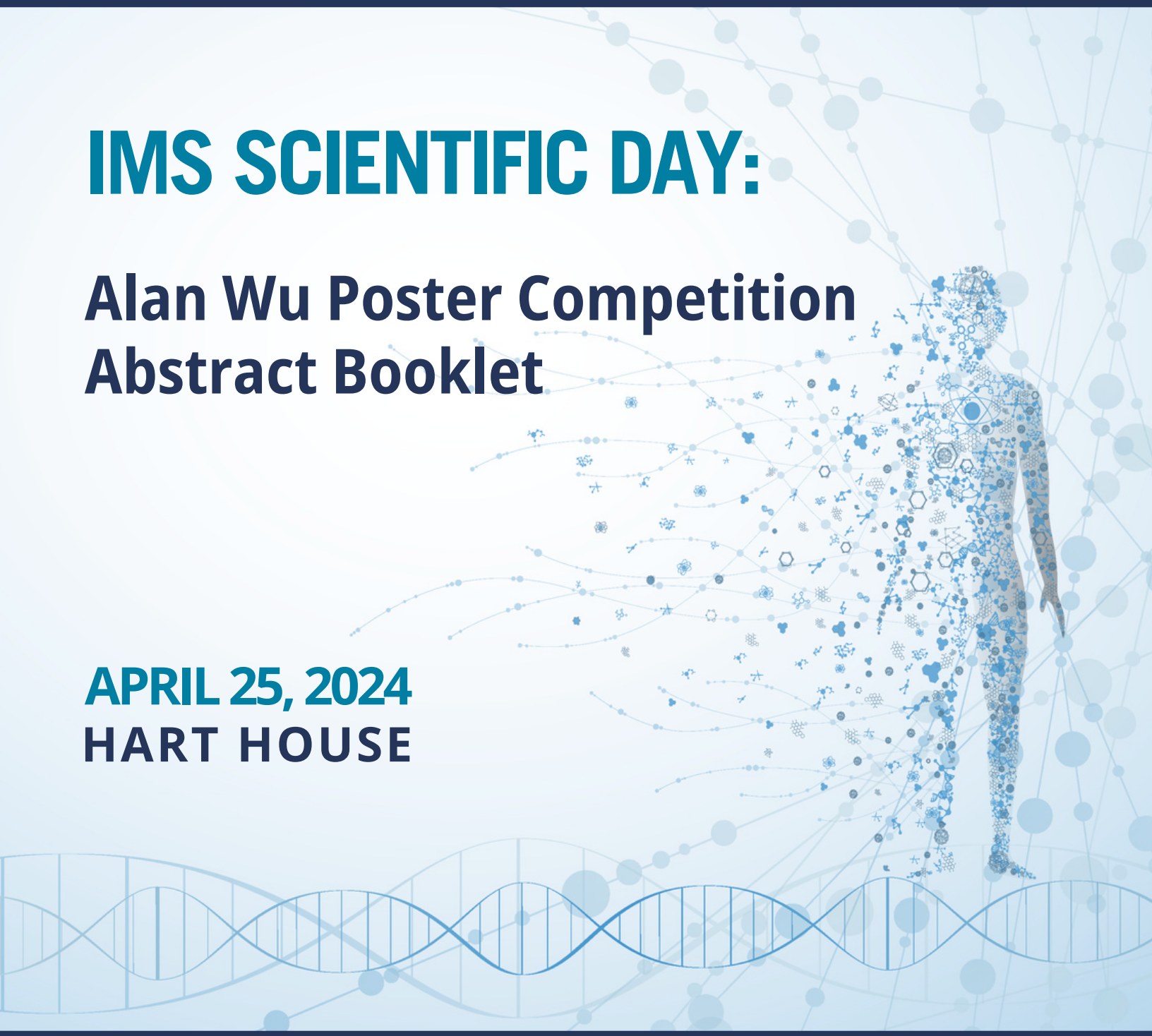
Institute of Medical Science
UNIVERSITY OF TORONTO

IMS SCIENTIFIC DAY:

Alan Wu Poster Competition Abstract Booklet



APRIL 25, 2024
HART HOUSE

Temerty Medicine

OVERVIEW

Agenda

8:00 – 8:30	**Registration & Breakfast**
8:30 – 9:45	**Alan Wu Poster Competition Session 1**
9:45 – 10:00	**Director's Report**
10:00 – 10:45	**Keynote Lecture**
10:45 – 11:30	**Research Panel**
11:30 – 12:45	**Alan Wu Poster Competition Session 2**
12:45 – 1:45	**Lunch Break**
1:45 – 2:45	**Laidlaw Competition**
2:45 – 3:00	**Coffee Break**
3:00 – 4:00	**Awards Ceremony**
4:00 – 5:00	**Reception**

Poster Locations & Judges

Topic	Group	Session & Room		Judges	
Neuroscience-Brain Health	A	1	East Common	Yat Man Tsang	Sara Sadat-Afjeh
Neuroscience-Brain Health	B	1	East Common	Alastair Flint	Lori Holden
Neuroscience-Brain Health	C	1	East Common	Neil Goldenberg	Michael Velec
Neuroscience-Brain Health	D	1	East Common	Alan Moody	Robert Grant
Neuroscience-Brain Health	E	1	Music	Monica Serban	Reinhart Reithmeier
Neuroscience-Brain Health	F	1	Music	Amy Boyle	Erica Vieira
Cancer	G	1	Music	Nicholas Neufeld	Chao Zheng
Cardiovascular-Respiratory-Musculoskeletal	H	1	Music	Julia Sorbara	Phyllis Billia
Endocrine/Gastroenterology	I	1	Debates	Erin Dickie	Linda Mah
Cancer	J	2	East Common	Jennifer Kwan	Nicolette Stogios
Infection-Immunology	K	2	East Common	Arun Tiwari	Sara Sadat-Afjeh
Cardiovascular-Respiratory-Musculoskeletal	L	2	East Common	Lori Holden	Michelle Nadler
Cardiovascular-Respiratory-Musculoskeletal	M	2	East Common	Andrew Sage	Samantha Anthony
Population Health-Education	N	2	Music	Victor Tang	Pushpal Desarkar
Population Health-Education	O	2	Music	Amy Boyle	Dmitry Rozenberg
Regenerative Medicine/Development	P	2	Music	Erica Vieira	Shree Bhalerao
Regenerative Medicine/Development	Q	2	Music	Yuliya Nikolova	Chao Zheng
Neuroscience-Brain Health	R	2	Debates	Shehryar Saharan	Katharine Dunlop
Other	S	2	Debates	Vanessa Goncalves	James Kennedy

Alan Wu Poster Groups

Session 1: 8:30 AM – 9:45 AM

Topic: Neuroscience-Brain Health

Name	Group	Poster Number	Location
Fellows, Elise	A	1	East Common
Pedro, Karlo	A	2	East Common
Kelardashti, Nikou	A	3	East Common
Falby, Madeleine	A	4	East Common
Clifford, Kevan	A	5	East Common
Cheng, Venice	A	6	East Common
Arbabi, Keon	A	7	East Common
Akbarian, Niki	A	8	East Common

Name	Group	Poster Number	Location
Dennis, Anthaea-Grace Patricia	B	1	East Common
Alhamdah, Yasmin	B	2	East Common
Lagacé, Micheline	B	3	East Common
Hamani, Michelle	B	4	East Common
Joghataie, Goldin	B	5	East Common
Mencia Ybarra Zavala, Marta	B	6	East Common
Baker, Kayla	B	7	East Common
Tassone, Vanessa	B	8	East Common

Name	Group	Poster Number	Location
Mehta, Dhvani	C	1	East Common
Demchenko, Ilya	C	2	East Common
Chandran, Ilakkiah	C	3	East Common
Kim, Esther	C	4	East Common
Ali Gami, Jasmine	C	5	East Common
Matin, Rafi	C	6	East Common
Li, Jerry	C	7	East Common

Name	Group	Poster Number	Location
Diaz, Joel	D	1	East Common
Sabac, Denise	D	2	East Common
Bartels, Hanne	D	3	East Common
Haikazian, Sipan	D	4	East Common
Buso, Chloé	D	5	East Common
Tamayo Velasquez, Valentina	D	6	East Common
Ma, Jennifer	D	7	East Common

Name	Group	Poster Number	Location
Laessing, Pamina	E	1	Music
Wang, Jessica	E	2	Music
-, Vittala	E	3	Music
Cameron, Avery	E	4	Music
Li, Janet	E	5	Music
Baer, Jenna	E	6	Music
Waye, Elizabeth	E	7	Music

Name	Group	Poster Number	Location
Kumari, Sonika	F	1	Music
Syed, Omer Ahmed	F	2	Music
Panganiban, Kristoffer	F	3	Music
Chen-Li, David	F	4	Music
Zhang, Molly	F	5	Music
Mahmood, Raesham	F	6	Music
Zaidi, Alina	F	7	Music
Snan, Lulia	F	8	Music

Topic: Cancer

Name	Group	Poster Number	Location
Wang, Justin	G	1	Music
Yuan, Baijiang	G	2	Music
Chehade, Rania	G	3	Music
Hanna, Lilian	G	4	Music
Trkulja, Kyla	G	5	Music
Zamani, Neda	G	6	Music
Corke, Lauren	G	7	Music
Pandya, Vishal	G	8	Music

Topic: Cardiovascular-Respiratory-Musculoskeletal

Name	Group	Poster Number	Location
Abdul-Samad, Karem	H	1	Music
Abrahim, Salma	H	2	Music
Vieira, Fernando	H	3	Music
Kim, Dorothy	H	4	Music
Khan, Hamzah	H	5	Music

Lee, Angela	H	6	Music
Fatah, Meena	H	7	Music

Topic: Endocrine/Gastronenterology

Name	Group	Poster Number	Location
Ahmad, Syed Zain	I	1	Debates
Bruce, Kyla	I	2	Debates
Yeung, Emily	I	3	Debates
Chan, Carmen	I	4	Debates
Ray, Prachi	I	5	Debates
Ganji, Niloofar	I	6	Debates
Novielli, Julia	I	7	Debates

Session 2: 11:30-12:45

Topic: Cancer

Name	Group	Poster Number	Location
Begum, Yeasmin Sultana	J	1	East Common
Maher, Abdula	J	2	East Common
Mau, Daniel Jing-On	J	3	East Common
Shariati, Sara	J	4	East Common
Plahouras, Christina	J	5	East Common
Aguiar, Stefan	J	6	East Common
Gandhi, Shreya	J	7	East Common

Topic: Infection-Immunology

Name	Group	Poster Number	Location
Fadlelmawla, Nahla	K	1	East Common
Kain, Taylor	K	2	East Common
Sebben, David	K	3	East Common
Amancio de Carvalho, Marcio Gabriel	K	4	East Common
Lees, Kaitlin	K	5	East Common
Mahassine, Ayoub	K	6	East Common
Aguilar, Fiorelle	K	7	East Common
Salvant, Elsa	K	8	East Common

Topic: Cardiovascular-Respiratory-Musculoskeletal

Name	Group	Poster Number	Location
Doherty, Christina	L	1	East Common
Ghosh, Sreemoyee	L	2	East Common
Alavi, Neeki	L	3	East Common
Gendron, Evelyne	L	4	East Common
Zhou, Xuanzi	L	5	East Common
Guo, Kunze (Mandy)	L	6	East Common
Deng, Mimi	L	7	East Common

