase of the cell cycle. Thus, the absence of primary cilia formation would allow cancer cells to divide and therefore proliferate more easily.

Studies have demonstrated the antitumorigenic role of the lactation hormone Prolactin and its receptor (PRLR/PRLR) signalling in breast cancer. Our preliminary data has been shown that SPICE 1 (Spindles and Centrioles associated gene1) is a constitutive interactor with PRLR and its possible role in in suppressing centrosome duplication and inducing ciliogenesis in breast cancer. Our global protein analysis has shown that SPICE1 interacts with several cilia proteins upon PRL stimulation. Moreover, several markers of the primary cilia are positively correlated with PRLR and SPICE 1 expression in breast cancer patients. Thus, we hypothesized that PRL/PRLR signalling through interaction with SPICE1 would increase cioliogenesis of the primary cilia, induce cell arrest, and suppress tumor development. One of these markers is IFT88 (Intraflagellar transport 88). The IFT complex protein allow the formation and maintenance of the cilia and it has been demonstrated that a defect in these constituents leads to abnormalities in the assembly of the primary cilia. Our work will focus on the identification of several markers and their correlation with the prolactin signaling pathway on the MCF7 and MDA231 cell lines.

Methods:
Western blot and immunoprecipitation will be performed to confirm IFT88 and SPICE 1 and PRLR. Additionally, the structure and the presence of the primary cilia will be studied by immunofluorescence using MCF7, MDA231 and SPICE-K.O cell line.

Results:
The aim of this project is to highlight the impact of prolactin on the formation, maintenance and structure of the primary cilia in cancer cells, mediated by the prolactin signaling pathway with the main player: SPICE 1. This new role of prolactin completes the action of tumor suppressor that it holds.

Conclusion:
Through this work defining the role of prolactin in primary cilia formation in breast cancer would help better understand its antitumorigenic role and its potential therapeutic value.

POSTER #31 - Mast Cell Homing for Enhanced Fracture Healing

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INTRODUCTION: Bone fractures are among the most common musculoskeletal injuries, and approximately 5-10% of patients experience complications such as delayed unions and non-unions. Consequently, such complications may require invasive revision surgeries, which are not always successful. Mast cells, commonly thought of as immunological villains, play a critical role in the inflammatory phase of bone healing and their granules have the widest range of pro-osteogenic growth factors and cytokines among immunocompetent cells. Inorganic polyphosphate (polyPs), an innate bioactive compound, is a polymer of repeating phosphate units of varying chain lengths. PolyPs are secreted by degranulating platelets at the fracture site and play an important role in blood coagulation. In addition, polyPs have been shown to recruit immune cells to the fracture site and modulate immune cell function *in-vitro;* however, their role in bone repair by means of immunomodulation has not yet been investigated. As such, we hypothesize that polyPs have the potential to enhance bone repair by homing mast cells to the fracture site, and by modulating their degranulation release profiles.

METHODS: We are developing a thermoresponsive poloxamer, poly-N-vinyl-caprolactam, and chitosan composite hydrogel tuned for controlled release of polyP at the fracture site. Various compositions of the hydrogel were prepared and tested for calibrating sol-gel transition temperature and time. The hydrogel was then doped with 1 mM polyP, and its release profile was determined by fluorescence measurements of polyP in solution. Furthermore, various concentrations of polyphosphates will be tested for their chemotactic potential towards mast cells using m-slide chambers and live cell imaging with subsequent single cell tracking.

RESULTS: Our hydrogel formulation shows sol-to-gel transition at physiological temperatures of 37°C within 60 seconds and remains stable for storage at 4°C. Preliminary release profile studies of our hydrogel show sustained release of polyP for 96 hours.

CONCLUSIONS: In this project, we are developing a biocompatible thermoresponsive gel with controlled release of polyP. We are in the process of evaluating the cytotoxicity and cell adhesion of murine bone marrow-derived stem cells and osteoblasts to validate this formulation for bone tissue engineering application. Furthermore, we aim to test this hydrogel for its ability to home mast cells towards it *in-vitro* by using live cell imaging and single-cell tracking. Taken together, this study will optimize and validate this polyP-releasing hydrogel for testing in a pre-clinical *in-vivo* murine fracture repair model developed by our group, for the ultimate goal of studying enhanced fracture repair mediated by polyP-guided mast cell homing and maturation at the fracture site. We anticipate the release of polyP will increase mast cell migration to the fracture site, thus increasing the degranulation of mast cells and release of pro-osteogenic growth factors and cytokines, thereby enhancing osteogenesis.

POSTER #32 - Digital Adherence Technologies for TB Treatment: Systematic Review Protocols and Preliminary Results

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Background: Tuberculosis (TB) is the second leading infectious cause of death (after COVID-19) worldwide, with 1.5 million deaths each year. Latent TB infection and active TB disease both require months of antituberculosis antibiotic treatment to eradicate infection. Non-adherence to treatment can lead to treatment failure, relapse, generation of drug resistance, ongoing transmission, and death. Directly observed therapy is a common approach to promote adherence, but is logistically challenging, expensive, and potentially intrusive. Digital adherence technologies (DATs) including phone-based technologies, digital pillboxes, and ingestible sensors can potentially provide more patient-centric approaches for supporting TB medication adherence, and improving outcomes. This project aims to synthesize all relevant evidence around currently available technologies to support TB treatment adherence, including associated implementation considerations, health outcomes, accuracy, and cost-effectiveness. We will present this information in four linked systematic reviews, with meta-analyses if/where appropriate.

Methods: We searched for relevant literature from 2000 to 2022 in MEDLINE/Ovid, Embase, Cochrane Central Register of Controlled Trials (CENTRAL), CINAHL, and Web of Science, as well as MedRxiv (preprints) and ClinicalTrials.gov (trial protocols). Key search terms denote TB (active or latent) and digital technologies (i.e., mobile phone, smartphone, video observation, medication monitors, text messaging, etc.). A broad search identified all possible qualitative and quantitative studies related to DAT implementation, using the RE-AIM framework, followed by narrower searches to focus on quantitative results addressing health outcomes, accuracy, and cost. Abstracts from the Union World Conference on Lung Health were also searched to identify any unpublished or previously unidentified studies from 2004 onwards. Studies were excluded if they did not involve the use of a DAT for TB treatment support, or if they did not report original data. Meta-analyses will be conducted if there is sufficient clinical and methodological homogeneity in study designs, settings, technologies, and measured outcomes. All stages of the review involved dual review by two team members, with disagreements resolved by a third member (senior investigator).

Preliminary Results: A total of 13,266 titles were identified by our initial search strategy of TB and DATs. After removing 4165 duplicate articles, a total of 9101 distinct titles and abstracts were screened for relevance. After excluding 8427 of these because they did not address TB and/or DATs, or did not report original data, 674 moved onto full-text eligibility screening and review. Thus far, 142 studies have been retained as eligible for data extraction related to DAT implementation, with smaller numbers retained for the reviews addressing health outcomes, the accuracy of dose recording, and costs. Standardized extraction of data from the included articles is now underway, and some initial results will be shared in this poster presentation.

Discussion: Synthesis of the included studies will provide information on the implementation, health outcomes, accuracy, and cost-effectiveness of identified DATs for TB treatment adherence, and highlight remaining evidence gaps. This review is essential to advance tuberculosis treatment programs, particularly in settings with higher TB incidence.

POSTER #33 - Associations of Diabetes Duration with Dimensions of Diabetes Management, Diabetes Knowledge and Glycemic Control Among Adolescents with Type 1 Diabetes Before Transfer to Adult Care

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Objectives: Transition to adult care is a challenging period for adolescents with type 1 diabetes (T1D). Evidence is lacking on whether diabetes duration influences comfort with diabetes management in preparation for this transition. The aim of the study was to examine the associations of diabetes duration with dimensions of diabetes management (self-efficacy, diabetes distress, transition readiness), diabetes knowledge, and glycemic control in adolescents with T1D before transfer to adult care.

Methods: Using a cross-sectional design, we conducted a secondary analysis of baseline data of adolescents (age 16-17 years) with T1D receiving care at a pediatric diabetes clinic at two academic hospitals in Montreal and enrolled in the Group Education Trial to Improve Transition (GET-IT-T1D). Participants completed validated questionnaires on self-efficacy (Self-Efficacy for Diabetes Self-Management Measure [SEDM], score 1 to 10), diabetes distress (Diabetes Distress Scale for Adults with Type 1 Diabetes [T1-DDS], score ≥3 indicates distress), transition readiness (Am I ON TRAC? For Adult Care questionnaire [TRAC], score ≥8 indicates readiness) and diabetes knowledge (L'Aide aux Jeunes Diabétiques Diabetes Knowledge and Skills Questionnaire [AJD DKS], score 0 to 50), as well as a HbA1c capillary blood test (%). Diabetes duration was obtained from medical charts. The primary outcome was self-efficacy. We examined associations of diabetes duration with self-efficacy, transition readiness, diabetes distress, diabetes knowledge and HbA1c using multivariate linear and logistic regression models adjusted for potential confounders (sex, socioeconomic status, insulin pump use, glucose sensor use, psychiatric comorbidity).

Results: Of 203 adolescents with T1D (96 males, 47.3%), mean diabetes duration (SD) at baseline was 7.57 (4.44) years. Mean SEDM score (SD) was 6.83 (1.62), mean TRAC Knowledge scale score (SD) was 42.57 (5.88) and mean AJD DKS Questionnaire score (SD) was 36.82 (3.60). 44 participants (22.1%) had a T1-DDS total score ≥3, indicating diabetes distress, whereas 22 (11.5%) had a TRAC Behaviour index ≥8, indicating transition readiness. Diabetes duration was not associated with self-efficacy, transition readiness, diabetes distress or diabetes knowledge in both unadjusted and adjusted models. Adolescents with a longer diabetes duration had higher HbA1c (adjusted β, 0.104; 95% CI, 0.050 to 0.159).

Conclusion: Whereas diabetes duration is not associated with diabetes management dimensions or diabetes knowledge, adolescents with longer diabetes duration are at risk for higher HbA1c and may need additional support to improve glycemic control before transition to adult care. Longitudinal studies would help to better understand the impact of diabetes duration at time of transition to adult care.

POSTER #34 - Immunohistochemical analysis of RPE cell viability atop drusen in enucleation and evisceration specimens

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Purpose: Drusen are lipoproteinaceous deposits located between the retinal pigmented epithelium (RPE) and Bruch's membrane. Soft drusen are amorphous, granular, large (>125μm) and are associated with age-related macular degeneration, a leading cause of vision loss. As drusen grow, they disrupt the RPE by pushing against the cells. The aim of this study is to evaluate RPE viability atop drusen in histopathological sections obtained from enucleated and eviscerated eyes.

Methods: 20 eyes with drusen were obtained from the MUHC-McGill University Ocular Pathology and Translational Laboratory, Montreal, Canada (2013-2019). Of the 20 eyes, 10 were enucleated (50%) and 10 eviscerated (50%) among which 4 were removed due to technical difficulties. The eyes were embedded in paraffin and histopathological sections were obtained. Initial studies were done to identify the macular area and drusen. These sections were then stained using an immunohistochemical panel aimed at RPE identification and viability assessment. The panel includes HMB-45, MelanA (melanocyte markers) and Cytokeratin 8/18 (CK8/18) (epithelial cell marker) for RPE identification. It also includes the polyclonal cytoplasmic stain Ki-67 (Cell proliferation marker), and monoclonal intranuclear stains p53 (cell damage marker), p21 (cell cycle arrest marker), and caspase-3 (apoptosis marker). Histopathological analysis was done in scanned sections (Zeiss AxioScan.Z1) in eyes with drusen. RPE atop the drusen were compared to the undisturbed RPE surrounding it.

Results: Histopathological analysis was performed. Analysis of H&E slides revealed changes in RPE morphology above and beside drusen. These changes include pyknotic nuclei, loss of cell contour, pigmentary dispersion, and cell discontinuity. The cells atop the drusen expressed positivity for HMB-45, MelanA, and CK 8/18. Similar results were observed in the undisturbed RPE surrounding the drusen. P53 and p21 expression did not vary significantly between disturbed and undisturbed RPE. Ki-67 expression decreased in RPE above drusen when compared to the undisturbed cells. Whereas Caspase-3 expression increased in disturbed RPE.