Name	Group	Poster Number	Location
Teshler, Lizabeth	M	1	East Common
McCaig, Abby	M	2	East Common
Dang, Steven	M	3	East Common
Djahanpour, Niousha	M	4	East Common
Main, Kimberly	M	5	East Common
Fernandez Campos, Beatriz	M	6	East Common
Sibai, Jad	M	7	East Common

Topic: Population Health-Education

Name	Group	Poster Number	Location
Hafuth, Sowsan	N	1	Music
Saberian, Lillian	N	2	Music
Russell, Cayley	N	3	Music
Iturmendi-Sabater, Iciar	N	4	Music
Chandran, Anuijan	N	5	Music
Patel, Suhani	N	6	Music
Abdouh ,Daniya	N	7	Music

Name	Group	Poster Number	Location
Perivolaris, Argyrios	O	1	Music
Huang, Kiko Zi Yi	O	2	Music
Ip, Vanessa	O	3	Music
Sino, Sara	O	4	Music
Dozet, Danijela	O	5	Music
Traubici, Benjamin	O	6	Music
Mathews, Angela	O	7	Music

Topic: Regenerative Medicine/Development

Name	Group	Poster Number	Location
Sun, Jiahui (Angela)	P	1	Music
Hussain,Tafsia	P	2	Music
Mahmoud, Dina	P	3	Music
Sun, Yilin	P	4	Music
Calderon Novoa, Francisco	P	5	Music

Name	Group	Poster Number	Location
Maksimoska, Vida	Q	1	Music
Ricardo, Samantha	Q	2	Music
Costigan, Caoimhe	Q	3	Music
Hachem, Laureen	Q	4	Music
Gao, Fei Yu	Q	5	Music
Raleigh, Matthew	Q	6	Music

Topic: Neuroscience-Brain Health

Name	Group	Poster Number	Location
Paleczny, Sarah	R	1	Debates
Hassan, Omar	R	2	Debates
Ashworth, Kristen	R	3	Debates
Taha, Hiba	R	4	Debates

Topic: Other

Name	Group	Poster Number	Location
Moffat, Gordon	S	1	Debates
DeVuono, Isabella	S	2	Debates
Siddiqui, Salsabil	S	3	Debates
W Benjamin, Stephanie	S	4	Debates
Quddusi, Ayesha	S	5	Debates
Elsaid, Sonja	S	6	Debates
Jamal, Omer	S	7	Debates
Yakubov, Rebeca	S	8	Debates

Contents

ABSTRACTS

A: Neuroscience & Brain Health

A.1: Associations between C-reactive protein and individual symptoms of depression in a lower-middle income country

Student: Elise Fellows, Supervisor: Ishrat Husain

Background: Data on associations between inflammation and depressive symptoms largely originates from high income population settings, despite the greatest disease burden in major depressive disorder being attributed to populations in lower-middle income countries (LMICs).

Purpose & Hypothesis: The purpose of this work is to assess the prevalence of low-grade inflammation in adults with treatment-resistant depression (TRD) in Pakistan, a LMIC, and to investigate associations between peripheral C-Reactive Protein (CRP) levels and individual depressive symptoms.

Methods: This is a secondary analysis of two randomized controlled trials investigating adjunctive immunomodulatory agents (simvastatin and minocycline) for Pakistani adults with TRD (N = 191). Logistic regression models were built to assess the relationship between pre-treatment CRP (≥ or < 3mg/L) and individual depressive symptoms measured using the Hamilton Depression Rating Scale. Descriptive statistics and regression analysis were used to assess treatment response for inflammation-associated symptoms.

Results: High plasma CRP (≥ 3mg/L) was detected in 87% (n = 146) of participants. Early insomnia (OR 2.33, 95% CI 1.16-5.25), early morning wakening (OR 2.65, 95% CI 1.29-6.38), psychic anxiety (OR 3.79, 95% CI 1.39-21.7), gastrointestinal (OR 0.38, 95% CI 0.14-0.86) and general somatic symptoms (OR 0.34, 95% CI 0.14-0.74) were associated with high CRP. Treatment with minocycline, but not simvastatin, improved symptoms positively associated with inflammation.

Conclusions: The prevalence of inflammation in this LMIC sample with TRD was higher than what has been reported in high income countries. Insomnia and anxiety symptoms may represent possible targets for personalized treatment with immunomodulatory agents in patients with elevated CRP. These findings require replication in independent clinical samples.

A.2: Machine learning-based cluster analysis identifies four unique phenotypes of degenerative cervical myelopathy patients with distinct clinical profiles and long-term functional and neurological outcomes

Student: Karlo M. Pedro, Supervisor: Michael G. Fehlings

Background: Degenerative cervical myelopathy (DCM), the predominant cause of spinal cord dysfunction among adults, manifests as a complex array of interrelated symptom and is characterized by considerable heterogeneity of clinical presentation and response to therapeutic intervention. Despite efforts to characterize the clinical presentation and functional course of patients with DCM, approaches to date have not been able to address the complex heterogeneity of these patients.

Purpose & Hypothesis: This study sought to use machine learning-based clustering algorithms to identify distinct patient clinical profiles and functional trajectories following surgical intervention. We hypothesize that in DCM, identifiable symptom clusters are present, potentially facilitating enhanced diagnostic precision and optimized therapeutic allocation within this patient cohort.

Methods: In this study, we implemented two clustering techniques to discern DCM subpopulations, utilizing aggregated data from three large DCM trials. Key covariates, including the Nurick score, NDI (neck disability index), neck pain, and motor and sensory scores, were employed for patient clustering through k-means and latent profile analysis (LPA). Various goodness-of-fit indices were evaluated to determine the optimal number of clusters. Outcome differences among identified phenotypes were assessed using ANOVA, followed by posthoc Tukey test. Multinomial logistic regression was utilized to identify significant predictors of group membership.

Results: A total of 1,047 DCM patients (mean [SD] age: 56.80 [11.39] years, 411[39%] females, 788[76.4%] Whites, with a mean [SD] symptom duration of 29.75 [46.20] months) had complete one year outcome assessment after surgery. Latent profile analysis identified four DCM patient phenotypes: “severe multimodal impairment” (n=286), “minimal impairment” (n=116), motor-dominant” (n=88) and “pain-dominant” (n=557) groups. Patients within these specific phenotypes exhibited a unique symptom profile and distinct trajectories of functional recovery. The “severe multimodal impairment group”, comprising of frail elderly patients, demonstrated the worst overall outcomes at one year (SF-36 PCS mean [SD]: 40.01 [9.75]; SF-36 MCS mean [SD], 46.08 [11.50]) but exhibited the most substantial neurological recovery after surgery (ΔmJOA mean [SD]: 3.83 [2.98]). The application of the k-means algorithm yielded a similar four-class grouping solution. A higher frailty score and a positive smoking status predicted membership in phenotype 1 (“severe multimodal impairment” group) (OR 1.47 [95%CI 1.07-2.02] and 1.58 [95% CI 1.25-1.99, respectively]), while undergoing anterior surgery and a longer duration of symptoms were associated with phenotype 4 (“pain-dominant”) group (OR 2.0 [95% CI 1.06-3.80] and 3.1[95% CI 1.38-6.89], respectively).

Conclusion: Unsupervised learning algorithms applied to DCM symptoms at baseline enabled the prediction of distinct patient phenotypes. The concept of symptom clustering, using multiple clinical and functional metrics, provides a valuable framework for uncovering novel DCM subpopulations, enhancing patient identification beyond the use of a single patient reported outcome measure such as the mJOA.

A.3: Brain-behavior relationships between alpha oscillations and behavioral phenotypes related to pain-attention interaction

Student: Nikou Kelardashti, Supervisor: Karen Davis

Background: Attention is one of the key factors influencing our experience of pain, yet the interaction between attention and pain is complex and not well understood. The Davis lab has previously defined two behavioral phenotypes related to pain-attention interactions. The first phenotype classifies individuals based on how pain impacts cognitive performance during an attention-demanding task. Individuals are designated as A-type (i.e., attention to task dominates) when they have better task performance (i.e., faster reaction time) during concomitant pain than in the absence of pain. Conversely, individual are designated as P-type (pain dominates) when they exhibit diminished task performance (i.e., faster reaction time) during concurrent pain compared to a no-pain condition. The second phenotype is determined based on an intrinsic attention to pain (IAP) score, which quantifies the degree to which a person attends to pain (high IAP) or mind-wanders away from pain (low IAP). MRI-based imaging has identified structural and functional brain features within the dynamic pain connectome (DPC)—a system that includes the ascending nociceptive pathway, descending antinociceptive pathway, default mode network, and salience network—in the A/P and IAP phenotypes. However, the fine temporal dynamics of brain activity is not discernible using the hemodynamic-based fMRI approach. An understanding of the brain dynamics that underlie pain-attention phenotypes can be obtained using magnetoencephalography (MEG) that has high temporal resolution to examine resting-state neural oscillations within the DPC with millisecond precision. The alpha oscillation band has been linked to attention. Peak alpha frequency (PAF)—the frequency in the alpha range (8-13 Hz) with the greatest power—has been linked to acute pain sensitivity and shown to be aberrant in individuals with chronic pain.

Purpose: The overall goal of this project is to explore brain-behavior relationships between alpha oscillations in the DPC and behavioral pain-attention phenotypes in healthy individuals.

Hypothesis: We tested the hypotheses that compared to A-type and low IAP individuals, P-type and high IAP individuals have 1) higher PAF power in the descending antinociceptive pathway and default mode network, 2) lower PAF power in the salience network and ascending nociceptive pathway, and 3) slower PAF.

Methods: Resting state MEG was acquired from 50 healthy individuals (age_{mean}±SD=26.9±6.4; 27F, 23M). Power spectra analyses were done for alpha band activity in regions of interest (ROIs) of the DPC. The A/P phenotype was designated based on cognitive interference task performance with and without concurrent pain. An IAP score was determined from an experience sampling task of mind-wandering away from vs attention to painful stimuli. A secondary analysis examined sex differences.

Results: There were no significant differences in PAF or PAF power between A- and P-type individuals. However, those with high IAP had significantly higher PAF power in the left thalamus compared to those with low IAP ($p=0.0112$, $d=-1.12$). Males with low IAP had higher PAF power than females throughout the DPC. There was no sex difference in PAF/power within A-, P-, or high IAP groups.

Conclusion: This study found that the tendency of individuals to mindwander away from pain is associated with PAF power in the thalamus, a key component of the ascending nociceptive pathway. The finding that males with a propensity to mindwander from pain have higher PAF power compared to females, highlights the importance of sex-specific considerations in understanding neural functions underlying the pain-attention interactions.

A.4: The collapse of degeneracy: how a loss of intrinsic biophysical diversity in deep subiculum pyramidal neurons paves the way for seizure activity

Student: Madeleine Falby, Supervisor: Taufik A. Valiante

Background & Purpose: A neurological insult has the potential to bring about an epilepsy phenotype, while at other times a healthy physiological state in the brain is preserved. This phenomenon is yet to be understood, presenting a challenge for the development of eective epilepsy therapeutics. The concept of degeneracy, the ability of distinct entities to compensate for one another and adapt to unpredictable changes, provides an elegant solution to explain the homeostatic protective mechanisms that maintain healthy functioning of the brain when threatened by epileptogenic insults.

Scientific Question & Hypothesis: However, a degenerate system requires heterogeneous components and may collapse when variability of these components is reduced. We, therefore, hypothesized that a loss of neuronal heterogeneity, a precondition to degeneracy, underlies the failure of homeostatic protective mechanisms leading to an increased vulnerability to epileptogenic insults and the emergence of seizure activity.

Methods: Using the kainic acid model of epilepsy, we investigated the heterogeneity of intrinsic biophysical properties of deep subiculum neurons, an essential participant in the propagation of seizure activity.

Results: By comparing whole-cell patch-clamp recordings, we found a significant loss of heterogeneity in the spike threshold property of subiculum neurons engaging in seizure-activity ($p < 0.0001$). This finding was not found to be influenced by inter-animal variability or accompanied by a significant difference in the mean threshold value.

Conclusions: The loss of heterogeneity in the threshold property suggests that deep subiculum pyramidal neurons are more selectively tuned to respond to a tight range of inputs. This may reflect a lack of flexibility to perturbations presented by seizure activity and explain the subiculum's role in propagating seizure activity. While the exact mechanisms underlying this loss of heterogeneity have yet to be investigated, this finding underscores a potential collapse of degeneracy that accompanies epilepsy.

A.5: Neurostructural effects of novel polygenic risk scores for molecular brain aging

Student: Kevan P. Clifford, Supervisor: Yuliya Nikolova

Purpose: Healthy and pathological brain aging are associated with distinct changes in brain structure. Abnormal trajectories of brain aging may, in part, be driven by deviations from typical gene expression patterns. Here, we evaluated the neurostructural effects of novel polygenic risk scores (PRSs) capturing shifts towards older-age-like expression in age-dependent genes (AGE-PRS) in a large non-clinical population.

Methods: Lists of age-dependent genes and corresponding cis-eQTL variants were based on in-house transcriptomic data from postmortem cortical tissue across the adult lifespan (n=209, 20-92y). Genetic and Freesurfer-extracted regional cortical thickness (CT) measures were obtained from the UK-Biobank (n=31,384, 44-73y). We computed 25 inter-related AGE-PRSs, comprising 123-2614 genes, based on 5 distinct thresholds for each gene's association with cis-eQTL variants nested within 5 thresholds for genes' associations with age. Linear regressions on regional CT (FDR-corrected across 62 regions) were conducted with PRS-AGE as the independent variable, controlling for demographics, 10 genetic components, and study site.

Results: The AGE-PRS comprising 725 age-dependent genes showed strongest phenotypic effects. It was associated with greater CT in the left precentral gyrus (pFDR<0.0001), left insula (pFDR=0.031), and right precentral gyrus (pFDR=0.041). Furthermore, across all PRS thresholds a distributed pattern of higher CT emerged in nominally significant regions, most frequently in the precuneus (n=15 AGE-PRSs), caudal middle frontal (n=13 AGE-PRSs), and superior temporal (n=8 AGE-PRSs) gyri.

Conclusions & Implications: Greater CT in frontotemporal regions co-occurring with genetic shifts toward older-age-like gene expression may reflect compensatory processes, or neurostructural phenotypes resembling those observed in major depressive disorder and initial stages of neurodegenerative disease.

A.6: Identifying TMS-EEG Neurophysiological markers using long interval cortical inhibition in treatment-resistant depression

Student: Venice Cheng, Supervisor: Daphne Voineskos

Background: About a third of individuals with major depressive disorder (MDD) do not respond to a standard dose of antidepressants. These individuals can be treated with a form of repetitive transcranial magnetic stimulation called intermittent theta-burst stimulation (iTBS) delivered to the left dorsolateral prefrontal cortex (DLPFC), with a 50% response rate. Long interval cortical inhibition (LICI) is a potential biomarker for responders to iTBS, as those with MDD have shown impaired inhibition.

Purpose & Hypothesis: The purpose of this study is to identify whether cortical inhibition could be a possible biomarker for iTBS treatment response by examining LICI in responders and non-responders pre- and post-treatment. We hypothesize that more inhibition will be present in responders at baseline, as inhibition can be an indicator of plasticity and enhanced response to treatment. We also hypothesize that LICI will show greater change post-treatment in responders when compared to baseline, given the deficits found in cortical inhibition in those with MDD.

Methods: 111 patients with MDD underwent TMS-EEG LICI measures before and after a course of iTBS. Responders had at least a 50% decrease in HRSD-17 scores post-treatment. LICI was elicited using a suprathreshold conditioning stimulus, followed by a suprathreshold test stimulus at 100ms intervals. The magnitude of LICI was determined using the ratio of peak-to-peak size of conditioned MEP over unconditioned MEP.

Results: A negative relationship was shown between response versus LICI at baseline (GLM: $F = -0.02$, $p = 0.020$), ethnicity (GLM: $F = -0.99$, $p = 0.037$), and sex (GLM: $F = -1.89$, $p = 0.025$). A significant change in LICI in responders from baseline to post-treatment was also found ($t(61) = -2.29$, $p = 0.0252$). A higher baseline LICI appears to be associated with non-response to therapeutic iTBS. Further analysis is required to determine the predictive value of LICI; however, these results reinforce the role of cortical inhibition as a marker of effective iTBS.

A.7: Sparse nonnegative matrix factorization identifies molecular subtypes of psychiatric and neurodegenerative brain disorders

Student: Keon Arbabi, Supervisor: Shreejoy Tripathy

Background: Psychiatric and neurodegenerative brain disorders are heterogeneous at the molecular level. Understanding how this heterogeneity may define patient-level differences is important for developing targeted therapies. Postmortem single-nucleus RNA-sequencing (snRNA-seq) cohort studies of major depressive disorder (MDD; PMID: 32341540, N = 71, 160k cells), schizophrenia (SZ; PsychENCODE, N = 140, 470k cells), and Alzheimer's disease (AD; ROSMAP, N = 414, 1.6M cells) provide the most detailed censuses of molecular changes in these disorders currently available.

Purpose & Hypothesis: snRNA-seq cohort studies will reveal distinct molecular subtypes of MDD, SZ, and AD.

Methods: For each dataset, we aggregated (pseudobulked) expression across cells from the same person and cell type, and subset to genes differentially expressed between cases and controls in each cell type. We then used sparse nonnegative matrix factorization (sNMF) to identify groups of genes (molecular signatures) dysregulated in groups of cases (patient subtypes). Molecular signatures were annotated (CHEA, GO, KEGG) and patient subtypes were associated with available demography (age, sex), clinical features (cognition, neuropathological burden), and genetics (variants, polygenic risk scores).

Results: Our results reveal cell-specific molecular signatures that are distinct between disorders, with the greatest subtype diversity observed in neuronal cells. In excitatory cells, 5 subtypes were identified in AD, 3 in SZ, and 2 in MDD. Notably, in AD, we identify a subtype strongly linked to TREM2 gene variants, neurogenesis, and cognitive decline. In all three disorders, multiple microglial subtypes are associated with inflammation, with a highly sex-specific subtype identified in MDD. Overall, our approach leverages the heterogeneity captured by snRNA-seq cohort studies to identify patient-level molecular subtypes linked with disease pathophysiology and clinical symptoms.

A.8: Genomic analyses of the relationship between neuroticism and cognitive decline in older adults

Student: Niki Akbarian, Supervisor: James L. Kennedy

Background: Mounting evidence suggests an association between neuroticism, the predisposition to experience intense and frequent negative emotions in response to sources of stress, and the risk of cognitive decline in older adults. Several theoretical models are postulated to explain the underlying mechanism of this relationship. Among these, the "common cause" theory proposes that neuroticism and cognitive decline may have a shared etiology, such as genetic variations that contribute to individual differences in both neuroticism and cognitive decline. However, the genomic relationship between neuroticism and susceptibility to cognitive decline remains an understudied area.

Purpose: This study aims to examine the relationship between genetic liability to neuroticism and cognitive decline in older adults.

Hypothesis: If the common cause model is true, the genetic liability to neuroticism will be positively associated with accelerated cognitive decline in older adults.

Methods: To test our hypothesis, data from a subset of UK Biobank participants (N = 11415, 4951 females, mean age = 63.4 (SD = 2.71)) aged 60 or above, with white British ancestry and no dementia history, were analyzed. Participants completed 4 cognitive tests of Fluid Intelligence for logic and reasoning, Symbol Digit Substitution for processing speed, Trail Making for executive function, and Pairs Matching for memory during the baseline (October 2014-April 2015) and the follow-up (February 2021-January 2022) assessments. To determine genetic liability of each participant to neuroticism, the polygenic risk score (PRS) of neuroticism was calculated using the summary statistic of neuroticism from Nagel et al. (2018) Genome-Wide Association Study and the clumping and thresholding method in Plink and R software. For statistical analysis, mixed effect modelling with "lmer" package in R software was utilized. Sex, age, baseline cognitive score, and first 10 principal components were added to the models as covariates. P values were adjusted via Tukey method and the significance threshold was set to $p<0.05$.

Results: Individuals with higher neuroticism PRS demonstrated lesser decline in the fluid intelligence score as the magnitude of decrease was significantly lower for individuals in the third quartile of neuroticism PRS than the second and the first quartile (3^{rd} quartile vs. 2^{nd} quartile: estimate=6.28×10^{-3}, $p<0.001$ | 3^{rd} quartile vs. 1^{st} quartile: estimate =1.26×10^{-2}, $p<0.001$ | 2^{nd} quartile vs. 1^{st} quartile: estimate =6.28×10^{-3}, $p<0.001$). Similar trend was observed for Symbol Digit Substitution (3^{rd} quartile vs. 2^{nd} quartile: estimate=1.32×10^{-2}, $p=0.002$ | 3^{rd} quartile vs. 1^{st} quartile: estimate =2.64×10-2, $p=0.002$ | 2^{nd} quartile vs. 1^{st} quartile: estimate 1.32×10^{-2}, $p=0.002$) and Trail Making (3^{rd} quartile vs. 2^{nd} quartile: estimate=-0.015, $p<0.001$ | 3^{rd} quartile vs. 1^{st} quartile: estimate=-0.031, $p<0.001$ | 2^{nd} quartile vs. 1^{st} quartile: estimate=-0.015, $p<0.001$) scores. However, no association was found between changes in Pairs Matching score over time and neuroticism PRS.

Conclusion: The results suggest that a higher genetic liability to neuroticism is related to a lesser decline in fluid intelligence, processing speed, and executive functioning over time in older adults. However, caution is warranted in interpreting these results due to limitations. Despite statistical significance, the effect sizes observed were small, thereby limiting the practical implications. Additionally, the sample comprised only individuals of British ancestry, potentially restricting the generalizability of the findings. Nevertheless, this study provides initial evidence suggesting a potential protective role of genetic predisposition to neuroticism against cognitive decline in older adults. Further research incorporating diverse populations is warranted to validate these findings.