Conclusions: The histopathological study of drusen and RPE is possible in both enucleation and evisceration patient samples. More specifically, the characteristics of damaged RPE cell above drusen were identified and described. Using our immunohistochemical panel, the cells overlying drusen were identified as RPE cells and found that MelanA, HMB-45, and CK8/18 remain positive in viable and damaged RPE. Damage to the RPE cells overlying soft drusen was quantified and qualified by the intranuclear and cytoplasmic stain positivity. Ki-67 expression revealed reduced proliferation of RPE cells above drusen whereas Caspase-3 expression revealed that apoptosis plays an integral role in the drusen-induced RPE cell damage.

POSTER #35 - Docetaxel improves hepatic lipid metabolism and reduces atherosclerosis

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Background. High density lipoproteins (HDL) remove cholesterol from cells. Desmocollin (DSC1) is a negative regulator of HDL biogenesis, and we have identified it as a contributor to the accumulation of cholesterol in the atherosclerotic plaque. After screening ~10 million small molecules, we have detected that the chemotherapy drug docetaxel (DTX) inhibits the DSC1 activity and promotes HDL biogenesis. We have shown that DTX reduces atherosclerosis in the apoE$^{-/-}$ mouse model. Here, we report on the metabolic effects of DTX in this model.

Methods. ApoE$^{-/-}$ mice were fed a high-fat diet and were divided into three groups after two weeks on a high-fat diet: baseline, DTX-treated and vehicle-treated. The baseline group consisted of mice sacrificed after two weeks on a high-fat diet. The other two groups received a subcutaneous implantation of an osmotic pump that was loaded with either 1 ug/ul of DTX or vehicle, and both groups were fed with a high-fat diet for an additional six weeks. At the end of a total of eight weeks, the mice of the DTX- and vehicle-treated groups were sacrificed. Blood and tissue samples of the mice from all three groups are analyzed.

Results. DTX reduced triglyceride levels observed in the apoE$^{-/-}$ mice, normalized non-esterified fatty acid levels that were elevated in the vehicle-treated group, decreased glucose levels that were elevated by the high-fat diet, and reduced total cholesterol, low density lipoprotein (LDL)- and HDL-cholesterol. DTX decreased all lipoprotein cholesterol in the blood but increased the HDL-/total cholesterol ratio, which is consistent with DTX promoting HDL biogenesis. Liver sections were stained with Oil Red O which identifies neutral lipids (triglycerides and cholesteryl esters). By examining the area stained, we observed that the DTX-treated mice showed more lipids than the baseline group, but considerably less compared to the vehicle group. In addition, by measuring the area covered by lipid-laden atherosclerotic lesions of the aortic surface area, DTX markedly reduced atherosclerotic lesions compared to vehicle.

Conclusions. The results show that DTX decreases atherogenic lipids (triglycerides, LDL- and total cholesterol) while increasing the HDL-/total cholesterol ratio. This atheroprotective lipid profile was also reflected by reduced lipid levels in the liver and the aorta. Consequently, DTX reduces atherosclerosis caused by dyslipidemia in an animal model. We suggest that DTX, a DSC1 inhibitor, may be used to treat atherogenic dyslipidemia and atherosclerosis at doses 100-1000 times less than used for cancer chemotherapy.

POSTER #36 - PARTAGE: BLOCKCHAIN DATA DONATION

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In developed countries nowadays, almost all hospitals and clinics keep patients' personal medical information in digital form while taking the necessary security measures and complying with all applicable legal requirements. Digital data offers great potential to drive real-world evidence (RWE) and artificial intelligence (AI) research in healthcare. But real-world data are not automatically available for research and more often than not they are inaccessible to researchers due to stringent security and privacy rules. Therefore, strategies to increase the amount of data available for research while preserving its security are needed. Data sharing is the act of an individual who contributes their health data for research purposes by providing their consent. People may voluntarily allow the transfer of their data generated for a clinical purpose to a collective research dataset where it may be used for a “broad secondary use” research purpose that is different than originally intended. This allows researchers to use otherwise private data for the benefit of society.

In our lab (kildealab.com), we are building a prototype blockchain-based patient-centered data-sharing system called the PARTAGE Research Portal. By allowing patients to share data from their phones directly to research studies using the Opal patient portal, we can simultaneously engage them in research and ultimately accumulate patient-shared data for research studies across multiple hospitals. However, we are conscious that the system needs to be secure and compliant with regulations. Therefore, we are investigating a novel approach for controlling personal medical data for use in research in a tamper-proof manner by storing just the data-sharing consents (from patients) and data-access privileges (of researchers) in a blockchain network. Rather than storing the actual data on the blockchain, we store hashes (i.e., digital fingerprints) of the data together with associated patient consent. In this way, we are using blockchain technology to enable the patient to control the transfer of and access to their data.

This summer project is specifically focusing on the most efficient way of storing hashes of patients’ data in the blockchain and matching them with the hashes generated by researchers when they wish to access the patient’s data. The main advantage of this method is that it ensures that the researcher only gets access to data that the patient explicitly shared using tamper-proof digital consent.

POSTER #37 - Web-based teaching tools for neuroscience

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Computational models of the brain allow us to better understand neuronal function from a single cell to an abstract level. However, complex manuscripts and modeling code written in languages such as MATLAB or Igor can be inaccessible or difficult to understand for students. To overcome this issue, I have translated the Hopfield Network, a more abstract concept of information processing, and the Backpropagation Action Potential model, which focuses on the single-cell level, to JavaScript, HTML, and CSS. This way, students without a coding background can access advanced computational neuroscience programs with just an internet browser.

The Hopfield network uses neuronal connections to enable pattern recognition, reflecting our brain's ability to recognize any pattern despite inconsistencies; however, in my simulation, the patterns will strictly be pixelated images. The neurons in this single-layer neuron recurrent network relate to each other, generating a weight value which helps the system attempt to revert a distorted image to the original. However, because more than one pattern is stored within these weights, there is a possibility for misrecognition or even false recognition.

The second part of the project focuses on the backpropagation in the single-cell model. This simulation is built upon the Huxley Giant Squid Axon Model of action potential generation and propagation. In the Backpropagation Action Potential model, the user can select a number of dendritic segments and a stimulation position by current injection. Because of the size difference between the soma and the dendrite, the dendritic current will have less impact on the soma's voltage change. Therefore, a user can see the backpropagation phenomenon as the spiking soma sends current back into the dendrites. This model coincidence detection in dendrites as well as state timing-dependent plasticity.

To summarize, my project will provide better access to these models to enhance students' learning experience. This user-friendly tool will not require any product licenses or coding background, eliminating the coding barrier between students and computational neuroscience.

POSTER #38 - Voice-based screening for SARS-CoV-2 exposure in cardiovascular clinics (VOICE-COVID-19-II): A Randomized Controlled Trial Protocol

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Background: The SARS-CoV-2 pandemic has disrupted the healthcare system, limiting healthcare resources such as the availability of healthcare professionals, patient monitoring, contact tracing, and continuous surveillance. As a result of this significant burden, digital telemedicine tools and artificial intelligence (AI) technologies have proliferated to increase the efficiency of healthcare and meet the unmet needs of a strained healthcare system. However, the increasing popularity of remote screening tools during the pandemic revealed a need for objective measures of reliability of the data acquired by these tools.

Objective: This paper describes the study design of an open-label, non-interventional, cross-over, randomized controlled trial assessing whether voice-based screening can detect SARS-CoV-2 in patients as accurately and efficiently as screening by healthcare coordinators.

Methods: A total of 52 patients visiting the heart failure clinic at the Royal Victoria Hospital of the McGill University Health Center, in Montreal, Quebec will be recruited. Patients will be randomly assigned, by block randomization, to first be screened for symptoms of SARS-CoV-2 by either digitally, by Amazon Alexa, or manually, by the research coordinator. Participants will then subsequently cross-over and be screened either digitally or manually. After completion of the SARS-CoV-2 screening, a survey will be presented by the coordinator to patients to assess their perceived user preference and Alexa app engagement. The primary endpoint is the inter-rater reliability on the accuracy of randomized screening data performed by Amazon Alexa versus to standard healthcare coordinator. The secondary endpoint is the perceived level of comfort and app engagement of patients with 5-point Likert scales and binary mode responses.

Results: The study is currently recruiting participants and the anticipated completion date is the end of Fall 2022.

Conclusions: The use of voice-based assistants could improve the provision of health services and reduce the burden on healthcare personnel. Demonstrating a high inter-rater reliability between Amazon Alexa and healthcare coordinators may serve future digital-based tools to streamline screening and delivery of care in the context of other conditions and clinical settings.

Keywords: Voice-based technologies, Amazon, Alexa, SARS-CoV2, COVID-19, digital screening

POSTER #39 - Monogenic diabetes in the UK Biobank (UKBB) cohort

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Background: Monogenic diabetes mellitus (MDM) is a rare form of diabetes caused by a mutation to a single gene. The most common form of MDM is maturity-onset diabetes of the young (MODY). Only dominant forms of MODY have been identified so far, and as much as 90% of cases are misdiagnosed as type 1 diabetes (T1D) or type 2 diabetes (T2D). Accurate diagnosis has therapeutic implications. We sought to screen the over 500,000 participants in the UK Biobank (UKBB) to identify misdiagnosed MDM.

Hypothesis/Objectives: We hypothesized that analysis of exome sequences would uncover cases of known MODY as well as evidence of recessive forms of the disease. The data generated will contribute to the Endocrine Genetics Lab's ADDAM study (Accurate Diagnosis in Diabetes for Appropriate Management) to better diagnosis, health outcomes, and quality of life for those affected by MDM.

Methods: We identified UKBB participants with BMI less than 30 and initial diabetes diagnosis before 25 years of age. Genetic risk for T1D was estimated using the T1D GRS2 (Genetic Risk Score 2). REVEL and InterVar were used to predict the pathogenicity of variants to the 14 known MODY genes of the lowest scoring individuals. Participants who are homozygous or compound heterozygous for Likely Pathogenic or Pathogenic variants will be investigated for evidence of recessive MODY.

Results: Of the 502,411 UKBB participants, 971 met our criteria for inclusion and had relevant genetic data available. After assessing genetic risk, 97 individuals were flagged for variant interpretation. We are currently analyzing the results and expect to find many MODY gene variants across multiple participants that are Likely Pathogenic or Pathogenic including individuals who are homozygous or compound heterozygous for such alleles.

Conclusions: Any MODY causing variants discovered in the UKBB will help grow our understanding of the disease. In combination with locally generated ADDAM data (225 exomes) we will look for novel genes with homozygous or compound heterozygous variants occurring in more probands than expected by chance alone, which will be further studied for evidence of a recessive MODY.

POSTER #40 - A Search for Autosomal Recessive Diabetes

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Introduction: Type 1 diabetes (T1D) is an autoimmune-mediated attack of insulin-producing β-cells of the pancreatic islets. Our research focuses on a rare form of the disease, called monogenic diabetes (also known as MODY), that follows a Mendelian form of genetic transmission, not due to autoimmunity. Already 14 autosomal dominant genes have been identified, thought to account for 1 to 4 percent of all diabetic cases (Li et al., 2022). However, there has been very little to no literature focusing on the autosomal recessive counterpart of monogenic diabetes. Why? Mostly because of a lack of family history suggesting monogenic etiology, that would trigger genetic investigation (Li et al., 2022).

Recent studies regarding WFS1, a gene responsible for Wolfram syndrome, a constellation of features, including optic atrophy and deafness, in addition to childhood-onset of diabetes mellitus and diabetes insipidus, have found certain recessive variants that cause non-syndromic diabetes mellitus. These mutations have been found to present in a similar proportion to the most common autosomal dominant MODY (Zhu et al., 2021).

Objective: The objective of our study (NCT03988764) *Accurate Diagnosis in Diabetes for Appropriate Management* (ADDAM) is to investigate how often diabetes is due to unusual genetic causes, by discovering new genes that interfere with the function of pancreatic β-cells. There is also incentive for disease management, since T1D is managed with insulin injections, but monogenic diabetes, in many cases, can be treated with simpler and more effective treatments (Li et al., 2022).

Hypothesis: We hypothesize that an equal proportion of diabetic cases are caused by autosomal dominant and autosomal recessive mutations.

Methodology: Whole exome sequencing will be performed for patients found negative for all three T1D autoantibodies tested (GAD65, IA-2 and ZnT8). The frequency of previously identified pathogenic variants will be compared to control exomes, allowing a measure of the proportion of monogenic diabetes among patients diagnosed as T1D. The exomes carrying unknown mutations are analyzed for pathogenic variants in novel genes. Identified candidate variants are confirmed through PCR and Sanger Sequencing. A REVEL score is used for variant pathogenicity result (Ioannidis et al., 2016).