B: Neuroscience & Brain Health

B.1: Identification of Parkinson's disease and mild cognitive impairment using neuroimaging and biofluid biomarkers: a study in the PPMI cohort

Student: Anthaea-Grace Patricia Dennis, Supervisor: Antonio P. Strafella

Background: Parkinson's Disease (PD) is a neurodegenerative disorder resulting in both motor symptoms, such as bradykinesia, rigidity, tremor, gait difficulties, and a variety of nonmotor symptoms, like cognitive impairment and behavioural complications. During PD progression, slowly progressive cognitive decline, termed mild cognitive impairment (MCI), may develop. PD and MCI have been explored with neuroimaging data biomarkers; however, relying on these biomarkers alone can sometimes be ineffective because of individual differences in brain activity. Thus, combining biofluid biomarkers, allowing also for proteomic differences, can help in a better biological definition of the disease.

Purpose & Hypothesis: Since current diagnostic tests focus on individual biomarkers, misdiagnoses can be frequent, thus resulting in inaccurate diagnoses. This research aims to combine neuroimaging and biofluid data as biomarkers for PD and MCI progression, with the goal of developing a more efficient method of predicting disease states and symptoms. Machine learning models created using DaT-SPECT imaging, beta-amyloid, tau, alpha-synuclein, or neurofilament light will perform with higher accuracy when distinguishing subjects by disease state or cognition than some diagnostic tests.

Methodology: Using the support vector machine and random forest machine learning techniques, models were created based on neuroimaging and biofluid biomarkers for a subset of PD and healthy participants from the Parkinson's Progression Markers Initiative (PPMI) dataset. To reduce error, ten models were trained and tested for each biomarker and technique and the average metrics were reported.

Results: So far, random forest models tend to be more accurate than support vector machine models. Random forest models using data derived from DaT-SPECT imaging performed with high accuracy in differentiating by disease state in subjects with MCI. Additionally, random forest models with DaT-SPECT imaging performed slightly better when differentiating by disease state in subjects with normal cognition. When DaT-SPECT imaging was applied to distinguish by cognitive state, both random forest and support vector machine models performed poorly. DaT-SPECT imaging may not have utility in differentiating subjects by cognition. This study found that random forest models differentiating PD subjects from healthy subjects performed better than some diagnostic tests when DaT-SPECT imaging data was used. When DaT-SPECT imaging was combined with proteomic concentrations derived from biofluids, random forest model performed with higher accuracy than when DaT-SPECT was used alone.

Next Steps: This study's next steps involve developing machine learning models for biofluid biomarkers and developing models that combine both neuroimaging and biomarkers.

B.2: Examining depression in older surgical patients: an observational cohort study

Student: Yasmin Alhamdah, Supervisor: Frances Chung

Background: Depression affects individuals across various ages and populations but is of particular significance in the older surgical population due to its adverse impact on cognitive function, surgical recovery, and overall quality of life. The aging population worldwide is experiencing an increased burden of surgery for various medical conditions. As this population continues to grow, understanding the prevalence and trajectory of depression in older surgical patients becomes paramount for comprehensive healthcare delivery. This is because depression can significantly impact the overall well-being of older individuals, affecting not only their mental health but also exacerbating physical health conditions.

Purpose & Hypothesis: This multicenter prospective cohort study aimed to determine the overall prevalence of depression preoperatively and postoperatively at 30-, 90-, and 180-days in older surgical patients. We also aimed to look at depression in cognitively impaired versus unimpaired individuals. We hypothesize that the overall prevalence of depression in older surgical patients will be high preoperatively. The prevalence of depression may exhibit an increase in the immediate postoperative period (30-days) compared to the preoperative baseline. Additionally, we anticipate observing a gradual decline in the prevalence of depression at the 90- and 180-day postoperative timepoints, reflecting a trajectory of recovery.

Methods: Participants $\geq$ 65 years undergoing elective, non-cardiac surgery were recruited from preoperative clinics at Toronto Western and Mount Sinai Hospitals, Toronto. Participants completed the 15-item Geriatric Depression Scale (GDS) through an online survey preoperatively and postoperatively at 30-, 90-, and 180-days. A cut-off of $\geq$ 5 was used to define overall depression. Participants also completed four cognitive screening tools: the Telephone Montreal Cognitive Assessment and Modified Telephone Interview for Cognitive Status over the telephone and the Ascertain Dementia Eight-item Questionnaire and Center for Disease Control and Prevention cognitive question through the online survey. Linear mixed-effects models were used for trajectory analysis.

Results: Among 307 participants (mean ± SD age: 72.9 ± 5.5; 56.0% female), 62 (20.2%) screened positive for preoperative depression. Forty-five (14.7%) had mild depression (GDS score 5-8), 11 (3.6%) had moderate depression (GDS score 9-11), and 6 (2.0%) had severe depression (GDS score 12-15). At 30-, 90- and 180-days postoperatively, the prevalence of depression was 25.1%, 15.1%, and 17.5% respectively. Those who were depressed had significantly lower mean GDS scores at 90- and 180-days postoperatively vs. preoperatively (5.38 ± 0.37 and 5.41 ± 0.35 respectively vs. 7.52 ± 0.28, $P \leq 0.05$). The mean GDS scores decreased over time in those who were depressed (*P for time <0.001*).

Conclusions: Depression is prevalent in older surgical patients. Our study contributes valuable insights into the prevalence and temporal trajectory of depression in older surgical patients. This creates an impetus for identifying and addressing perioperative depression to enhance perioperative care and facilitate improved surgical recovery.

B.3: Neonatal dysglycemia and resting-state networks measured with magnetoencephalography at early school-age after neonatal encephalopathy

Student: Micheline Lagacé, Supervisors: Emily Wy Tam, Cecil D. Hahn

Background: Long-term impacts of dysglycemia during neonatal encephalopathy (NE) require investigations. Magnetoencephalography (MEG) is a neurophysiological imaging tool measuring rhythmic neuronal activity with a high spatial and temporal resolution, allowing investigation of resting-state networks (RSN).

Purpose & Hypothesis: Assess the relationship between dysglycemia in NE and RSNs at early school-age using MEG. Neonatal hypoglycemia and hyperglycemia will affect RSNs differently at early school-age.

Methods: A prospective cohort of NE survivors following continuous glucose monitoring during the first 72h of life were assessed at 5.6±0.4 years. Resting-state MEG data were acquired using the *Inscapes* movie. MEG analysis used the FieldTrip toolbox and a standard MNI MRI template. Neural oscillatory activity was defined by the averaged power spectral density estimated for five canonical frequency bands for seven RSNs. A multiple linear regression model assessed relationships between oscillatory activity and RSNs with neonatal glycemia while correcting for sex, socioeconomic status, severity of hypoxic-ischemic encephalopathy and brain injury on MRI.

Results: Resting-state MEG data was available for 33 participants (neonatal hypoglycemia 13, hyperglycemia 7). The regression model showed an inverse relationship for maximal and minimal neonatal glycemia on gamma activity, respectively a positive and negative relationship. This effect was present in all RSNs, with the largest effects found in dorsal attention and default mode networks, followed by motor and language.

Conclusions: Both minimal and maximal glucose levels during NE are associated with long-term changes in localized processing and excitatory-inhibitory neural processes, across brain networks, particularly the dorsal attention, motor, language, and default mode networks.

B.4: Comparing GPi DBS programming clinicaly versus with neuroimaging-based software

Student: Michelle Hamani, Supervisor: Alfonso Fasano

Deep brain stimulation (DBS) is a neurosurgical procedure involving the implantation of electrodes that regulate abnormal brain impulses through targeted neuromodulation. DBS is primarily used for movement disorders including Parkinson's Disease, a neurodegenerative disorder affecting the dopaminergic neurons of the basal ganglia. Several visits to a healthcare provider are necessary to reach the optimal electrical settings, and thus the success of this therapy depends on precise electrode placement and configuration. However, the programming of these electrodes can be challenging and time consuming due to the number of possible combinations and patient anatomical variation. GUIDE XT (Boston Scientific) is a programming software that uses neuroimaging to enable more advanced visualization and programming. Thus, the purpose of this study was to compare motor symptom improvement and time taken to program between the software and clinical approach. It was expected that GUIDE XT would yield similar improvement in much less time.

This study relied on a double-blind randomized crossover design, where 7 patients that underwent GPi DBS with a Boston Scientific model were selected. The investigator programmed the patient via the software and compared this program to their clinical settings acutely and during a one-month follow-up. Each patient arrived without having taken their levodopa dose, and were randomized into either condition, where MDS-UPDRS III, gait analysis, and side effect annotation were performed. The same set of experiments and randomization were repeated subsequent to levodopa administration.

The results displayed similar motor improvement in the clinic but worsening of symptoms with GUIDE XT during the follow-up. Nevertheless, programming via the software was significantly less time-consuming.

Overall, since the use of GUIDE XT was less time intensive and does not require a professional to program, it may be suggested as a method for initial programming, after which the clinical-based approach could be implemented for fine-tuning and adjustments.

B.5: Post-fall cognitive and neuropsychiatric issues in neurodegenerative disease patients

Student: Goldin Joghataie, Supervisor: M. Carmela Tartaglia

Background: Falls are the most common mechanism of injury faced by millions of patients with neurodegenerative diseases (ND) each year. Falls are also the number one cause of concussions. However, there are large gaps in literature regarding fall related cognitive and neuropsychiatric symptoms (NPS) and there is no known information on how concussion symptoms present in neurodegenerative disease.

Purpose: We aimed to compare patients with NDs who have experienced falls, with those who have not, regarding cognitive and neuropsychiatric symptoms (as they are also common post concussion symptoms), using available data from the Ontario Neurodegenerative Disease Research Initiative (ONDRI) database.

Hypothesis: We hypothesized that patients with NDs who have experienced a fall will have greater cognitive deficits and worse neuropsychiatric symptoms than patients without falls.

Methods: We used data from the Ontario Neurodegenerative Disease Research Initiative dataset (ONDRI) on 504 individuals in five ND types. We compared frequency and severity of different NPS, and performance in 24 tests in five major cognitive domains, between patients with and without falls in the past 12 months. We tested for sex, age and educational background differences for both NPS and cognitive symptoms in patients as well as their caregivers. In addition to differences in patient and caregiver sex, we looked at the number of hours caregivers lived with patients.

Results: Comparing those who experienced falls in the last year (n=169; mean-age=68.3±9; 36%Female), to those who had no falls (n=314; mean-age=68.7±7; 32%Female), there was significantly higher total NPS severity ($p=0.0061$), frequency of anxiety ($p=0.00026$); and anxiety severity ($p=0.002$) in addition to other symptoms such as depression, apathy, appetite and night-time behaviours. Patients who had a fall also had significantly lower ($p<0.001$) scores in attention and working memory, executive function, language and visuospatial domains, this was not the case in non-fallers.

Conclusions: ND patients with falls had significantly worse NPS and cognitive function. Such issues post fall event must be assessed, as they can be important prognostic factors in disease stage, impact baseline treatment for NDs, possibly worsen symptoms from previously undiagnosed neuropsychiatric disorders or post concussion symptoms. Ignoring post fall symptoms significantly increases risk of misdiagnosis that can significantly hinder patient quality of treatment and life.

B.6 Predictors of cerebellar hemorrhage and cerebellar hemorrhage volume in preterm infants

Student: Marta Ybarra Zavala, Supervisors: Emily Tam, Steven Miller

Background: Cerebellar hemorrhage (CbH) contributes to motor and cognitive disabilities in preterm infants. A complicated neonatal course including hemodynamic disturbances seem to be related to an increased risk for CbH.

Objective: To define risk factors related to cardiovascular insufficiency in preterm infants for CbH, Cbh volume and location.

Methods: Early-life (median post-menstrual age [PMA] 33 weeks, IQR: 31.7- 34.4 weeks) and term-equivalent age (TEA) (median PMA 41.1 weeks, IQR: 39.6- 43.3 weeks) brain MRIs were performed in a prospective multisite cohort of very preterm infants born <32 weeks) gestation. Presence of CbH (T1, T2 and SWI) was assessed by a pediatric neuroradiologist. Total CBH volume was calculated based on the manual segmentation performed by two trained raters. Hypotension requiring treatment, presence of a PDA requiring treatment, and the SNAPII Score (quantifying illness severity in first 12 hours of life) together with other comorbidities were recorded. Uni- and multivariable regression analyses were conducted. Presence of CbH, presence vermis hemorrhage and CbH volume were outcomes variables.

Results: 309 very preterm infants (27.6± 2 weeks) were included. 60 infants presented CbH (19.4%), vermis involvement was observed in 14 infants. Infants with CbH had lower GA and rate of C section, and higher rates of prolonged mechanical ventilation, NEC stage 2 or higher, coagulopathy, PDA, IVH and higher SNAP-II score ($p<0.05$). On univariable regression, variables related to hemodynamic disturbances were associated with CbH, vermis involvement and CbH volume ($p<0.05$). Multivariable regression showed that hypotension requiring inotropes was independently associated with the presence of CbH (OR 3.93, 95%CI 1.65-9.37, P=0.002) and vermis involvement (OR 5.33, 95%CI 1.18-24.04, P=0.029). Although hypotension requiring inotropes was also associated to CbH volume (0.36 log mm^3 ,95% CI 0.17 - 0.54, $p< 0.001$), this model does not explain a large amount of variance in CbH volume.

Conclusion: This study highlights the important role that hemodynamic disturbances play in CbH with significant hypotension requiring treatment with inotropes being an independent risk factor for CbH. Factors associated to larger CbH volumes need further attention.

B.7: Identifying the role of GABAergic cells in the periaqueductal grey and the preBötzinger complex in breathing and respiratory depression by opioids

Student: Kayla Baker, Supervisor: Gaspard Montandon

Background: Breathing is an essential and automatic process that is maintained by brainstem regions that are vulnerable and can be targeted by drugs such as opioids to cause respiratory depression. Opioids are widely prescribed analgesic medication, but this respiratory side effect can be lethal and limits the use of opioid analgesics safely and effectively. To better understand how opioids depress breathing it is critical to first identify the neural circuits that control respiratory rhythms and how they overlap with analgesia. Two key brain regions in this pathway are the preBötzinger Complex (preBötC) which is involved in generating inspiratory activity, and the periaqueductal grey (PAG) which is involved in analgesia. Inhibitory GABA cells in these brain regions express the mu-opioid receptor meaning they can be targeted by opioids, but the role of these GABA cells in the control of breathing and respiratory depression is unknown.

Purpose & Hypothesis: GABAergic neurons in the preBötC and PAG express mu-opioid receptors but their roles in breathing, analgesia, and respiratory depression are unknown. We aim to identify the role of the GABAergic preBötC and PAG cells in breathing using *in vivo* optogenetics and hypothesize that optogenetic activation of inhibitory preBötC and PAG neurons will depress breathing.

Methods: To stimulate GABA neurons, we stereotaxically injected a cre-dependent adeno-associated virus expressing either the excitatory channelrhodopsin or the inhibitory archaerhodopsin in the region of interest of vesicular GABA transporter (vGAT)-cre mice. We then measured breathing using diaphragm activity in anesthetized mice and whole-body plethysmography in freely behaving mice while we stimulated inhibitory cells with blue light and inhibited cells with green light. We measured nociception during photostimulation using the tail-flick assay.

Results & conclusions: We found that preBötC vGAT cell excitation depresses breathing and inhibition can trigger inspiratory activity. These data suggest that the inhibitory preBötC cells are involved in modulating inspiration timing. Determining the role of GABA cells in coordinating respiratory rhythms will allow us to better understand what is occurring in the brain when breathing is depressed such as in opioid-induced respiratory depression.

B.8: Remotely delivered physical activity program for treatment-resistant depression: protocol for a pilot randomized controlled trial

Student: Vanessa Tassone, Supervisor: Venkat Bhat

Background: Depression affects approximately 280 million people worldwide. More than one-third of individuals will not respond to at least two antidepressant medication trials, meeting the criteria for treatment-resistant depression (TRD). Alternative therapeutic modalities, such as physical activity (PA), are garnering interest in depression research. Several studies have reported that PA can improve mood and mental health in both clinical and non-clinical populations. However, few studies to date have examined the effects of PA interventions as an add-on to pharmacological treatment in TRD. We will conduct a pilot randomized controlled trial (RCT) evaluating a remotely delivered, one-on-one, individualized PA program (MoveU.HappyU; MUHU) in adult participants with TRD.

Purpose & Hypothesis: *Aim 1:* To estimate the feasibility of randomizing adult participants with TRD to (1) treatment as usual (TAU) with the addition of a remotely delivered, one-on-one, individualized PA program (MUHU) or (2) TAU for 4 weeks based on rates of recruitment, adherence, trial completion, and data collection. We will also assess acceptability of the PA program based on responses to an exit survey and semi-structured qualitative interview. We hypothesize that: we will be able to recruit the planned sample (with the rate of at least 2 participants per month); rates of adherence, trial completion, and data collection will be >80%; and participants will report satisfaction with the content and delivery of the intervention. *Aim 2:* To estimate the standard deviations of the 17-item Hamilton Depression Rating Scale, Patient Health Questionnaire-9, Generalized Anxiety Disorder-7, and the World Health Organization-Five Well-Being Index, as well as their within-person correlations between baseline and the end of the intervention period, and between baseline and the end of the follow-up period. *Aim 3:* To evaluate the effect of the PA program on digital physiological passive data collected through a wearable device (Oura Ring).

Methods: Over 2 years, we will conduct a single-site (St. Michael's Hospital, Unity Health Toronto) pilot trial in 30 sedentary adults (aged 18-65) with TRD randomized to receive (1) a PA program (MUHU) add-on to TAU, or (2) TAU. The PA program will consist of weekly meetings with a program trainer wherein participants will engage in 30 minutes of behavioural change coaching and 30 minutes of a structured PA program. Participants randomized to the PA program will also be instructed to independently complete 120 minutes of PA per week during the 4-week intervention period. Participants in the TAU group will not receive any instructions pertaining to PA. The intervention period will be followed by 6 weeks of observation. Throughout the study, both groups will receive the same active and passive digital monitoring via self-report questionnaires and a wearable device (Oura Ring), as well as traditional monitoring (i.e., scales administered by a blinded rater).

Conclusions: This pilot trial will provide estimates of rates of recruitment, adherence, trial completion, and data collection, while also providing insight into participant satisfaction, which will determine the feasibility and acceptability of a large multi-centre RCT. The collected data will also provide clinical parameter estimates that will inform the design and implementation of this RCT, including the sample size calculation. Effects of the PA program on digital physiological passive data (e.g., sleep, activity, and readiness information) will also be established. Ultimately, this line of research will facilitate the development of behavioural treatments for TRD and improve its outcomes, including improvement in quality of life.

C: Neuroscience & Brain Health

C.1: Repetitive transcranial magnetic stimulation of the insula and prefrontal cortex for the treatment of cannabis use disorder: preliminary results

Student: Dhvani Mehta, Supervisors: Tony George, Victor Tang

Background: Cannabis use disorder (CUD) is present in 3-4% of the general population, yet effective therapeutic options are limited. Repetitive transcranial magnetic stimulation (rTMS) is a non-invasive neuromodulatory technique that may be a promising treatment for CUD.

Objectives: We are conducting a rater-blind, randomized, proof-of-concept clinical trial to evaluate the feasibility and tolerability of 20 sessions (once daily, 5 days/week) of high frequency (HF) or low frequency (LF) bilateral rTMS using a parabolic coil stimulating the prefrontal cortex (PFC)/insula for the treatment of CUD.

Hypothesis: It is hypothesized that this treatment will be feasible in terms of having a ≥50% study completer rate, and that both HF and LF rTMS will reduce cannabis use outcomes and executive dysfunction post-rTMS, as well as induce changes in PFC/insula connectivity.

Methods: A total of N=46 will be recruited and randomized to receive 20 sessions of either LF (1Hz) or HF (10Hz) rTMS targeting the PFC/insula. Clinical outcomes, including cannabis use outcomes and psychiatric symptoms, are assessed weekly. Executive function and resting-state functional magnetic resonance imaging are evaluated pre/post rTMS. For this preliminary analysis, pre/post changes in clinical outcomes for all participants, irrespective of group allocation, were evaluated in a blinded manner using a repeated measures ANOVA.

Results: Twelve participants have completed the study thus far. Nine (75%) have undergone all 20 sessions and tolerated rTMS at the full dose without adverse effects. Of the 3 dropouts, 1 cited discomfort, while 2 withdrew due to non-study-related reasons. Statistically significant reductions ($p<0.05$) in cannabis consumption, cannabis craving, anxiety, and depressive symptoms were observed following 20 sessions of rTMS treatment.

Conclusions: In this preliminary analysis, bilateral rTMS (LF or HF) applied to the PFC/insula appears to be feasible and well-tolerated amongst CUD participants, and may reduce cannabis consumption and craving, as well as improve psychiatric symptoms. The differential effects of HF vs. LF stimulation will be analyzed at study completion.

C.2: Non-invasive temporal interference stimulation of the subgenual anterior cingulate cortex: study design

Student: Ilya Demchenko, Supervisors: Tom A. Schweizer, Venkat Bhat

Background: The stimulation of deep brain structures has been historically only possible using invasive technologies. Temporal interference (TI) is a novel, non-invasive technique that can potentially overcome this limitation.

Purpose & Hypothesis: Here, we present an experimental protocol for delivering TI to the sgACC for the first time, where we hypothesize that 130 Hz TI will modulate the functional activity of the sgACC and the negative attentional bias.

Methods: Thirty healthy participants who score high on psychometric measures of sadness will be randomly assigned to receive either a 20-minute 130 Hz TI or sham stimulation. Before and after the stimulation, participants will complete the Ambiguous Faces Interpretation Task involving the presentation of stimuli of morphed faces (neutral, sad, happy, angry). Participants will be asked to categorize the stimuli into the four respective emotions and rate their intensity. Pre- vs. post-stimulation functional magnetic resonance imaging (fMRI) scans will be acquired.

Expected Results: Participants receiving TI will demonstrate a significant increase in fMRI outcomes of sgACC blood-oxygen-level-dependent signal variance and functional connectivity. Behaviourally, target engagement of the sgACC will correspond to decreased interpretation bias toward sad stimuli, quantified as a decreased total number of sad faces and slower reaction times.

Conclusions: This study will be the first to demonstrate the non-invasive target engagement of the sgACC with TI. If successful, this project will lay the groundwork for conducting future human experiments with TI targeting the sgACC and exploring the use of this technology for the treatment of psychiatric mood disorders, such as major depression.

C.3: Transition in epilepsy: the healthcare practitioner perspective

Student: Ilakkiah Chandran, Supervisor: Danielle Andrade

Background: Approximately 1.1 million children with epilepsy become adults every year. Moving from the pediatric to adult health care can be troublesome, and transition guidelines have been suggested, although not implemented in most places. The International League Against Epilepsy (ILAE) has set a Transition from Pediatric to Adult Care Task Force, and their first goal was to review the current situation in different parts of the world.