Expected results and impact on society: We expect to discover variants in novel genes responsible for autosomal recessive monogenic diabetes. Certain candidate genes have already been selected for further investigation. ADDAM will help children and adults misdiagnosed as Type 1 Diabetes to receive more accurate and effective treatment for their condition. It will also contribute to improvements in early detection of monogenic diabetes through exome sequencing.

POSTER #41 - Δ3, Δ2-enoyl-CoA isomerase 1 (ECI1) expression in prostate cancer tissue provides additional prognostic information after Transurethral Resection of the Prostate (TURP)

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Background: Prostate cancer is the most frequently diagnosed cancer among Canadian men. Although distinguishing aggressive from indolent prostate tumors is important to optimize the therapeutic approach and avoid unnecessary treatments, accurately risk-stratifying patients remains challenging. Tissue biomarkers are promising tools to improve risk stratification and identify patients who may benefit of new targeted therapy. The *ECI1* gene resides in the 16p13.3 gain region associated with prostate cancer progression and poor clinical outcome. It encodes Δ3, Δ2-enoyl-CoA delta isomerase 1 (ECI1), a mitochondrial enzyme involved in beta-oxidation of unsaturated fatty acids. Our lab has recently shown that ECI1 overexpression expands the maximal mitochondrial respiratory capacity of cancer cells which lead to increased tumor growth and metastasis in nude mice. In addition, high ECI1 expression in prostate tumors was associated with poor outcome after radical prostatectomy. However, the association between ECI1 expression and the outcome of patients with advanced prostate cancer remains to be determined. This research project studies the ECI1 expression in tumor samples from patients who underwent a palliative operation to relieve urinary obstruction called Transurethral Resection of the Prostate (TURP).

Hypothesis and Objective: The hypothesis is that there is an association between ECI1 expression in prostate cancer tissue and the survival outcome of patients with advanced prostate tumors. The objective is to determine if high levels of ECI1 expression are associated with lower survival in a cohort of TURP patients.

Method: Immunohistochemistry (IHC) is used to detect ECI1 in prostate tumors of a cohort of 300 TURP patients with clinicopathologic data and represented by duplicate cores on a Tissue Microarray (TMA). The staining is quantified by computing the H-score which is used to stratify patients in two risk groups for the Kaplan Meier survival analyses. IHC for PTEN, ERG, SPINK and AR has previously been carried out on this TMA and the results are available to examine the prognostic impact of combining ECI1 staining with these biomarkers.

Results: A preliminary analysis of a subset of the cohort (n=170) shows that high expression of ECI1 is associated with shorter overall survival (log-rank, P=0,037). Moreover, patients who were expected to have a favorable clinical outcome based on an individual biomarker's status such as PTEN positive and ERG negative were further risk stratified by ECI1 (PTEN positive subgroup: n=117, P=0,017; ERG negative subgroup: n=120, P=0,008).

Conclusion: The data so far suggests that high levels of ECI1 expression are associated with lower survival in patients with advanced prostate cancer following TURP procedure and that the combination of ECI1 with other known biomarkers improves risk-stratification. This study is to be continued on the entire TURP cohort to fully assess the prognostic value of ECI1 alone and in combination with other biomarkers.

POSTER #42 - Assessment of Quality of Life Among Patients with Coronary Microvascular Dysfunction

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Background: Women with Coronary Microvascular Dysfunction (CMD) are reported to have poor clinical outcomes including higher rates of heart failure, hospitalizations for acute coronary syndrome, sudden cardiac death, myocardial infarction, stroke, adverse prognosis, and poor quality of life. However, which factors determine quality of life (QoL) in women with CMD remains poorly understood.

Objective: Our aim is to assess the determinants of QoL among women with CMD.

Methods: The study involved 44 women with CMD and 50 healthy volunteers. Women were defined as having CMD if they had a clinical diagnosis of chest pain and non-obstructive coronary artery disease (NoCAD) and were excluded from having non-cardiac causes of chest pain, epicardial spasm, coronary artery dissection and coronary slow flow. Baseline clinical and sociodemographic characteristics were obtained for all participants. Women with CMD completed the Seattle Angina Questionnaire (SAQ), a 19 item questionnaire that evaluates the frequency of angina (SAQ Angina Frequency score), the disease-specific effect of angina on patients' physical function (SAQ Physical Limitation score), the burden of coronary artery disease on patient's QoL (SAQ Disease Perception score), the change in frequency of angina at patient's most strenuous level of activity (SAQ Angina Stability score), and the patients' satisfaction with current treatment (SAQ Treatment satisfaction score). Individual scores range between 0 and 100, where 100 is indicative of better quality of life.Descriptive statistics and multiple linear regression analysis will be used to assess the determinants of overall quality of life in women with CMD.

Preliminary findings and expected results: Compared to healthy volunteers, women with CMD are more likely to be taking anti-anxiety medications (9.1% vs 2.0 %) and anti-depressant medications (22.7% vs 2.0 %). Higher disease perception score, physical limitation score, Angina Stability Score, and Angina Frequency Score were associated with higher SAQ Summary score. Variations in SAQ treatment satisfaction score were not associated with variation in SAQ Summary score. We suspect that women with CMD present with greater number of comorbidities and take a higher number of medications compared to healthy controls. Furthermore, we expect that age, level of education, depression and physical limitations of angina to be the/our determinants of quality of life among women with CMD.

Conclusion: In this study, we found that a lower physical limitation score, angina frequency score, disease perception score, and angina stability score were associated with lower QoL. Understanding the determinants of QoL will allow healthcare providers to better address the needs of women with CMD.

POSTER #43 - Generating Patient-Specific iPSC-Derived Endothelial Cells for Creating a Hypoxic Disease Model

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Background: Myocardial infarction (MI) continues to be a major cause of death and morbidity worldwide, causing long-term heart failure (HF) in patients that survive. Patients who suffer an MI are at risk of getting HF, a disease that progresses over time as the human heart undergoes maladaptive remodeling post-injury, and the terminally differentiated cardiomyocytes and cardiac vascular cells are unable to repair the damaged myocardium. Recently, induced pluripotent stem cells (iPSCs) have been studied due to their proliferative properties and ability to differentiate into various lineages. Endothelial cells (ECs) are specifically of interest as they play a significant role in cardiac homeostasis through lining the heart's blood vessels and regulating a plethora of processes such as vascular permeability and tone, angiogenesis, and platelet adhesion, among other mechanisms. Currently, no interventions or drugs specifically target damaged ECs, making injury irreversible. This preliminary project involves the generation and characterization of patient-derived iPSC derived-Endothelial Cells (iPSC-ECs) from cardiomyopathy patients and healthy controls to better understand the role hypoxic injury plays in EC dysfunction.

Methodology: We generate iPSC-ECs from the peripheral blood of patients with cardiomyopathies (n=2) and healthy controls (n=2). The PBMCs we collect are reprogrammed into iPSCs via electroporation with episomal vectors (Oct4, Sox-2, Lin28, klf4, L-Myc) and proceeded with an optimized EC differentiation protocol (STEMCELL Technologies). Furthermore, we aim to produce a patient-specific in-vitro iPSC-EC injury model via hypoxia by subjecting iPSC-ECs to 24 hours of 0% oxygen, mimicking an MI-like cell damage phenotype. The injury is then characterized through cell viability, cell metabolism, and cell migration assays, as well as a Western Blot to quantify different markers of damage and cell death.

Results: Our preliminary characterization results demonstrated that iPSC-ECs expressed CD31, CD34, CD45, and ICAM1 markers via immunocytochemistry, and not the iPSC pluripotency markers - OCT4, and TRA-1-60, which indicates that the iPSC-ECs have successfully differentiated into pure ECs from iPSCs. Qualitatively upon differentiation, the generated iPSC-ECs for both the control and diseased patient, go from a round cell morphology to fish-shaped and elongated cells with a dark cell body. Both the Alamar Blue assay and Crystal Violet assay measured the metabolism and viability of the cells, respectively, and both showed that hypoxic injury has significantly lowered the viability and metabolism of iPSC-ECs. In addition, the migration assay, a hallmark of angiogenesis, has shown general trends of hypoxic ECs migrating at a slower rate and lower total closure of the gap compared to the normoxic cells.

Discussion: The iPSC-ECs have a significantly more diseased phenotype in hypoxia, demonstrated by the three different assays, and characterization has proven the purity of ECs generated, thus concluding that hypoxia might be a suitable model to use for iPSC-EC injury. A preliminary disease model of patient-specific iPSC-ECs could lead to the testing of angiogenesis-related experiments such as tubular formation assays, vessel-delivery-related drugs for pharmacological testing, or as a screening tool for novel regenerative therapies.

POSTER #44 - Prolactin and its effects on the Hippo pathway

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Background: The Hippo tumor suppressor pathway is a key regulator in organ size and is often dysfunctional in cancer. It works through an upstream kinase cascade that extracts the oncogenic Yes associated protein (YAP) transcription factor from the nucleus into the cytoplasm for phosphorylation and ubiquitin mediated degradation. Initiation of the kinase cascade is performed by many signals including G protein coupled receptors (GPCRs), cell-cell adhesion complexes and various soluble cytosolic proteins. To date, not many extracellular soluble ligands have been proven to activate the pathway. The peptide hormone prolactin (PRL), its receptor (PRLR) and its downstream Jak2/STAT5 signaling pathway controls various physiological mechanisms in the mammary gland and tissue. During pregnancy, serum levels of PRL rise and induce a morphogenic program to prepare the mammary gland for lactation by carefully controlling the differentiation of mammary epithelial cells (MECs). PRL induces the formation of tight junctions, apico basal polarity and cell-cell adhesions, all three of which are dysregulated or even completely absent in breast cancer. In this study, PRL's involvement in activating the Hippo pathway is described.

Methods: Subcellular fractionation, western blotting and immunohistochemical techniques were used to prove that PRL induced YAP nucleocytoplasmic translocation and degradation in vitro. MCF7 breast cancer cell line was treated with 250 ng/mL PRL solution at varying time points up to 8 hours. Subcellular fractionation and SDS-PAGE analysis were performed to collect, purify and quantify critical changes in proteins involved in Hippo signaling when treated with PRL. In addition, two-dimensional immunofluorescence was performed to visualize and confirm YAP cytosolic accumulation during treatment.

Results: From the experiments described above, it was shown that within an 8-hour period, PRL induced substantial nuclear clearance of YAP into the cytosol and subsequent degradation through western blot evidence of its phosphorylation.

Conclusions: PRL's involvement in the Hippo pathway further strengthens its role as a differentiation and anti-tumorigenic factor in breast cancer.

POSTER #45 - Screening for antibiotic activity in bacterial isolates from the Canadian High Arctic

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The rise in antibiotic resistance is a global health crisis making common infections and minor injuries deadlier. At the same time, the number of antibiotics available to fight resistant pathogens is decreasing due to a lack of new antibiotics discovered in recent decades. The discovery of new antibiotic is now critical. Most of the antibiotics currently used are derived from natural products, namely compounds naturally produced by microbes, such as bacteria, to fight off other microorganisms. Unfortunately, attempts discovering antibiotics from synthetic compounds have failed in the past. This has led researchers to return to natural product discovery using new methods and bioprospecting in new environments. Studying unexplored environmental niches, such as soil and sediments in the Canadian High Arctic, is a possible way to find bacteria that produce novel antibacterial compounds. This extreme environment has great microbial diversity, which results in evolutionary pressure to select for bacteria that produce antimicrobials as a defense mechanism. Previously for this project, MIMM 212 undergraduate students screened bacteria isolated from Canadian High Arctic soil against safe relatives of the ESKAPE pathogens (*Enterococcus faecium*, *Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa,* and *Enterococcus* species) through the Tiny Earth program. Then, to avoid the rediscovery of known antibiotics, the strains demonstrating antibiotic activity were counterscreened through a dereplication platform, namely an antibiotic resistance platform (ARP) of susceptible *E. coli* strains containing plasmids encoding resistance genes to known antibiotics. The goal of this project is to screen the 2018 and 2021 Tiny Earth strains that passed the ARP for antibiotic activity against the ESKAPE pathogens themselves. We hypothesise that the Arctic bacteria are producing novel antibiotics with activity against at least one ESKAPE pathogen. To start exploring this question, we first developed a replica plater stamping assay, which consists of growing a lawn of the ESKAPE pathogen and then stamping the Arctic strains with the replica plater on top. With this method of co-culture, zones of inhibition can be observed when an Arctic strain has antibacterial activity against a pathogen. Each of the Arctic isolates were tested against a standard “lab” strain and a “clinical” strain for each ESKAPE pathogen to better characterize their antibacterial activity. To date, 12 2018 Arctic isolates were tested using the replica plater stamping assay. Seven showed activity against Methicillin-susceptible *Staphylococcus aureus* (MSSA) and Methicillin-resistant *Staphylococcus aureus* (MRSA). No robust activity was seen against the other pathogens by any of the strains. Also, in previous assays, we saw no activity from 2017 Arctic isolate “MNAAK”, against *P. aeruginosa,* but with this replica plater stamping assay we observed consistent antibiotic activity. These preliminary findings suggest that some isolates from the Canadian High Arctic are worth pursuing for further investigation as they have robust antibiotic activity against multiple pathogens. Furthermore, it suggests that the replica plater stamping assay is a great screening tool that allows us to identify isolates with activity not seen in other co-culture screening assays.

POSTER #46 - Regulation of TGF-B in chondrocytes

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Introduction: Among the cytokines, transforming growth factor beta (TGF-β) is a critical mediator of cartilage repair and maintenance. Dysregulation of TFG-β signalling and responses in chondrocytes have been found to be involved in osteoarthritis (OA). OA is a highly prevalent degenerative joint disease that affects more than 528 million people worldwide. OA is a disabling condition for which there is currently no effective disease-modifying therapy. Previous studies have shown CD109 to be a novel TGF-β co-receptor and have concluded that CD109 is a potent negative regulator of TGF-β signalling in the skin. The current study has three objectives as follows: 1) to identify CD109 as a modulator of the balance between TGF-β signalling via ALK1 versus ALK5 signalling pathways, 2) to identify the functional implication of CD109 in OA progression in vivo in murine articular chondrocytes, and 3) to develop cartilage-specific CD109 knockout mice and challenge them with the destabilization of the medial meniscus (DMM) surgery to obtain a better understanding of the role of CD109 in OA progression in vivo.

Methods: Articular cartilage tissue was harvested, and primary chondrocytes were obtained from CD109 knockout (KO) and wild-type (WT) mice. Western blot and immunohistochemistry (IHC) techniques were used to examine the TGF-β signalling components in cartilage tissue and chondrocytes by determining the level of ALK5 as opposed to ALK1, and the level of Smad2/3 as opposed to Smad1/5. Additionally, the function of chondrocytes was determined by examining the expression of collagenase (MMP-13), aggrecanase (ADAMTS-5), collagen (type II versus type I), and aggrecan at the protein and mRNA levels by western blot, immunocytochemistry (ICC) or real-time PCR, and IHC.

Results: The results showed that articular chondrocytes collected from CD109 KO and WT mice demonstrated a similar expression of type I collagen and proteoglycan. Additionally, type II collagen, ALK5, and aggrecan expression increased significantly in the CD109 KO mice. Finally, a loss of CD109 expression in the knockout mice displayed decreased ALK1 levels and TGF-β1-induced Smad1 phosphorylation, and also inhibited MMP13 and ADAMTS5 protein levels in murine primary articular chondrocytes.

Conclusion: CD109 plays a critical role in the differential regulation of ALK5 versus ALK1 pathways in murine articular cartilage and chondrocytes. More specifically, CD109 decreases the TGF-β/ALK5/Smad2/3 signalling pathway while increasing the TGF-β/ALK1/Smad1/5 signalling pathway. CD109 also promotes an OA-like phenotype in murine articular chondrocytes and cartilage by inhibiting ECM protein production and promoting protease expression. Therefore, the results of this study provide novel insights into the mechanism by which TGF-β regulates chondrocyte function and may lead to strategies for therapeutic intervention to improve cartilage repair.

POSTER #47 - ATF7 as a potential epigenetic regulator of the lung inflammatory response in Cystic Fibrosis

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Cystic fibrosis (CF) is a lung disease associated with lung infection and inflammation, leading to irreversible structural lung damage as well as impaired lung function. My research project aims to investigate the stress-dependent epigenetic regulator cyclic AMP-dependent transcription factor 7 (ATF7), that might be involved in the tuning the inflammatory response to pathogens exacerbating CF. I hypothesize that prior activation of ATF7 by environmental stressors (other infections, noxious substances) will lead to a change in the epigenetic landscape of epithelial cells, changing the threshold of activation and/or the magnitude of the pro-inflammatory mediators' synthesis in response to Pseudomonas aeruginosa, an important CF pathogen.

During my summer project, I will test this hypothesis using an in vitro system of lung epithelial cells (Beas-2B) and neutrophil-like cells (differentiated HL60). I will first assess the expression of ATF7 in Beas-2B cells using immune-blotting. I will create a CRISPR-mediated knockout of ATF7 in Beas-2B and HL60 cells. I will use two viral delivery systems for CRISPR-mediated deletions in lung epithelial cells developed by my co-supervisor, Dr. Gregory Fonseca. The loss of expression of ATF7 will be assessed using immuno-blotting. The final step will be to challenge the [WT] and [ATF7-/-] cells with two stressors (H2O2 and flagellin), let them recover for a week prior to exposure to P. aeruginosa-diffusible material. The last step will be to map the epigenetic landscape of challenged [WT] and [ATF7-/-] using ATAC sequencing.

A precise protocol was used to create this viral vector containing the CRISPR-Cs9 machinery. First, I cloned a plasmid containing the information for the sgRNA. This backbone plasmid was developed by the lab of Dr. Fonseca. I then ligated sequences which are specific for my gene of interest (GOI), ATF7, using restriction enzymes. The sequence is then verified by colony-PCR, and finally Sanger sequencing. Once the plasmid is confirmed to contain the sequence of interest, I produce my viral vectors using Hek293 cells, and I then put my plasmids in them (the one I created plus two other ones which code for other important machineries of Cas9). This then yields my viral vector.

The completion of this project should determine whether ATF7 plays a role in regulating the epigenetic landscape of lung epithelial and neutrophil-like cells in response to stressors relevant to lung physiology. For the moment, I do not have concrete results. However, preliminary results showed that ATF7 is expressed in Beas-2Bs, using immunoblotting. I also showed all steps of the way that I cloned a plasmid containing a sg-RNA specific for my gene of interest, ATF-7. Results of sequencing arriving soon will confirm those results. The next steps, which should be done by the end of the Summer, will show I created this model, and can be used for further experiments.

POSTER #48 - THE SHORT-TERM EFFECT OF RED MEAT VERSUS PLANT-BASED MEAT ALTERNATIVES ON CARDIOVASCULAR RISK MARKERS: A RANDOMIZED CONTROLLED TRIAL PROTOCOL

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Background
Cardiovascular disease continues to be a leading cause of death both worldwide and in Canada. Amongst others, diet remains a key modifiable risk factor for the development of cardiovascular disease. Trimethylamine N-oxide (TMAO) is a gut microbiota-generated metabolite that has been shown to be associated with atherosclerosis and cardiovascular disease. This association is dose-dependent, with a positive correlation between circulating TMAO and major adverse cardiovascular events. The purpose of the Finding Optimal Oral Diet-1 (FOOD-1) trial is to directly evaluate the short-term impact of plant-based meat substitutes versus red meat-based diets on circulating TMAO levels.

Methods
The FOOD-1 trial is planned as a randomized, single-blinded, cross-over trial that will recruit forty healthy adults. Approximately 38 participants will be randomly assigned in 1:1 ratio to a plant-based meat alternative (Beyond Meat) or a red meat-based diet for 6 days and later cross-over to the alternate intervention. Baseline data including demographics, comorbidities, and dietary history will be captured. Plasma and urine samples will be collected following each intervention period. Figure 1 graphically illustrates the study protocol. The primary outcome is change in plasma TMAO. Changes in plasma TMAO levels between the plant-based meat alternative and the red meat-based diets will be analyzed using a linear mixed-effects model, adjusting for the order and phase of randomization. A paired t-test and analysis of covariance will be used to validate the significance of the comparison.

Discussion
This study is designed to investigate the role that plant-based meat alternatives may have in impacting short term changes in serological markers of atherosclerosis and cardiovascular disease. Reductions in serum TMAO levels after dietary changes from red meat to plant-based meat alternatives, and vice versa, will highlight the causal relationship between markers of cardiovascular disease and the consumption of red meat. These results will provide greater insight into how different dietary patterns influence cardiovascular disease risk and may inform primary prevention strategies to reducing cardiovascular disease burden.

POSTER #49 - Role of the transcriptional activator Yap in initiation and resolution of gastric cell metaplasia.

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Background: Gastric cancer represents the third leading cause of cancer-related deaths worldwide. Consequently, understanding the pathogenesis of the disease has become a major priority. One of primary causes of gastric cancer is chronic infection with the bacterium Helicobacter pylori, which induces inflammation that leads to lesions within the gastric epithelium resulting in loss of acid-secreting parietal cells. The loss of parietal cells, known as oxyntic atrophy, is compensated by a hyperproliferative response, as well as induction of metaplastic cell lineages. Thereby, resulting in abnormal mucus-producing cells at the base of the gastric glands, known as spasmolytic polypeptide/trefoil factor 2-expressing metaplasia (SPEM). Although the link between SPEM and the development of gastric cancer is well accepted, the mechanisms underlying initiation and resolution of SPEM remains poorly understood.

Recent progress and Hypothesis: Previous work at our lab has demonstrated an essential role of the Hippo signaling effector Yap in driving intestinal crypt regeneration and transcriptionally reprograming intestinal stem cells. Using conditional knockout mice, we have recently shown that Yap and its homologue Taz are required for resolution of SPEM induced by high dose tamoxifen (HDT)-dependent oxyntic atrophy. In addition, similar work has shown that SPEM is dependent on activation of IL-13 expressing type II innate lymphoid cells (ILC2). Based on these findings, we propose that Yap and Taz may be implicated in regulating the type II immune circuit underlying SPEM initiation and/or resolution. To address this hypothesis, this project will pursue the following objectives.

Objectives and Methodology:
1) Role of Yap/Taz in regulating type 2 immunity during the metaplastic response resulting from parietal cell injury Parietal cell injury will be induced *in vivo* by intra-peritoneal injection of tamoxifen in Clusterin-CreERT; Yapfl/fl; Tazfl/fl mice. Stomach tissue will be harvested at various timepoints post-tamoxifen injection for formalin fixed paraffin-embedded tissue processing and analysis. The infiltration of Gata 3 positive ILC2s will be studied by immunohistochemistry. RNAscope will be performed using multiple probes to assess macrophages recruitment. Assays will be visualized on a confocal microscope at the RI-MUHC imaging platform. QuPath, an open-source software for digital pathology and whole slide image analysis will be used for quantification of imaging data.

2) Investigate the role of Yap and Taz on growth and metaplastic response in IL-13 dependant gastric organoids. Since IL13 has been shown to be sufficient to drive a SPEM related gene signature in gastric organoids, corpus glands will be harvested from tamoxifen-treated Clusterin-CreERT; Yapfl/fl; Tazfl/fl mice and grown in gastric organoid media with/without IL-13. Finally, RT-qPCR assays will be performed to monitor the levels of SPEM-associated genes such as Muc6, Tff2, Mist1 etc.

Conclusion: We anticipate that these studies will shed light into the function of Yap and Taz in regulating type 2 immunity during metaplastic response after parietal cell injury. Finally, we expect that Yap and Taz play a role in growth and regeneration in IL-13 dependent gastric organoids therefore giving insight into mechanisms of SPEM.

POSTER #50 - Investigating the biological and clinical impact of ectopically misexpressed Cancer Testis genes

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The interest in non-melanoma skin cancers (NMSCs) has risen drastically due to its increased prevalence in our population. NMSCs affect about 80,000 Canadians every year. This can be attributed to a variety of factors, including environmental and genetic causes. Although there are numerous NMSCs, the two dominant forms are basal cell carcinoma (BCC) and cutaneous squamous cell carcinoma (cSCC). Even though there have been significant improvements in NMSCs prevention and control, further research is required to improve our understanding of NMSCs. Recent studies have shown a link between HPV infection and UV radiation-induced DNA damage in promoting tumorigenesis in cSCC. Lately, novel techniques have been developed to tackle advanced NMSCs. Initial screening analysis revealed the ectopic expression of Cancer Testis (CT) genes in cancer samples, most notably in BCCs samples. Results identified *PRAME* as the most prevalent misexpressed CT gene, followed by *HORMAD1*. Gene expression profiles were performed via RT-PCR. Our research targets the genetic causes of NMSCs and that's why we have been investigating genes of interest like *PRAME and HORMAD1*. The molecular expression of *PRAME and HORMAD1* is being further examined in new patient samples and in cSCC and BCC cell lines to establish their cellular localization and to determine the link between expression levels and response to treatment. It is believed that these genes influence the development of NMSCs, and thus targeting them could be of great therapeutic use.