Purpose & Hypothesis: This study aimed to understand the perspectives and attitudes of healthcare practitioners on the challenges of transitioning epilepsy patients to adult care. By understanding these perspectives, we aim to identify the barriers preventing satisfactory transition while examining the elements needed for successful transitions.

Methods: A questionnaire was distributed to practitioners worldwide through ILAE Chapters in 8 languages. The responses were then analyzed through descriptive analyses and qualitative summaries.

Results & Implications: 306 practitioners from 55 countries responded to our questionnaires. 52% of whom were adult neurologists, and 45% were child neurologists. The availability of an adult neurologist with knowledge of the condition was the most described factor to impact patient transition. The three most common practitioner-perceived barriers to building and sustaining transition programs were the lack of multidisciplinary teams, patients' attachment to the childcare system and the limited education and training available for epilepsy transition. This survey highlights the views of many practitioners globally about the transition in epilepsy. It identifies various barriers, aiding policymakers in targeting specific issues to make structured and sustainable transition programs possible.

C.4: Comparing childhood-onset and adult-onset post-traumatic stress disorder: results from the National Epidemiologic Survey on Alcohol and Related Conditions-III (NESARC-III).

Student: Esther Kim, Supervisor: Bernard Le Foll

Background: Posttraumatic stress disorder (PTSD) can develop following exposure to a traumatic event and includes a collection of symptoms that altogether significantly affect quality of life. Nearly half of US adults will experience at least one traumatic event in their lifetime with more than 5% developing PTSD. In children, two-thirds of the population experiences trauma or adverse childhood experiences (ACEs) with approximately 10% of children 17 years and younger diagnosed with PTSD. ACEs have been associated with negative long-term health consequences including increased risk of developing PTSD.

Purpose: The purpose of this study is to understand the underlying differences in childhood-onset (<18) and adult-onset (≥18) PTSD and gain novel insight in how the age of onset of PTSD can play a role in the development of PTSD symptoms and comorbidities.

Hypothesis: Individuals with childhood-onset PTSD will have worse outcomes in symptoms, binge-drinking behavior, and comorbidities compared to individuals with adult-onset PTSD due to influences of ACEs, which are associated with PTSD development, and additional negative health consequences.

Methods: Using the data collected from the National Epidemiologic Survey on Alcohol and Related Conditions-III (NESARC-III) on individuals who met the DSM-5 criteria for PTSD (N = 1,348), we will compare childhood-onset and adult-onset PTSD with regard to demographic, symptoms of PTSD, binge-drinking behavior, and comorbidities.

Results: Based on previous literature on the effects of ACEs in PTSD development, the childhood-onset group will have worse outcome in all clinical variables than the adult-onset group. Evidence suggests that ACEs may also influence drinking behavior, and development of additional psychiatric diagnoses.

Conclusion: This study demonstrates the differences between childhood-onset and adult-onset PTSD and can aid further studies exploring childhood-onset PTSD, to provide novel insight into various treatment options.

C.5: Utilizing multi-echo resting-state fMRI to characterize the long-term brain function sequelae in youth with a history of an enterovirus (EV-71) CNS infection

Student: Jasmine Ali Gami, Supervisor: Tarek Rajji

Background: An Enterovirus (EV) infection, particularly the EV-71 strain, is a common pediatric infectious disease. EV-71 is known to cause illnesses ranging from a low-grade fever, and hand-foot-and-mouth disease, to a severe central nervous system (CNS) disease. With a global presence, this life-altering infection poses a serious public health threat across the Asia-Pacific region and beyond. EV infection outbreaks have also emerged in Canada from time to time over the past few years. Among the manifestations and sequelae, the long-term impact of the EV CNS infection remains a critical concern with neuropsychiatric sequelae. Specifically, numerous studies have shown the correlation between an EV-71 CNS infection and the development of attention-deficit/hyperactivity disorder (ADHD) and associated emotional-behavioural problems later in life. However, no published evidence has ever studied the longitudinal brain development in this unique population of youth who develop ADHD following EV-71 CNS infection earlier in life (EV-71-ADHD henceforth).

Objective: Our proposed research will investigate the longitudinal brain features of youth with a pediatric EV-71 CNS infection earlier in life. Specifically, our research will set on addressing the following question: "Are there abnormalities in functional connectivity within the midbrain that would contribute to the development of EV-71-ADHD?"

Hypothesis: A number of neuroradiological reports suggest that an EV-71 CNS infection tends to involve the brainstem. Meanwhile, the midbrain is integral for the secretion of dopamine, which is a neurotransmitter whose dysregulation could contribute to ADHD. Notably, midbrain pathology is not directly reported in idiopathic ADHD. Taken together, we thus hypothesize that youth with EV-71-ADHD, relative to idiopathic ADHD, EV-71 without ADHD, and neurotypical control, would have altered intrinsic functional connectivity based on the midbrain.

Methodology: This will be a naturalistic longitudinal cohort study of youth who had a previous EV-71 CNS infection 6-18 years ago. sixteen of them developed ADHD later in life. Youth with idiopathic ADHD and neurotypical youth will be used as control groups. We will utilize multi-echo resting-state functional magnetic resonance imaging (rsFMRI) to examine the functional connectivity of dopamine-producing structures within the midbrain such as the Ventral Tegmental Area (VTA) and the Substantia Nigra (SN).

Significance: The significance of our research is two-fold: 1) Characterizing longitudinal brain features of youth with an EV-71 CNS infection may facilitate the development of targeted intervention and rehabilitation strategies to enhance the quality of life and reduce disabilities in infected youth; 2) By filling the knowledge gap of longitudinal brain features, our results may lay a foundation to explore possible long term brain and cognitive impairments following emerging viral infectious diseases.

C.6: Effects of deep brain stimulation of the centromedian thalamic nucleus on GABA in a mouse model of epilepsy

Student: Rafi Matin, Supervisor: George M. Ibrahim

Background: Despite the best available medical interventions, individuals with refractory epilepsy continue to experience debilitating seizures. Deep brain stimulation (DBS) offers a promising avenue, involving the surgical implantation of electrodes to modulate neural circuitry through electrical stimulation. While DBS targeting the centromedian nucleus of the thalamus (CM-DBS) has shown clinical promise for refractory epilepsy, its neurobiological mechanisms remain elusive, hindering personalized treatment development. Furthermore, while previous studies demonstrate DBS's ability to increase γ-Aminobutyric acid (GABA) levels, a critical inhibitory neurotransmitter in epilepsy, this has not been explored in the context of CM-DBS.

Purpose: In this study, we used mouse models to determine whether CM-DBS increases GABA levels within the CM network. Additionally, considering clinicians adjust stimulation settings to optimize therapeutic efficacy, we assessed the effects of various CM-DBS settings on GABA levels.

Hypothesis: We hypothesized that CM-DBS with clinically effective settings would increase GABA levels within the CM-network.

Methods: C57BL/6J mice (n=18, healthy control) and Cntnap2-KO mice (n=18, epilepsy model) were randomly assigned to 4 experimental groups: 1) CM-DBS high frequency (clinically effective stimulation), 2) CM-DBS low frequency (clinically ineffective stimulation), 3) Sham (receiving DBS electrode but no stimulation), 4) Naive (no surgery). Microdialysis was concurrently performed during CM-DBS to assess changes in GABA levels at the CM. Additionally, tissue samples from various brain regions within the CM network (i.e., thalamus, basal ganglia, hippocampus, amygdala) were collected following stimulation. GABA levels from the microdialysis and tissue extraction samples will be quantified with mass spectrometry.

Results: Although the experiments are complete, analysis with mass spectrometry and results are pending. Our data will enable us to evaluate the impact of stimulation on GABA and confirm whether clinically effective stimulation parameters correlate with increased GABA in the CM network.

Conclusions: Our findings will provide insight into how CM-DBS achieves its therapeutic effects. Future studies can further explore additional mechanisms at the molecular, cellular, and network levels to obtain a comprehensive understanding of this technique. Ultimately, this line of research will enhance our understanding of how CM-DBS achieves seizure suppression and facilitate tailored treatment optimization for patients.

C.7: Regional hippocampal signatures of accelerated brain aging in trigeminal neuralgia

Student: Jerry Li, Supervisor: Mojgan Hodaie

Background: Does pain modify aging? Machine learning offers powerful avenues to identify correlates of biological wellbeing that can be influenced by chronic pain. Trigeminal neuralgia (TN) is the most common form of chronic neuropathic facial pain. TN serves as a unique and valuable model to better understand the possible dynamic plasticity associated with chronic pain, as it is highly amenable to surgery. Previously, abnormalities in the hippocampus and insula of patients with TN normalized following pain relief from surgery. Patients with TN also had significantly greater brain age than healthy counterparts, with hippocampal regions substantially contributing to this estimation.

Purpose & Hypotheses: Given the known impact of chronic pain on cognitive and affective processes – both intrinsically linked to the hippocampus and aging – we hypothesize that hippocampal subfields may be effective predictors of brain age. Furthermore, given that the hippocampus can recover following successful surgery, accelerated brain aging may also be mitigated.

Methods: Multiple support vector regression (SVR) models were trained on the brains of 522 healthy subjects from Cam-CAN to predict their chronological age, or "brain age". Using FreeSurfer 7.1, T1-weighted MRI scans of whole brains were segmented into constituent and hippocampal subfield volumes, upon which separate SVRs were trained and optimized. The regression effect in final age predictions was corrected using a linear regression of the training results. Afterwards, models predicted the age for 123 TN patients. Performance metrics, such as the correlation coefficient (r), coefficient of determination (R^2), and mean absolute error (MAE) are reported.

Results: An SVR built on hippocampal subfields performed nearly as well as one built on whole-brain segmentations. Another SVR built on a combination of these volumes outperformed all previous models (r = 0.94; R^2 = 0.88; MAE = 5.64). This combined model was used to predict the ages of individuals with TN (r = 0.81; R^2 = 0.62; MAE = 7.14) and found their mean brain age, 62.10 ± 0.92 years, to be significantly greater than their actual mean age of 59.59 ± 1.32 years (q = 0.022). Surgical responders had significantly greater brain age than chronological age (q = 0.046), whereas non-responders did not (q = 0.134).

Conclusions: Across a plethora of diseases, a brain age greater than chronological age robustly correlates with negative health trajectories. These include increased risk of developing chronic illnesses, cognitive decline, and mortality. Accordingly, we present another instance in which patients with TN, a debilitating manifestation of chronic neuropathic facial pain, appear to have brains that are biologically older. The open-access methods employed in this study promote quicker validation and translation of brain age into clinical tools for prognostication and optimization of therapeutic avenues to alleviate burdens on healthcare professionals and patients, alike. A future direction would be to compare performance between different models and neural networks.

D: Neuroscience & Brain Health

D.1: Early neural activity and effective connectivity related to visual body perception in adolescents with anorexia nervosa: a pilot study

Student: Joel Diaz, Supervisor: Jamie Feusner

Background: Disturbance in the experience of one's weight or shape is a core symptom of anorexia nervosa (AN), with high prognostic and therapeutic relevance. Recent EEG studies investigating visual body perception in healthy controls have observed specific brain waves—theta frequency oscillations—in key electrodes that correspond to the location of a body processing region of the brain (i.e., the extrastriate body area), which is sensitive to individual body parts but insensitive to their global configuration.