POSTER #51 - Effects of folic acid supplementation on mice kidneys

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Background: Since 1996, folic acid has been authorized as supplements in food in Canada, and since 1998, all Canadian flours are supplemented with folic acid. This has been a very efficient measure in order to fight NTDs (neural tube defects). Indeed, it has been shown that folic acid deficiency in pregnant women can lead to NTDs. Nowadays, physicians suggest to take folic acid as a supplement to pregnant women and women that want to be pregnant. These measures have helped to reduce these disorders by half. However recent studies have shown that an excess of folic acid could be toxic. A cohort study shows us that we would observe an increase int the number of CAKUT (congenital abnormalities of the kidneys and the urinary tract) in children born from mothers with a folic acid supplemented diet (FASD). This is why we are interested in the effect of an excess of folic acid on the kidneys. Moreover, the pathway of folic acid implies MTHFR. It is an enzyme involved in the nucleic acid synthesis as well as in the methylation of DNA. We observe a common polymorphism in the population (5-20 %) of this enzyme that is C677T. We also wonder if this polymorphism could increase negative effects of an overconsumption of folic acid.

Hypothesis: Excess of folic acid is toxic for kidney development and/or for kidneys.
Excess of folic acid lead to a decrease of the amount of MTHFR (or just active form of MTHFR). The polymorphism C677T of MTHFR gene is responsible of a decrease of folic acid metabolization in folic acid supplemented diet. We have less MTHFR / it is less active with FASD (folic acid supplemented diets) and a TT genotype.

Experiments: Count of glomeruli : embedding kidneys in paraffin then section them at microtome. Then stain them with H&E. We can count glomeruli manually or by an automated way : Qupath deep learning.
We assume that the diet and / or the genotype influence the amount of glomeruli per kidney. We are expecting less glomeruli in mice with FASD than in CD. This amount could be even smaller if the mice are TT. Western Blot α-MTHFR : We assume that the diet and / or the genotype influence the amount of MTHFR or active form of MTHFR in kidneys. We should have less MTHFR (or active MTHFR) in kidney's extracts from FASD mice or / and in TT mice. Kidney culture : dissecting embryo kidneys from Hoxb7GFP mice at E12-E13 and put them in culture. Make a control medium (DMEMF12 + FBS 5 %) and a medium supplemented with folic acid (DMEMF12 + FBS 5% + folic acid), 10 times more than control medium. Observe the development of kidneys over time and watch the branching of the kidney. We expect to see abnormalities in kidneys that are in medium with more folic acid. We expect differences in development that will not be compensated over the time. We don't have significative results for the moment. But we expect to have in off data for the beginning of August.

POSTER #52 - Opal OncoBuddy and OncoConseil Programs

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Background
Many cancer patients find their experience with the disease difficult. Some are reluctant to tell their family and friends about the illness; others simply don't feel understood. The existing support programs offered are not always fitting for patients' needs. Not all kinds of support are perceived as helpful. However, peer support, in which a new cancer patient gains insight from the hindsight of a former cancer patient, has been shown to help patients in a way that professional support cannot. With this in mind, the Opal Health Informatics Group is working on creating a peer support algorithm for the Opal app that is capable of matching patients for peer support according to their needs and preferences.

Methods
In this summer research project, we are looking at what is most important from the point of view of patients when looking for effective peer support. We started by searching in the peer-reviewed and gray literature for criteria that various matching services use to match people, such as dating apps, Indian marriage websites, and other forms of peer support programs. With this research, we designed a preliminary semi-structured interview guide to interview cancer patients at our centre regarding their preferences. Using this preliminary guide, we interviewed some of our patient partners, who are cancer survivors working on our research team. Based on these mock interviews, we have identified some problems and improved the guide accordingly. Before the end of this summer project, we plan to conduct and code the official semi-structured interviews with cancer patients, who will be recruited using the Opal app in the coming weeks. From the data collected, the informatics side of our research team will create an algorithm for integration into Opal. The algorithm will be able to match a future cancer patient with a potential peer volunteer (OncoBuddy) or match them with written advice that is most suited to their needs that was left behind by a former patient (OncoConseil).

Results
To date, we have reviewed the peer-reviewed and gray literature and conducted three mock interviews with our patient partners. We are currently recruiting participants for the official interviews. We aim at interviewing at least six patients before the end of the summer.

POSTER #53 - Morphological Reconstructions of Vip Interneurons in the Motor Cortex

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Inhibitory interneurons (INs) play a critical role in the brain by shaping and controlling cortical circuits. As such, disruptions in IN signaling can lead to imbalances in cortical activity that are thought to be implicated in many disorders, such as epilepsy. INs are categorized based on their expression of distinct molecular markers, their morphology, and their physiological properties. Of the different IN types, vasoactive intestinal peptide-expressing (Vip) INs are particularly poorly described. For example, there is little known about how Vip IN activity leads to changes in its inputs or outputs, a concept known as plasticity. Yet, Vip INs have a key disinhibitory role in the brain by controlling the activity of other INs and can thus lift the brake on activity in the brain. It has been shown, for example, that inhibiting Vip IN activity reduces seizure susceptibility and duration. Vip INs may thus be a key point for controlling seizures.

For this reason, our lab aimed to characterize the short-term plasticity of Vip INs in the mouse motor cortex. We found that Vip IN inputs displayed a surprising diversity in short-term dynamics. Therefore, for my project I aimed to perform morphological reconstructions of Vip INs to explore whether the heterogeneous short-term dynamics at Vip IN inputs could be attributed to differences in cell morphology.

To accomplish this, I used a program called Neuromantic to trace and reconstruct Vip INs in layer 2/3 of the mouse motor cortex. The Vip INs had been previously filled with biocytin during electrophysiological experiments, were immunostained with Streptavidin Alexa Fluor 488 or 647, and then imaged using confocal microscopy. In all, 3D reconstructions of Vip INs can thus provide insight on the morphological characteristics of these INs. Statistical analyses will subsequently be performed to assess whether Vip IN morphology is associated with the observed heterogeneous short-term dynamics at Vip IN inputs.

POSTER #54 - Computational models for cell-cell interaction inference from single-cell spatial data

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Background: Understanding the mechanisms of cell-cell interactions in multicellular systems is essential for the diagnosis and treatment of various diseases. For example, a better understanding of the signaling networks that drive the pathogenesis and metastasis of various cancers can help develop novel diagnostic tools and targeted treatment methods. Recent progress in spatially resolved transcriptomics technologies, such as 10x Genomics Visium and Slide-seq, have allowed researchers to profile gene expression in captured locations of tissue samples at near single-cell levels of resolution. However, the identification of spatial domains with similar spatial expression patterns and the reconstruction of cell-cell interactions from spatially resolved single-cell data remains an important challenge in SRT studies.

Methods: Many existing methods for spatial domain identification and cell-cell inference from single-cell data do not take into account spatial information. Without knowing the physical relationship between different cells, it is difficult to correctly infer cell-cell interactions within a broader tissue context. As a result, these methods may fail to identify spatially consistent domains within heterogeneous tissue samples and miss certain active ligand-receptor pairs.To overcome these limitations, we aim to develop a graph convolution-based deep neural network framework that can be used to infer cell-cell interactions and identify spatial domains. Our model uses a graph convolution layer integrated in a variational autoencoder model to learn a latent embedding of the single cell data that aggregates gene expression and spatial information together. Graph convolutional networks (GCN) are a variant of convolutional neural networks which operate directly on graph structured data, allowing each node to aggregate the information of its neighbours. The variational autoencoder model then learns a latent embedding of the data that can be used to infer cell-cell interactions and identify spatial domains within the heterogeneous tissue samples.

Results: Our results show that our model can use spatial information and gene expression data to learn a latent embedding of the data. Compared with methods that do not take spatial information into account, our method allows us to identify more spatially consistent domains.

POSTER #55 - The effect of adipocyte-specific CCN5/WISP2 overexpression on β-cells in high-fat diet and streptozotocin-induced diabetes

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Type 2 diabetes, a disease in which the body develops insulin resistance and in which pancreatic β-cells produce less insulin, currently lacks effective treatment options. The need for methods to promote pancreatic β-cell survival and proliferation in order to treat type 2 diabetes has called for further examination of their regulators. Recent research has revealed that the growth factor CCN5/Wnt inducible signaling protein 2 (part of the CCN5 family of proteins, which have signaling and regulatory roles) functions in cultured pancreatic β-cell proliferation and survival. In fact, Dr. Liu's lab has demonstrated the presence of CCN5 expression in resting pancreatic β-cells, and that murine β-cell proliferation and survival rates in vitro benefit from recombinant human CCN5 protein. CCN5 overexpression also promotes the proliferation of insulinoma cells, while CCN5 deficiencies cause increased insulin resistance. Thus, we hypothesize that CCN5 improves metabolic function and promotes β-cell survival, proliferation, as well as insulin secretion in vivo, reducing type 2 diabetic development. This study aims to confirm whether CCN5 stimulates β-cell survival and proliferation, therefore attenuating type 2 diabetes, and to determine the mechanism through which it acts.

For this experiment, two groups of transgenic mice are being evaluated. Firstly, CCN5-knockout mice are being analyzed for the effects of a loss of CCN5 protein. Secondly, mice with adipocyte-specific CCN5 overexpression (aP2-CCN5 mice) are being evaluated to establish whether CCN5 overexpression will promote β-cell survival and attenuate diabetes. In order to induce hyperglycemia in wild type and transgenic mice, streptozotocin (STZ) injections along with a high-fat diet are being used. By evaluating the development of β-cells and β-cell-related health outcomes of these groups of transgenic mice, then by comparing them to each other and to wild type mice, the effects of CCN5 overexpression may be elucidated.

Currently, obstacles faced in the study include successfully inducing diabetes in mice, and the optimal STZ dosage and injection schedule are yet to be determined. Furthermore, female mice require different dosages as they have been shown to possess decreased sensitivity to STZ injections compared to male mice. However, one method that has been successful in inducing diabetes is through using a high-fat diet for 3 weeks preceding injections, then using a 50 mg/kg dose of STZ in 1M sodium citrate. The difficulty in inducing diabetes may be attributed to CCN5 overexpression, which may be hindering the effects of STZ. Thus, further experimentation will be required to achieve the desired diabetic phenotype in transgenic aP2-CCN5 mice. This will then allow the mechanism of action of CCN5 on pancreatic β-cells to be investigated, potentially providing novel methods to treat and prevent β-cell dysfunction characteristic of type 2 diabetes.

POSTER #56 - Montreal Derm FilEZ: Validation of an Interactive Online Dermatology Educational Platform for Dermatology Residents

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Background: Dermatology residents in Canada are required to pass a rigorous 6-hour written royal college examination composed of approximately 200 long and short answer questions. With over 3,000 dermatological pathologies to account for, residents are often given the enormous task to self-study the material whilst completing their clinical training. The current gold-standard learning tool is a 2,880 pages textbook. Prior research has proven that a combination of interactive teaching methods, such as audio, visual, writing and active recall, can increase knowledge retention up to 90%. As such, a new revolutionary free online interactive learning platform for dermatology residents called Montreal DermFilEZ was created with the aim of improving long-term knowledge retention compared to traditional textbook reading. The aim of this study is to validate this new learning tool.

Methods: Approximately 40 dermatology residents across Québec were randomized into two groups: the control group and the experimental group. The control group was asked to study three main pathologies using the gold-standard textbook, and the experimental group was asked to study using the DermFilEZ platform. A pre-questionnaire is sent at the start of the study and a post-questionnaire is sent 3-months later. This post-questionnaire will test for their long-term retention, but also obtain their input on the interactive learning platform.

Results: Data collection is still ongoing, but based on the current literature, the research team believes that residents who use the interactive teaching platform will outperform their colleagues who only use textbook studying and that it will be overall a better enjoyable experience.

Conclusions: This study will aid in improving the education of future generations of physicians, which ultimately has positive effects on patient outcomes.

POSTER #57 - The Ultraviolet Irradiation on Keratinocyte Cells Induces the Reactivation of the Retrotransposon LINE-1 Expression Leading to Cell Senescence and Aging

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Background: Narrow band ultraviolet B (NB-UVB) and Broad Band ultraviolet B (BB-UVB) lights are commonly used in phototherapy for the treatment of Atopic Dermatitis (AD). Interestingly, the UVB light exposure in keratinocyte cells can lead to genomic instability, which is a harmful cellular event. Retrotransposons, which comprise ~40% of the mammalian genome, have played an important role in evolution through their transposable activity. The largest, and the only currently active human group of mobile DNAs are the *LINE-1* retrotransposons, which are non-LTR (Long Terminal Repeat) retrotransposons, constitute ~17% of human genome and encode two proteins *ORF1p* and *ORF2p*, that function together to insert mutations into the genome. Recently, the unusual expression of LINE-1 has been correlated with genomic instability.

Hypothesis: We hypothesize that *LINE-1* reactivation occurs at a high rate in response to UVs exposure, which significantly contributes to genomic instability/DNA damage leading to cell senescence and aging. Therefore, *LINE-1* activation may serve as a robust marker of stress response to UVs radiation in skin keratinocytes and its regulatory pathways may be potentially targeted to prevent/modulate aging and cells senescence.

Results and Methods: First, using immunofluorescence, we confirmed the early UV-induced *LINE-1 ORF1* protein overexpression in two normal keratinocyte cell lines N/TERT and HaCaT (1 hours after UVs exposure). Moreover, to investigate the effect of UVs radiation on *LINE-1* reactivation and cell senescence mechanism, we shone NB-UVB or NB-UVB light on these two cell lines *in vitro*, and measured the mRNA expression of *LINE-1* and multiple senescence markers after 1, 3, 6 and 24 hours following unique and/or several UVs exposures using RT-qPCR (reverse transcription quantitative real-time PCR). Furthermore, to demonstrate that the senescence markers expression is related to LINE-1 expression, we performed an *in vitro* chemical transfection of *LINE-1* expressing vector in these keratinocytes, and measured the expression of senescence markers after 1 to 5 days of *LINE-1* overexpression by RT-qPCR.

Conclusion: Our preliminary results show that the UVs radiation induces the LINE-1 reactivation, DNA damage and that a long-term exposure leads to cell senescence and aging. With our more recent experiments, we aim to show that this senescence is the consequence to LINE-1 reactivation. As a long-term aim, we would like to create organotypic 3D Skin cultures as an in-vitro model to study the efficiency of different sun creams in protecting against *LINE-1* reactivation and senescence upon UVs exposure. Finally, the findings of our experiments can provide critical evidence to help discourage people from exposing themselves to the sun and may foster the development of alternative treatments.

POSTER #58 - Methods for Separating and Characterizing Extracellular Vesicles Contributing to Atherosclerotic Plaque Instability

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Introduction: Atherosclerosis, a major cause of stroke, is the formation of a plaque in the carotid artery that feeds blood to the brain. The two main categories of plaques are stable and unstable, whose compositions are different, however, their formation remains unknown. The release of extracellular vesicles (EVs) from plaque cells can possibly lead to the development of plaque instability. EVs, which are cell-derived vesicles, act as messengers to exchange intracellular content between cells. Interestingly, patients with cardiovascular events have been associated with an elevated level of EVs in plasma. Yet, the role of EVs in the context of atherosclerotic plaque composition and instability remains to be investigated. Herein, we aim to compare and identify the best techniques to isolate and characterize EVs from stable and unstable atherosclerotic plaques obtained from men and women with severe carotid atherosclerotic disease.

Methods: Plaque samples were surgically removed from patients who were clinically indicated to undergo carotid endarterectomy. Plaque stability was assessed based on histological analysis performed by vascular pathologists. Plaque was enzymatically digested to create a single cell suspension. The supernatant was collected and split into samples to test different EV isolation techniques. The enzymes used were collagenase (I, III, and IV), dispase, elastase, DNase, and hyaluronidase to dissociate the collagen and fibrin within the plaque. The isolation techniques of EVs that will be compared are ultracentrifugation, size exclusion chromatography (SEC), and ultrafiltration combined with SEC. The characterization techniques conducted will be Nanoparticle tracking analysis (NTA), Western blot, Cytoflex, and electron microscopy.

Expected Results: The expected result is that ultrafiltration combined with SEC will perform better than ultracentrifugation and SEC alone. Ultrafiltration uses a porous membrane to separate particles by size, allowing EVs to separate from large or small particles. Subsequently, the sample is loaded on a column for SEC, which is highly effective in removing soluble protein and small impurities. This technique has proven to achieve higher yield in other specimens of EV and better preservation of biophysical properties of EVs compared to ultracentrifugation, alongside a lower protein/vesicle ratio, leading to higher purity. Cytoflex is expected to yield the best characterization results. It uses fluorescence to detect the vesicle number, size, and molecular cargo at the single EV level with high specificity. Furthermore, unstable plaques are expected to contain higher EV content than stable plaques, as stable plaques contain a large fibrous cap and less inflammatory cells, less likely to produce inflammatory cell derived EVs. However, unstable plaques have a larger necrotic core containing more inflammatory cells, and are prone to rupture leading to thrombosis, resulting in a higher production of procoagulant and inflammatory cell derived EVs.

Discussion: The goal of this project is to compare and contrast the different separation techniques to understand which will give a better yield and purity of EVs and compare and contrast the characterization techniques to appreciate EVs as accurate and precise as possible, from its size, concentration, and cargo. This project would lead to comprehend how EVs contribute to the development of plaque instability.

POSTER #59- Validation of protein interaction with cytoplasmic C-terminal domain of claudin 8 and its variants

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With 24 unique members in vertebrates, claudins are one of the main components of tight junctions, which play an important role in maintaining cell polarity and in controlling molecular diffusion through the paracellular space. Members of the claudin family share common characteristics of tetraspan proteins: two extracellular loops and intracellular N- and C-terminal domains. Of note, the C-terminal domain is known to interact with different cytoplasmic scaffolding proteins and components of the actin cytoskeleton. Previous projects in our lab showed that the removal of Cldn3, -4, and -8 during neural tube formation in chick embryos disturbs neural tube closure. Additionally, overexpression of human Cldn3 and Cldn8 variants in chick embryos causes neural tube defects. To explain the relationship between claudins and neural tube formation, a former Ph.D. student of our lab used embryo extracts to perform GST pull-down experiments and identified a range of different intracellular proteins that are bound to the C-terminal tails. In this project, I am validating the ability of these candidate partners to interact with claudins. Specifically, I will validate the interactions with wild-type Cldn8 and four cldn8 variants, two of which cause neural tube defects when overexpressed in chick embryos and two that do not. I am performing GST pull-downs followed by western blot analysis. Immunofluorescence will be used to examine the protein localization in neural ectoderm in vivo. Validation of the candidate interaction partners of the Cldn8 C-terminal domain and its variants will contribute to our understanding of the function of Cldn8 during neural tube closure.

POSTER #60 - Genotype-phenotype correlations in peroxisome disorders: D-bifunctional protein deficiency

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Introduction and objectives: D-bifunctional protein (DBP), encoded by *HSD17B4* gene, plays an essential role in fatty acid metabolism by catalyzing the second (hydratase activity) and third (dehydrogenase activity) steps of β-oxidation of fatty acids and fatty acid derivatives in peroxisomes. Individuals with DBP deficiency (DBPD) can manifest a wide spectrum of disease symptoms and severities, including cerebellum ataxia, sensorineural hearing loss, vision loss, neuromuscular hypotonia, and developmental delay. Up until now, DBPD has been classified in four different groups, based on whether the hydratase and/or dehydrogenase units of DBP are affected, and this is thought to predict overall disease severity and lifespan. However, it is not clear whether these different groups of DBPD are associated with different overall disease severities. Thus, in this research project, we aim to verify if this classification holds true, or if it is the severity of the mutations, rather than the specific DBP enzymatic units affected, that is associated with the severity of the disease. To address this, we have conducted a medical record review DBPD patients, modelled the effects of their mutations onto DBP 3D protein structure, and evaluated possible correlations between clinical severity and protein structure predictions.

Methods: We reviewed medical records from 16 patients with DBPD enrolled in our Natural History Study on Peroxisomal Disorders (*ClinicalTrials.gov* Identifier: NCT01668186) and used the publicly available crystallized DBP structure and Chimera protein visualization software to model the *HSD17B4* missense mutations found in our patients.

Results: From the medical records available in our patient cohort, we collected information on longitudinal clinical course, including clinical findings, medical interventions and tests, imaging, laboratory, and molecular findings. From this information, we classified our patients into severe (n=6), intermediate (n=7) and mild (n=3) disease severities. We have found that the severe group had neonatal seizure and hypotonia, earlier onset of hearing and vision loss, and abnormal VLCFA level, compared to the intermediate and mild patients who manifest their symptoms at a later stage of life. In addition, we simulated missense mutations (n=13) in *HSD17B4* found in our patients on DBP protein structure using Chimera. We performed prediction analyses to evaluate whether interactions, clashes, H bonds, hydrophobicity and density of the modified residue and its surroundings were affected. We will use these results to predict the severity of our patients' mutations on DBP structure and function.

Future work: In the second part of this summer research project, we will perform correlation analyses between the severity of clinical findings (including symptoms and levels of biochemical markers) in our DBPD patients and the severity of their mutations on DBP structure and function. We hypothesize that our data will allow to determine if it is the current severity classification (based on location of mutations only), or the consequences of the mutations on the protein structure (regardless of their locations), that accurately predicts overall disease severity in DBPD. The results from this study will inform care management guidelines for DBPD patient based on their genetic results and improve the clinical understanding of DBPD.

POSTER #61 - Defining the role of prolactin as a regulator of cell cycle arrest/progression in hormone receptor-positive (HR+) breast cancer cells

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Background: While mainly being known for its role in lactation and development of breast tissue, prolactin (PRL) is proving to be essential in the regulation of breast cell proliferation and differentiation. Breast cancer cells of the most differentiated HR+ breast cancer subtype, lines, such as MCF-7, are known to possess the prolactin receptor (PRLR) and are characterized by less aggressive features such as more differentiated traits and slower/more organized cell division. Evidence suggests the importance of ubiquitination in the progression and regulation of cellular division. Targeting specific proteins for degradation such as CDKs and/or Cyclins can precisely arrest or maintain the progression of mitosis. Here, we hypothesize that the PRL/PRLR signalling pathway suppresses breast cancer proliferation via the induction of cell cycle arrest.

Methods: Immunofluorescence and Western blots were performed to assess the correlation between the stimulation of the PRLR by PRL and the number of ubiquitinated proteins circulating in MCF-7 cells. The role of PRL in regulating the progression of the cell cycle in MCF-7 cells will be observed by Fluorescence-activated cell sorting (FACS). MTT cell-proliferation assay will also be performed using MCF-7 cells to assess the contribution of PRL in inhibiting the proliferation of breast cancer cells.

Results and conclusions: This project will define the role of PRL in the progression of the cell cycle in MCF-7 cells. Data obtained by immunofluorescence, western blotting, FACS analysis and MTT cell-proliferation assays would highlight the importance of PRL/PRLR in maintaining normal mitosis and further support an anti-tumorigenic role of PRL in breast cancer.

POSTER #62 - Development and characterization of a murine organoid-derived model of colorectal cancer

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Background: Colorectal cancer (CRC) is the second leading cause of cancer malignancies worldwide with annual deaths reaching 1 million. The increasing prevalence and risk of CRC prompt more advanced therapeutic research to improve prognosis and prolong survival. *In vitro* tumour models have been widely used for drug screening platforms and provide extensive elucidation of the mechanism of tumour growth and metastasis. Immortalized two-dimensional (2D) cancer cell lines originating from resected tumour tissues have been the primary method to generate *in vitro* cancer models due to the low handling cost and convenience of use. Despite such benefits, 2D cell lines fail to recapitulate the *in vivo* parental tumour and unnatural growth on monolayer surface, hence resulting in poor reproducibility in the clinical setting. Recent advancement of three-dimensional (3D) multicellular organoid promises new opportunities for cancer research and drug discovery due to its capability to resemble *in vivo* tumour physiology, heterogeneity, and genetic identity. Derived from patient's tumour tissues and supplied with medium mimicking the original tumour environment, *in vitro* organoid culture could provide higher accuracy and sensitivity towards drug treatment and address the gaps between cancer genetics and patient trials.

Recent progress and objectives: Many studies have shown that aberrant activation of *KRAS* as well as deletion of the *APC* and *TP53* tumour suppressor genes are key drivers of CRC progression. To establish a murine model of CRC, our lab has generated mice carrying the Cre deleter line Villin-CreERT crossed to Apcfl/fl, Trp53fl/fl, and lox-Stop-lox-KRasG12D alleles (AKP mice). Tamoxifen administration of these mice allows for Cre-dependent deletion of Apc, p53, and overexpression of oncogenic Kras throughout the intestinal epithelium. From these mice, we have recently generated AKP organoids from both the small and large intestines. Our objective for this research project will be to:

1. Functionally characterize the gene expression and growth characteristics of AKP organoids relative to wild-type organoids.
2. Employ a lentiviral-based CRISPR-Cas9 delivery system to knockout Smad4 in AKP organoids in order to study the contribution of TGF-β signalling in CRC.
3. Assess the sensitivity of AKP organoids to novel chemotherapeutic compounds in collaboration with Dr. Danuta Radzioch (RI-MUHC).
4. Perform preliminary experiments to generate an *in vivo* model of CRC liver metastasis through intrasplenic and/or orthotopic engraftment of AKP organoids, hence generating tailored CRC mouse models for further drug testing and clinical investigation.