Purpose & Hypothesis: The purpose of this study was to examine electrophysiological markers of abnormal perceptual processing in AN. Findings from this pilot study can help us better understand abnormalities in visual processing related to body image disturbance, which can aid in novel treatment strategies such as perceptual retraining and noninvasive brain stimulation. We hypothesized that individuals with AN will show greater event-related increases in theta oscillations in right occipito-temporal brain regions when viewing images of bodies compared to controls.

Methods: Twenty-three adolescent girls (16 AN, 7 controls; aged 10-19 years) were recruited. 125-channel EEG recordings were acquired while participants performed a two-choice matching task consisting of blocks of images of bodies, houses, and shapes. Adaptive mixture ICA was used to decompose EEG signals into independent components (ICs). Artifactual ICs were removed, and source localization was performed by fitting equivalent current dipoles to ICs using a template head model. ICs were clustered across participants by dipole location and a right occipito-temporal cluster was identified by scalp map. Time-frequency analysis was performed, and cluster-based permutation tests were used to compare group differences for each image type.

Results: For bodies, a single positive cluster ($p=.021$, corrected) was found which consisted of increased theta (4-8 Hz; 50-250 ms) and alpha (8-13 Hz; 50-175 ms) activity in AN compared to controls. No significant group differences were identified for shapes or houses. Results from this pilot study suggest individuals with AN demonstrate abnormal body part-based perceptual processing (as measured by increased theta and alpha activity) when viewing images of bodies. These findings provide insights into distinctive oscillatory patterns of abnormal visual body perception that could serve as a measure of treatment effects or be used to identify at-risk individuals early on (e.g., adolescents).

D.2: Pilot analysis of semi-supervised biopsychosocial subtyping in psychiatric treatment-seeking youth

Student: Denise Sabac, Supervisor: Daniel Felsky

Background: Youth seeking treatment for mental health concerns experience an elevated risk of developing severe mental illness into adulthood. Psychosis Spectrum Symptoms (PSS) may signal early risk, and understanding the biological and cognitive profiles of those with PSS could yield novel interventions. Existing data-driven, biopsychosocial studies aiming to characterize PSS are limited by data availability and often only explore single data modalities. In addition, subtyping analyses, which may permit precision medical approaches, typically rely on arbitrary decisions for data inclusion, processing, and workflow that affect final clustering solutions and contribute to reproducibility issues.

Purpose: Here we apply a novel clustering pipeline - Meta association-boosted Similarity Network Fusion (MabSNF) - to the discovery of patient subtypes enriched for PSS in treatment-seeking youth.

Hypothesis: We sought to demonstrate the ability of MabSNF to generate stable patient subtypes from a combination of biological, social, and psychological measures, hypothesizing that these subtypes would cut across traditional diagnostic groups.

Methods: MabSNF combines three approaches: 1) abSNF, which fuses patient similarity matrices across a large number of input features and types in a weighted manner, 2) meta-SNF, which performs exhaustive fusion and clustering based on combinations of workflow hyperparameters and inputs, and 3) weighted correlation network analysis, which distills the landscape of clustering solutions into representative meta-clusters based on Rand indices. We analyzed a subset of 202 participants from the Toronto Adolescent and Youth CAMH Cohort Study (ages 11-25y, mean=18.6y), considering 244 input features across four data types: sociodemographics, cognition, cortical thickness and subcortical volumes, and white matter microstructure. Feature weights for network fusion were assigned based on association with PSS.

Results: MabSNF generated a landscape of 630 clustering solutions. Solutions excluding white matter structural integrity data were most significantly associated with PSS. Experiment-wide representative solutions centered on two defined clusters (C1 n=108, C2 n=94). Top-contributing features were cortical thickness measures and performance on cognitive tests, with C2 showing greater average thickness, lower cognitive performance, and significantly higher PSS (p=0.0006).

Conclusions: These analyses demonstrate the potential of MabSNF when applied to a population of treatment-seeking youth, highlighting cortical thickness and performance on the NIH Toolbox and Little Man Task as important biomarkers of PSS.

D.3: Cortical effects of cochlear implantation in children with prelingual asymmetric hearing loss

Student: Hanne Bartels, Supervisor: Karen Gordon

Purpose: The objective of the present study was to investigate whether the behavioral and cortical effects of early cochlear implantation are different for children with deafness in the left versus right ear.

Background & Hypothesis: As children with asymmetric hearing loss (AHL) face various academic challenges, cochlear implants (CI) have become the standard treatment. However, achieving true binaural hearing continues to be a challenge. It is unknown whether the cortical and behavioral effects of AHL are different for children with left versus right-sided deafness, or whether the protection provided by the CI might be different. We hypothesize that left-sided deafness (a right-ear advantage) results in stronger effects on cortical processing of 1Hz stimuli.

Methods: To test this hypothesis, 69 children who received a CI following limited durations of prelingual unilateral severe hearing loss [mean (±SD) = 2.07 (±1.52)] were included in this study. Children had normal hearing in the contralateral ear [(n = 42 (60.9%)] or used a hearing aid for a mild to moderate hearing loss [PTA < 60dB, n = 27 (39.1%)] and 39 (53.6%) had a left cochlear implant. Cortical auditory evoked potentials (CAEPs) could be measured in 61 children (average of 2.85 recordings per child) using multi-channel electroencephalography at initial (≤ 2 weeks, n = 33), early (1-3 months, n = 49) and after chronic CI use (≥3 months, n = 96). Stimuli consisted of 36ms trains of acoustic clicks/biphasic electric pulses at a rate of 250 Hz, repeated at 1 Hz and were presented unilaterally to the CI and acoustic hearing ear (AH-ear) alone, as well as bilaterally. Localization of cortical sources of activity was performed using the time-restricted artifact and coherent source suppression (TRACS) beamformer. Cortical lateralization and aural preference were calculated, with more positive values indicating stronger right hemispheric dominance and contralateral ear preference, respectively.

Results: Immature P1 peak dipole moments were significantly larger in the right compared to the left auditory cortex ($p < 0.001$), and for stimulation of the AH-ear and bilateral condition compared to the CI-ear alone ($p < 0.001$ and $p = 0.005$, respectively). Peak latency decreased with time ($p < 0.001$) and was shorter for stimulation of the AH-alone than CI-alone ($p < 0.001$) or bilateral stimulation ($p = 0.001$). At initial CI-use, cortical lateralization [median (IQR) = 11.14% (-21.73% - 40.19%)] and aural preference [median (IQR) = 0.24% (-27.9% - 23.14%)] showed considerable variation among children, with no correlation to side of deafness or stimulation mode. Interestingly, chronic CI-use revealed a shift in hemispheric dominance across all conditions. Linear regression indicated a significant inverse relationship between initial CL values and evolution of CL over time, with more pronounced right and left hemispheric lateralization resulting in steeper negative and positive slopes, respectively ($p < 0.001$).

Conclusions: By investigating the cortical effects of cochlear implantation and comparing outcomes between left- and right-sided deafness, this study helps improve our understanding of the benefit of a CI in children with AHL. Our results demonstrate that processing of CAEPs to low-rate click-trains is variable among children at initial CI-use, and chronic CI-stimulation leads to a shift in hemispheric dominance. To comprehend the causes driving these shifts, and how these relate to side of deafness, further explorations are needed to explain variability among children and identify factors that determine initial ipsi- versus contralateral lateralization.

D.4: Real-world efficacy of maintenance ketamine infusions in treatment-resistant unipolar and bipolar depression

Student: Sipan Haikazian, Supervisor: Joshua D. Rosenblat

Background: Intravenous, subanesthetic-dose ketamine have demonstrated rapid and robust antidepressant effects in patients with treatment-resistant major depressive disorder (TRD) and bipolar depression (TRBD). Most analyses have analyzed the effects of acute ketamine infusions, i.e., multiple infusions administered in 2-3 weeks. However, few studies have analyzed the effectiveness of maintenance ketamine infusions following an acute course. Furthermore, no such analysis has been conducted in the TRBD patient population.

Purpose: To investigate the real-world efficacy of maintenance ketamine infusions in sustaining antidepressant effects seen with acute infusions in patients with TRD and TRBD.

Hypothesis: Maintenance ketamine infusions will prolong the antidepressant effects observed after a course of acute infusions in both patient populations.

Methods: In this retrospective study conducted in a private clinic located in Mississauga, Ontario, Canada, patients with TRD (n = 99) and TRBD (n = 24) received four subanesthetic doses of intravenous (IV) ketamine in a two-week period. Subsequently, patients received multiple booster ketamine infusions at a variable dosing schedule, and depressive, suicidal, and anxious symptoms were assessed before each infusion. Mixed model analyses were subsequently performed.

Results: After four acute infusions, significant changes in depressive symptoms were noted, as measured by the Quick Inventory for Depression Symptomatology Self-Report-16 total score ($p < 0.05$). In addition, there was no significant change in depressive symptoms over the maintenance infusions in patients with either disorder (TRD: $F = 0.194$; TRBD: $F = 0.473$, $p > 0.99$ for both), suggesting a sustained antidepressant effect. Significant improvements in self-reported suicidal and anxiety symptoms were also noted ($p < 0.05$), which were maintained throughout subsequent infusions ($p > 0.30$ for both symptoms). Ketamine was well-tolerated by most patients, with no long-term iatrogenic or addiction concerns being noted.

Conclusions: Real-world effectiveness of maintenance ketamine infusions for TRD and TRBD was observed, suggesting that ketamine can be an effective pharmacotherapy for relapse prevention of mood symptoms.

D.5: Investigating the pathophysiology and treatment of computer screen intolerance in patients with persisiting concussion symptoms

Student: Chloe Buso, Supervisor: Charles Tator

Background: About one-third of patients who sustain a concussion with persisting concussion symptoms (PCS) suffer from photosensitivity and computer screen intolerance (CSI), which significantly impair their ability to use a computer, cell phone screen or watch television. Many workers and virtually all students are required to use a computer screen daily. However, during the recovery process, working on a screen can exacerbate symptoms and interfere with a patient's recovery, including the return to work or school. In this study we evaluated a Flicker-Free screen and compared it to a conventional screen that flickers as a possible therapeutic intervention for concussion patients with CSI.

Purpose & Hypothesis: This study aims to determine if patients with CSI are less symptomatic after reading or watching a video on a Flicker-Free screen than a conventional computer screen that flickers. We hypothesize that CSI patients will experience a greater number and severity of symptoms on the conventional Flicker Screen than on the Flicker-Free Screen.

Methods: Concussion patients with CSI were randomized into two groups: Group 1 used the Flicker Screen during visit 1 and the Flicker-Free Screen for visit 2; and Group 2 used the two screens in reverse order. At each visit, subjects performed a reading and video task, and symptoms were recorded using the Sports Concussion Assessment Tool version 3 (SCAT3) at baseline, after reading, and after the video.