Conclusions: The development of AKP organoids will provide a unique model to investigate the stepwise processes underlying CRC metastatic progression, as well as a platform for anti-cancer drug testing.

POSTER #63 - MEG3 as potential tumor suppressor gene in gastrointestinal stromal tumors

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Introduction: Gastrointestinal stromal tumors (GISTs) are rare tumors that arise from the interstitial cells of Cajal. These tumors can occur anywhere in the gastrointestinal tract (most frequently in stomach and small intestine), and gain-of-function mutations in the *KIT* or *PDGFRA* genes are almost universal oncogenic driver events in these tumors. GISTs are also characterized by frequent loss of chromosomes 14, 1p, 22, and 15. In this project, we sought to identify candidate tumor suppressor that may be affected by these frequent chromosomal losses.

Methods: We profiled DNA copy number alterations, DNA methylation and RNA expression of 59 primary untreated GISTs to identify potential tumor suppressor genes affected on chromosomes 14, 1p, 22 and 15. We validated selected findings by *in situ* hybridization on tissue microarrays with fully annotated 225 primary untreated GIST specimens. For selected targets, we identified known target genes regulated by these tumor suppressors, and performed supervised clustering and correlation analysis to identify possible genes affected by the candidate tumor suppressor genes.

Results: Chromosome 14 loss was the most frequent DNA copy number alteration in our cohort. This aberration was detected in 49% (n=27) of cases with DNA copy number data available. In this study we focused on candidate tumor suppressor genes that may be affected on this chromosome. Joint analysis of DNA copy number data, RNA-seq data and DNA methylation data pointed to two candidate tumor suppressor genes that are downregulated in cases with loss of chromosome 14: long non-coding RNA *MEG3* and *DICER1*. By *in situ* hybridization, complete loss of *MEG3* expression was observed in 131 cases (58%), and partial loss was observed in 50 (22%) of cases. Loss of *MEG3* expression was significantly associated with gastric origin of the tumor (two-sided Chi-square test p=0.0024) and low/moderate risk for metastasis (two-sided Chi-square test p = 0.0038).

We identified 35 target protein-coding genes regulated by *MEG3* using LncRNA2Target and LncTarD databases. Eighteen of these genes are known to be downregulated and 17 genes are known to be upregulated by *MEG3* in other types of cancer. We calculated Pearson correlation between the expression levels of these genes and MEG3 in GISTs, and we identified *Bax, BMP4,* and *FOXO1* as the genes with the highest correlation with *MEG3*. *Bax* showed negative correlation with *MEG3* ($r = -0.49$, $p < 0.001$), and *BMP4* and *FOXO1* showed positive correlation with *MEG3* ($r = 0.33$ and $r = 0.37$, respectively; $p < 0.01$).

Conclusions: Our results show that expression of *MEG3* is frequently lost in GISTs, especially in gastric cases and in primary tumors with low/moderate risk of metastasis. This suggests that *MEG3*-associated molecular program may be activated in GISTs of smaller size and lower mitotic activity, and may play a role in the initial phase of tumor growth. We also identify *Bax, BMP4* and *FOXO1* as the potential target genes regulated by *MEG3* in GISTs. This study offers one of the first in-depth characterization of *MEG3* lncRNA expression in GISTs, and warrants further studies on the role of this tumor suppressor gene in these tumors.

POSTER #64 - Exploring the Gap: A Survey of the Digital Divide at the Montreal Children's Hospital

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Introduction: Mobile health apps have gained popularity for patient care and communication. However, inequitable access to digital technology (the so-called "digital divide") risks widening health disparities if inclusivity is not a foremost consideration in the design of such tools. There is limited data on the digital divide among families of children treated in a tertiary care setting. The collected data will inform the design of an upcoming mobile health app, *Children's Journey*, at the study site.

Methods: We developed a survey to assess the level of internet access and smartphone use. This was specifically among caregivers visiting the pediatric surgical clinics and the emergency department at the Montreal Children's Hospital (MCH). We developed a questionnaire using experience-based co-design, through a series of structured team interactions with patient partners and lab group members, followed by cognitive testing and piloting. The survey included 3 domains: digital access, digital skills and familiarity, and a concept test for our upcoming mobile health app. Questions were adapted from various validated instruments. Paper surveys and QR-coded posters for online surveys were placed at the above sites in the hospital. Numerical data were analyzed descriptively and open-ended questions were thematically encoded.

Results: Of the 204 respondents, 2% (5) reported not having internet or smartphone access and 2% (5) of people with internet rated their connectivity as 'bad' or 'very bad'. The reason for not owning a smartphone or internet was primarily due to expenses followed by a lack of interest. Of the 5 people who did not own a smartphone, 3 had a member in their household who did own one, while all 5 were above the age of 45. Additionally, 13% (25) of participants were uncomfortable using their smartphones, 5% (11) were unaware of how to download an app, and 7% (15) did not know how to use any of the most common smartphone apps or features. When introducing our app in the concept test section of the survey, 95% (183) of participants affirmed that they would use our app if available and that it would be necessary for them and their families to have it. Of the 49 short text responses regarding potential app concerns, 31 included app security and confidentiality as a source of concern. Frequent app suggestions by participants included the ability for efficient communication with their physicians, appointment scheduling, task or appointment reminders, updated wait-room wait times, and most commonly, high user-friendliness and app simplicity.

Conclusions: Although most families reported sufficient internet access and digital comfort to use mobile health apps, some struggled with prohibitive costs and device access. Respondents highlighted the need for added user functionalities embedded within the app, and some reported concerns with the sharing of medical information. The study has informed our ongoing app development and has identified social and educational interventions for improving both local internet access and comfort with mobile health technology.

POSTER #65 - Histologic assessment of mTOR pathway hyperactivation in resected focal cortical dysplasia type II

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Background: Focal cortical dysplasia is a malformation of the cerebral cortical development that is associated with intractable epilepsy. Symptoms usually appear early in childhood, although they have been documented to occur as late as in the patients' sixties. It is the most common cause of drug-resistant epilepsy in children, and the most common epilepsy-associated cause for neurosurgery. FCD is classified in three types (I, II and III) and many subtypes based on histological findings and the presence or absence of accompanying lesions. FCD type II presents with both architectural and dysmorphic abnormalities, i.e. cortical dyslamination and dysmorphic neurons, plus or minus balloon cells (large eosinophilic cells showing signs of abnormal development and differentiation). Somatic mutations resulting in upregulation of the mTOR pathway have been found to underly FCD type II. In addition, mTOR dysregulation has been found in other intractable seizure disorders, such as tuberous sclerosis complex (TSC) and hemimegalencephaly (HME), which also show disorganized cortical lamination and cytomegaly of histology. Thus, these disorders together form a group called mTORopathies.

Objective: This study aims to compare the clinical and histological features of mutation-positive and mutation-negative FCDII patients. By doing so, we hope to advance our understanding of the pathogenesis of FCD type II.

Methods and Results: This retrospective study will be done on a sample of 14 patients with FCD type II who have undergone surgical resection of their lesions between 2015 and 2019 and have been screened for mTOR pathway genes mutations. Cortical samples, formalin-fixed and embedded in paraffin, have been retrieved from the pathology laboratories of the facilities where the surgeries took place.

Histological slides will be prepared from the patient's samples, after which six different immunohistochemistry stains will be performed: phosphorylated S6 protein as a downstream marker or mTOR pathway hyperactivation, neuronal nuclear antigen (NeuN) as a mature neuronal marker, neurofilament H as a dysmorphic neuron marker, vimentin (intermediate filament present during epithelial-to-mesenchymal transition) as a balloon cell marker, glial fibrillary acidic protein (GFAP) to mark astrocytes and finally, HLA-DR/DQ/DP as a marker of activated astrocytes. The mTOR hyperactivation will be analyzed semi-quantitatively by using a scoring chart, which will include the intensity of the staining as well as the proportion of cells stained within one cell line. By performing the mTOR pathway stain and the various cellular type stains on consecutive sections, we will also be able to evaluate in which cell lines the hyperactivation occurs. The inflammatory environment will be evaluated by highlighting the presence of activated astrocytes and microglia. The astrocyte marker being non-specific to activated astrocytes, we will use histological features to assess their activation.

Data will be extracted from the patients' charts for multiple variables regarding their clinical presentation. The clinical and histological features will be analyzed to try and determine the distinctive characteristic of mutation-positive and -negative patients. Results to come.

POSTER #66 - Morphological and gap-junction characterization of developing astrocytes in visual cortex

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Background: The astrocyte, a type of glial cell, is crucial in a variety of essential central nervous system functions, including metabolic support, and synaptic transmission. Studies have shown that astrocytes may play a role in regulating spike-timing dependent plasticity (STDP) through the tripartite synapse model where astrocytes bidirectionally communicate with pre- and post-synaptic cells, which remains to be further explored. It also remains unclear how different aspects of astrocytes change throughout development. My project aims to characterize astrocytes over development based on their gap-junction and morphological properties.

Methods: Acute brain slices were obtained from postnatal day 3 (P3) to P30 wildtype C57BL/6J mice. After incubation in Sulforhodamine 101 (SR101), a synthetic dye that specifically stains astrocytes, astrocytes in L5 of the visual cortex were patched under 2-photon (2p) microscopy. The astrocytes were filled with internal solution containing biocytin via a patch pipette. The slices were then immunohistochemically stained with Alexa Fluor-conjugated streptavidin and imaged with confocal microscope to acquire images for gap-junction coupled cell counting and morphological reconstruction over development. Additional images acquired from 2p imaging were pooled into the morphometry dataset, as well as for SR101 cell counting to analyze astrocyte density across development.

Results: Gap-junction coupling of neighbouring astrocytes increased over development. Sholl analyse on reconstructed astrocytes, grouped into three age groups, P1-10, P11-20, and P21-30, demonstrated that astrocyte branch densities increased with age. Also, astrocyte branches extend further at earlier ages and the maximal branch extent shrinks when they get older. Astrocyte size was found to be stable across ages, even though cortical thickness increased with age. SR101 cell counting data illustrated that the astrocyte density per area remained stable, and the total number of astrocytes in the cortex was calculated to increase with age.

Conclusion: Our study adds insights to the investigation of the developmental profile of astrocytes, important for better understanding of the cellular and neuron-glia interactional properties of astrocytes. The next aims of our project will focus on exploring the role of astrocytes in STDP.

POSTER #67 - Clinical, Radiological, Etiological, and Genetic Features in a Cohort of 70 Patients with Schizencephaly

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Background: Schizencephaly is a rare congenital brain malformation, characterized by a cleft in the cerebral hemisphere lined in abnormal gray matter, that can manifest in a wide range of neurological problems, from epilepsy to motor and cognitive impairments. Due to its rarity (with an estimated incidence of 0.54 to 1.54 per 100,000 live births), the literature on schizencephaly is sparse with most reported studies being only case reports or small case series with mainly pediatric patients.

Objective: The aim of this study is to characterize the clinical, radiological, and genetic features of individuals with schizencephaly, in order to determine the etiologies as well as the specific imaging and clinical features that may help predict patient neurodevelopmental and epileptic outcomes.

Methods: We perform a retrospective cohort study of one of the largest groups reported with 71 individuals that received radiological confirmation of schizencephaly from 1998 to 2020 at the Montreal Children's Hospital, Montreal Neurological Institute, Centre Hospitalier Universitaire (CHU) Sainte-Justine, and Gaslini Children's Hospital (Genoa, Italy). We are reviewing patient charts for pregnancy and perinatal risk factors (in utero CMV infection, and substance use), epilepsy traits (age at onset, seizure type, electrophysiological features, treatment, and response), neurodevelopment characteristics, detailed radiological findings (localization, cleft type, and associated brain malformations), and genetic results (Array CGH, karyotype, and COL4A1). These aforementioned variables will then be compared to imaging features using logistic regression analyses in order to identify any correlations between a patient's radiological characteristics, prenatal risk factors, genetic traits, and clinical course. Then, these results will be contrasted with the existing literature.

Results: This cohort includes 31 children and 36 adults, of which 25 are biological males and 46 are females. To date, data collection has been completed for 17 patients. Several risk factors associated with schizencephaly are also commonly reported in this cohort, including young maternal age, monochorionic pregnancies, maternal health problems during pregnancy, lack of adequate prenatal care, substance use during pregnancy, and premature deliveries. Furthermore, the current results also suggest that patients with unilateral and closed clefts have the best neurodevelopmental and epileptic outcomes.

Conclusions: The correlations between imaging characteristics, outcomes, genetic features, and etiological factors may shed light on the application of these factors as predictors of developmental and clinical outcomes, as well as the determination of the etiology of the disease. Moreover, the large number of adult patients included in this cohort allows for a better understanding of the long-term impact of the complex symptomatology highlighting the essential points to consider in managing schizencephaly patients.

POSTER #68 - Validation of Interaction Partners of the Claudin Subtypes Cytoplasmic Tail

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Tight junctions are found between epithelial cells and are essential for paracellular transport of ions and other small molecules. The main component of tight junctions, claudins (Cldn), are a family of tetraspan proteins that contain 2 extracellular loops, one that controls the selectivity of ion passage and a second that mediates transdimerization with claudins on adjacent cells. Additionally, the cytoplasmic C-terminus tail of claudins is known to interact with other protein families, such as cytoskeletal proteins, scaffolding proteins, and proteins responsible for post-translational modifications. Claudins are present from early in development and have been found to be essential in forming the neural tube, the structure that gives rise to the brain and spinal cord. Specifically, claudin-depleted embryos show defects in apical constriction of individual cells and convergent extension of the epithelial sheet, resulting in open neural tubes. However, the selective removal of individual claudin subtypes elicit phenotypes with varying degrees of severity. In previous lab experiments, selective Cldn3 and Cldn8 removal with C-CPE showed the most severe phenotype, with failure of neural tube closure in all treated embryos. However, selective removal of Cldn4 showed overexpression of Cldn8, but a fully closed neural tube. Therefore, the difference in phenotype between Cldn subtype removal suggests unique interaction partners for each Cldn subtype in neural tube development. Through mass spectrometry of GST-fused Cldn tails incubated with embryo extract, interaction candidates for Cldn1,3,4,8, and 14 were identified. This project aims to validate these findings by performing a GST pulldown assay of GST bead-fused claudin tails incubated with embryo extract, allowing for the isolation of Cldn-bound interaction partners. My preliminary data show that claudin subtypes preferentially interact with unique partners, and we hypothesize that this accounts for the variation of phenotype upon each claudin's selective removal from the tight junction. Validation of these interaction partners can help understand the genetic causes of neural tube defects and allow for the development of therapeutics targeting claudin-associated diseases.

POSTER #69 - Development of an in vitro colonoid system to examine inflammatory memory in intestinal stem cells

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The intestine acts as dynamic immunological interface between the internal milieu and the external environment. This surveillance is mediated by traditional immune cells working in concert with non-traditional immune regulators such as epithelial cells to maintain a robust protective barrier. The vast diversity of environmental stimuli requires gut epithelial cells to respond broadly, yet effectively. This responsiveness may be accomplished through malleable epigenetic changes to intestinal stem cells (ISCs) that imprint a “memory” of historical inflammatory stimuli. As a result, ISC-derived progeny may become more “fit” to tolerate future insults. However, the factors responsible for inducing inflammatory memory in ISCs are only beginning to be identified. To fill this knowledge gap, we have developed an ex vivo organoid system wherein primary ISCs are grown in culture to mimic the in vivo morphology of the gut epithelium. This highly-controlled system allows for interrogation of factors that may directly imprint memory-like activity on epithelial cells as well as changes in gene expression and morphology. To test the ability of immune-derived factors in regulating inflammatory memory responses, we focused on interleukin (IL)-17, a cytokine that can paradoxically wield protective or pathogenic activity in a context-dependent manner during intestinal inflammation. Using our reductionist approach, colon-derived organoids were stimulated with recombinant IL-17 and, at various time points after challenge, gene expression analysis was performed. We observed that IL-17 stimulated robust induction of anti-microbial factors similar to previous reports. We are now using this system to examine the memory response to different secondary challenge and parse the protective versus pathogenic functions of IL-17 on the intestinal epithelium. These results may help inform clinical strategies to seek to improve intestinal resilience across the lifespan.

POSTER #70 - Utilizing Synchronous Healthcare Delivery to Optimize the Use of Guideline Directed Medical Therapies in Patients with Type 2 Diabetes: Results From the DECIDE-CV Clinic

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Introduction: The high burden of comorbidities among patients with Type 2 Diabetes (T2DM) may contribute to the low use of guideline directed medical therapies (GDMT) that improve cardiovascular outcomes, including sodium glucose cotransporter-2 inhibitors (SGLT2i) and glucagon-like-peptide-1 receptor agonists (GLP1RA).

Hypothesis: The DECIDE-CV clinic at McGill University (Montreal, Quebec, Canada) is a novel synchronous healthcare program whereby patients with T2D are assessed at each visit simultaneously by a cardiologist, endocrinologist, and nephrologist to enable rapid GDMT implementation. We hypothesized that synchronous healthcare delivery would increase SGLT2i and GLP1RA use among multimorbid patients with T2D.

Methods: We conducted a pre/post analysis of GDMT use throughout patient follow-up in the DECIDE-CV clinic. We evaluated the first 76 patients from 2020-10-26 to 2022-04-18 and used Canadian diabetes/ cardiovascular guidelines and provincial medication coverage criteria to assess eligibility for SGLT2i and GLP1RA. A 2-sample test for proportions compared use of GDMT at baseline and follow-up.

Results: At baseline, the mean age of patients was 68.5 years old, 79% were male, 33% were non-white minorities, 50% had chronic kidney disease, 64% had heart failure, 34% had history of prior stroke/transient ischemic attack and 58% had atherosclerotic cardiovascular disease. The median eGFR was 60.1 ml/min/1.73m^2 (IQR 40.7, 93.8), median NT-proBNP was 434 (IQR 123, 1425), and median HbA1c was 7.3% (IQR 6.8, 8.7). At baseline only 37% were prescribed a SGLT2i and 3% for GLP1RA despite being guideline eligible and having medication coverage. After the first visit, the use of therapies significantly increased to 90% for SGLT2i and 39% for GLP1RA. At the end of follow-up period of 122.25 days (± 100.5 days), 98% were prescribed a SGLT2i and 57% were prescribed a GLP1RA (P-value comparing proportion GDMT < 0.001; Figure 1).

Conclusions: Among patients eligible for GDMT, the initial use of SGLT2i and GLP1RA was low. Our model of synchronous healthcare delivery, in a multi-comorbid and diverse population, significantly increased the use of SGLT2i and GLP1RA.

POSTER #71 - Protein-protein interaction mapping of LIMD1 and CdGAP in HEK293 cells

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Background: Triple negative breast cancer (TNBC) accounts for approximately 15-20% of all breast cancers and it is characterized by the lack of expression of estrogen receptors, progesterone receptors and human epidermal growth factor receptor 2 (HER2). TNBC is therefore very hard to treat due to the lack of these receptors, and there are currently few successful treatments. It has been seen previously that the Rac1/Cdc42 GTPase-activating protein, CdGAP, is overexpressed in breast cancer patients, particularly in TNBC patients, and this overexpression is associated with poor patient outcome. Conversely, depletion of CdGAP can inhibit primary tumor growth and lung metastasis *in vivo*. More recently, the GAP-independent and transcriptional activities of CdGAP via interactions with other proteins are being studied further. Once such protein interaction is with LIMD1, which is a novel CdGAP interactor that was discovered by proteomic analysis. LIMD1 is a tumour suppressor gene that is downregulated in breast cancer and which has a wide variety of roles in cells, such as actin regulation and scaffolding at focal adhesions and cell-cell junctions. LIMD1 consists of two main regions: the pre-LIM domain and the LIM region, which contains 3 LIM domains. It was previously determined that the R1172 residue located at the C-terminus of CdGAP is essential to interact with LIMD1, but it is unknown which region of LIMD1 binds CdGAP. The function of this interaction between CdGAP and LIMD1 is also currently unknown.

Objective: The purpose of this study is to characterize the interaction of CdGAP and LIMD1 by determining which region of LIMD1 is binding to CdGAP. This will provide greater understanding into the function of this interaction and why it may be significant in breast cancer.

Materials and Methods: In this study we will overexpress truncating mutants of Xpress-tagged LIMD1 such as LIMD1 WT, LIMD1 Δ1-467 or LIMD1 Δ472-676. HEK293 cells will be cultured and co-transfected with one of the three LIMD1 constructs and GFP-CdGAP. Cells will then be lysed for protein extraction. Co-immunoprecipitation will be performed to determine which region of LIMD1 interacts with CdGAP and the protein interaction will be analyzed by western blot.

Anticipated Results/Conclusion: We hypothesized that the LIM domains of LIMD1 interact with CdGAP, based on previous findings that another LIM domain-containing protein, Ajuba, interacts with CdGAP via its LIM domains. These results will aid in further characterizing the interaction between CdGAP and LIMD1, which may provide insight into the purpose of their protein interaction. As both proteins are known to be found at focal adhesions, we can speculate that their interaction may be important for controlling focal adhesion dynamics. These findings ultimately seek to aid the discovery of novel drug targets for TNBC and therefore lead to the development of more successful treatment.

POSTER #72 - Characterization of left ventricular function by three-dimensional echocardiography in neonatal encephalopathy

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Background: Despite therapeutic hypothermia (TH), many newborns with neonatal encephalopathy (NE) develop brain injury/mortality. These neonates often present with cardiac dysfunction, considered to be associated with adverse outcomes. An enhanced understanding of their cardiac alterations by three-dimensional echocardiography (3D-ECHO) may help identification of cardiovascular-related targets to further improve their management and outcomes.

Objectives: To describe the left ventricular (LV) function by 3D-ECHO of NE newborns compared to age-matched healthy newborns. To describe the evolution of these parameters in time as well as their association with blood cardiac markers (Troponin and Creatine Kinase [CK]) obtained on the same day as the 3D-ECHO.

Study Design: This is a single-center prospective study of neonates with moderate or severe NE on amplitude integrated electroencephalogram treated with TH between October 2019 and November 2021. All had a research 3D-ECHO performed on day of life (DOL) 2 (during TH). Those with confirmed brain injury also had serial ECHO scans performed on DOL 3 (during TH), DOL 4 (after TH) and DOL 10. Brain magnetic resonance imaging (MRI) were performed on DOL2 and 10 and were scored by a masked neuroradiologist. 3D-ECHO analysis was performed by a masked trained extractor on TomTEC Arena software (LV package). Cardiac biomarkers were measured on the same day as the 3D-ECHO. Generalized linear mixed effect models with repeated measures were used.

Results: Of 120 neonates undergoing TH during this period, 16 were included and 10 had brain injury on MRI. Demographic and clinical characteristics were similar between groups (table 1). LV end-diastolic volume was higher in neonates with brain injury (4.6 [1.2] vs 3.4 [0.9] mL; p=0.04) – Table 2. A higher stroke volume was observed in the same group (1.8 [0.5] vs 2.5 [0.7] mL; p=0.047). Higher peak global circumferential strain (pGCS) was observed in neonates with brain injury (-26.6 [3.6] vs –21.3 [4.0]% ; p=0.01). With regards to trends in time (table 3), LV end-diastolic volume was found to be associated with DOL at evaluation (b=0.10 [0.01 - 0.19] mL increase for each DOL; p=0.04), as well as stroke volume (b=0.600 [0.002 - 0.120] mL increase for each DOL; p = 0.40) and left ventricle muscle mass (b=0.112 [0.001 - 0.224] grams increase for each DOL; p = 0.049). When correcting for day of life at evaluation, we found an association between pGCS and same day troponin levels (b=-2.71 [-4.92 - -0.49] ng/L; p=0.017), as well as CK levels (b=-3.02 x 10-4 [-4.12 x 10-5 - -5.62 x 10-4] U/L; p = 0.02). Similarly, the peak apical rotation (b=-2.24 [-4.41 - -0.70] degrees; p = 0.4) and the peak basal rotation (b=1.20 [0.04 - 2.36] degrees; p = 0.04) were associated with the same day blood troponin levels.

Conclusion: By comparing asphyxiated newborns with and without brain injury, we found differences in 3D-ECHO LV markers of function on DOL2. 3D-ECHO markers did increase with time and associated with same day cardiac biomarkers. Subtle LV alterations may contribute to ongoing brain injury in the postnatal setting of infants with NE.