Results: This study is ongoing, I am currently blinded, and thus refer to the two screens as Screen 1 and 2. The sample size to date comprises 44 patients (11 males, 33 females, median age = 39 years old, interquartile range (IQR) = 21, median number of previous concussions = 2, IQR=4). On Screen 1, there was a significant increase in the total number ($p = 0.0057$) and severity of symptoms ($p=0.0001$) after reading; no significant differences were found after the video. On Screen 2, there was a significant increase in the total number ($p = 0.0051$) and severity of symptoms ($p=0.0081$) after reading, and a significant increase in the total number of symptoms ($p = 0.0135$) after the video. However, there was no significant difference in the number or severity of symptoms between Screens 1 and 2. The general linear model confirmed that age, gender, and number of previous concussions were associated with the total number and severity of symptoms on Screen 1 and Screen 2 after reading.

Conclusions: To date, the Flicker-Free computer screen has not been proven to be a useful therapeutic intervention for CSI patients. Additional patients are required to complete this study which should be completed in a few weeks. We are pursuing additional measures to elucidate the treatment and the pathophysiology of CSI.

D.6: The development of a machine learning predictive and alert system for adverse events in psychiatry

Student: Valentina Tamayo Velasquez, Supervisor: Bernard Le Foll

Background: Early Warning Scores (EWS) have been widely implemented in various areas of healthcare. This methodology has not been applied in psychiatry, despite the severe adverse events such as restraint, seclusion, and patient/staff harm, or psychological suffering. Machine learning (ML) presents the potential to close this gap by creating predictive tools for patients who are at risk for deterioration and to prevent adverse events.

Purpose: To determine the factors that contribute to psychiatric patient deterioration and adverse events and assess if we can build a sufficiently accurate ML model to predict patient deterioration in acute mental health settings.

Hypothesis: The identification of factors contributing to adverse events, allows for the development of a ML-based EWS and alerting system that can predict deterioration and adverse events with acceptable predictability.

Methods: A ML-based model was developed by using Electronic Medical Record (EMR) data from patients admitted to a large public mental health facility between January 1st, 2020 to December 31st, 2022. A scoping review, grey literature search, and consultations with healthcare professionals yielded 80 static and dynamic data elements from the EMR that were included in the model.

Results: The discrete data elements were integrated into the retrospective data sampling to assemble an anonymized patient record corresponding to adverse event occurrences. A boosting and Long Short-Term Memory (LSTM) ML model were developed with a recall value of 70-75% and an AUC ROC of 0.73-0.74. The predictive EWS tool utilizes a deep learning model, which will predict the likelihood of an adverse event in the coming 24 hours based on a patient's EMR history. Silent testing and model refinement are ongoing.

Conclusion: As healthcare facilities strive to optimize patient care and workplace safety, ML can be used to predict deterioration in acute mental health settings with acceptable accuracy. These alerts can be used by clinicians to modify treatment to avoid or reduce deterioration and suffering.

D.7: Exploring pain processing as a potential biomarker for suicide risk: an fMRI study

Student: Jennifer Ma, Supervisor: Sakina Rizvi

Background: Suicide rates have not significantly decreased in the past decade. Current theory points to capability for suicide (CS) to explain why ideation does not universally precede attempt. Pain tolerance is a key feature; it is thought that distributional factors (e.g. lower pain sensitivity) and repeated experiences, resulting in acquired fearlessness about pain and death (e.g. self-injury, traumatic experiences), increases CS. Notably, there are no reliable predictors distinguishing suicide ideators from people at high risk of attempt, and no studies have investigated physical pain-invoked neural activity within a suicide-risk population.

Aims: To identify neural networks underlying CS and pain perception by: 1) Correlating neuroimaging with CS measures in suicide ideators, with and without a previous attempt; 2) Correlating brain function during a physical pain task with CS; 3) Understanding pain processing neural circuitry as a potential target to identify high risk individuals for suicide attempt.

Hypothesis: Higher CS scores will be associated with higher pain thresholds on a pressure pain task, due to lessened sensitivity. Suicide capability scores will be negatively correlated with connectivity of brain regions relevant to suicide and pain perception.

Methods: We enrolled participants in a pilot cross-sectional study (ages 18-70; n=5 suicide ideators without previous attempt, n=3 ideators with attempt history, n=10 controls). Participants completed an fMRI pressure pain task, involving 3 stimuli blocks via a vascular pressure cuff adjusted based on individual perception, after which participants rated intensity and unpleasantness. We conducted analyses on regions relevant to pain processing–anterior cingulate cortex (ACC), dorsolateral pre-frontal cortex (DLPFC), and anterior (aIC) and posterior insula (pIC) – and exploratory whole-brain using FMRIB Software Library (FSL). Between-group differences were assessed with one-way ANOVA in SPSS.

Results: Pressure pain responses between a collapsed suicide ideator group and controls exhibited medium-to-large effect sizes in all highlighted regions, except the DLPFC (d[aIC]=1.0, d[ACC]=0.6, d[pIC]=0.5, d[DLPFC]=0.1). Among ideators, whole-brain analysis revealed stronger insular and precentral gyrus pain-invoked activation ($p<0.001$, $p<0.001$), and reduced ACC, precuneus and middle frontal gyrus activity ($p<0.001$, $p<0.001$, $p=0.022$). Further, suicide attempters had stronger activation in the precuneus and precentral gyrus compared to ideators without attempt history ($p<0.001$, $p<0.46$), whereas ideators had greater activity in the middle frontal gyrus ($p<0.001$), where the DLPFC is located.

Conclusion: Differences in pressure pain-invoked neural activation among suicide-risk groups identify the pain network as a promising target for innovative assessment and intervention methods.

E: Neuroscience & Brain Health

E.1: Identifying suicidal subtypes using computational modelling

Student: Pamina Laessing, Supervisors: Sean Hill, Andreea Diaconescu, Peter Dayan

Psychiatric conditions are ubiquitously heterogeneous, making it critical to find subtypes with differing characteristics. Suicidal ideation involves at least two subtypes, with distinguishable cognitive and behavioural deficits, such as stress-responsivity and impulsivity. We examined whether computational modelling could separate these and/or other subtypes in relevant clinical populations from their performance in an aversive cognitive learning task.

We analysed choices and reaction times from two clinical suicidality groups comprising 129 veterans and 50 individuals with major depressive disorder (MDD), all assessed for suicidality, impulsivity, and anxiety. The behavioural data were analysed with a range of reinforcement learning models, hierarchically fitted to identify potential subgroups in the populations.

Hierarchical modelling arranged the veterans and MDD populations into 3 and 2 subgroups, respectively. We found distinct clinical correlations characterizing each group after controlling for age and sex. Better performing groups in both populations exhibited low to moderate correlations of lifetime suicidal ideation and cognitive control (in the veteran population) with performance biases and learning rates. Lower scoring groups showed low to moderate correlations of performance measures with impulsivity, anxiety, and hopelessness in both populations.

The computational subgrouping of clinical populations based on behavioural patterns is promising as a way of separating informative subtypes of suicidality. Further analyses are required to establish their clinical utility, but the identification of subgroups with poorer/biased performance throughout the task, which correlated with impulsivity, is consistent with a stress-sensitive phenotype of suicidal ideation.

E.2: CRB1 mutation alters photoreceptor development in human retinal organoids

Student: Jessica Wang, Supervisor: Brian Ballios

Background: Crumbs cell polarity complex component 1 (*CRB1*) mutations account for ~10% of all genotypes associated with two common inherited retinal diseases, causing uncurable vision loss in children and young adults. This vision loss is caused by a decrease in the number of light-sensitive photoreceptor cells.

Purpose: This study evaluates the role of Hippo/YAP and NOTCH pathways in retinal cell proliferation/ development of human *CRB1*-associated retinal disease (CD).

Hypothesis: CD leads to a prolonged proliferative state and increased cell death, lowering the photoreceptor population.

Methods: Human retinal organoids (ROs) were generated from induced pluripotent stem cell lines derived from a patient with homozygous *CRB1* mutation (p.Q120X; p.Q120X) and a healthy donor. Immunofluorescent staining for cell type-specific markers (retinal cell progenitors: CHX10, PAX6; photoreceptor/ bipolar progenitors: CRX, OTX2; photoreceptors: recoverin; cones: ARR3; rods: NRL, rhodopsin; ganglion cells: BRN3a; Muller glia: SOX-9) was performed. RNA from bulk ROs was extracted to measure the expression of the NOTCH (*NOTCH1, NOTCH2, Hes1, Hey1, Hey2*) and Hippo/YAP (*STK3, STK4, YAP1*) pathways via qRT-PCR.

Results: Both healthy and CD ROs displayed sequential differentiation consistent with *in vivo* development. ROs were positive for early retinal cell markers (BRN3a, PAX6, CHX10, OTX2, CRX) at week 8 and later markers (ARR3, NRL) at week 13. However, CD ROs expressed cone outer segment (ARR3), unlike healthy ROs at week 8, suggesting differences in photoreceptor development timing. Additionally, initial qRT-PCR revealed significantly lower expression in the NOTCH and Hippo/YAP pathways in CD ROs compared to healthy ROs at week 8.

Conclusion: CD ROs showed differences in photoreceptor development compared to healthy ROs. Further study will clarify the effects of NOTCH and Hippo/YAP signaling in CD to facilitate the discovery of novel therapies.

E.3: The effect of weight loss on brain age in schizophrenia

Student: Vittala, Supervisor: Sri Mahavir Agarwal

Introduction: Individuals with schizophrenia (SCZ) often have metabolic comorbidities, such as type 2 diabetes, and experience a reduced life expectancy due to cardiovascular diseases. Obesity, a common comorbidity in SCZ, can negatively affect brain health. However, there is limited understanding of how metabolic disorders impact brain structure in individuals with SCZ, and the effects of weight changes following pharmacological interventions have not been explored. In this study, we will investigate changes in brain morphology, specifically brain-age, in overweight or obese individuals with or without diabetes who have been diagnosed with SCZ.

Objective: To assess the changes before and after a 12-week period of pharmacological treatment targeting metabolic dysfunction.

Hypotheses: 1) a change in BMI will be positively associated with the change in brain age between baseline and endpoint; 2) there will be no significant difference in the strength of the correlation between the medication and placebo groups.

Methods: This analysis includes 48 participants, aged 18 to 65, from three double-blind studies investigating interventions for antipsychotic-induced metabolic dysfunction: TAO study (NCT01794429, 9 on medication and 8 on placebo), Metformin for prediabetes/diabetes study (NCT02167620, 11 on medication and 8 on placebo), and Topiramate in clozapine study (NCT02808533, 12 on medication). We collected brain structural MRI, metabolic measures, cognition data, and body mass index at baseline and week 12. We utilized a convolution neural network-based classifier that was trained and tested to estimate the brain age of each participant using brain anatomical T1 image.

Results: The BMI alteration demonstrated statistical significance within the whole sample ($p < 0.001$), as well as in both the medication ($p = 0.005$) and placebo groups ($p = 0.008$). Likewise, significant changes were found only in total and HDL (high-density lipoprotein) cholesterol levels across all three groups in terms of metabolic parameters. However, none of the groups exhibited any substantial changes in psychopathological scores or cognitive data between the baseline and endpoint assessments. Multiple regression analysis revealed a positive correlation between BMI change and alterations in brain age for the whole sample (beta = 0.263; $t = 1.85$; $p = 0.05$) and the medicated group (beta = 0.372; $t = 2.12$; $p = 0.04$), but not in the placebo group (beta = -0.106; $t = -0.40$; $p = 0.69$). However, no significant difference in the correlation strength was observed between the medication and placebo groups ($p = 0.12$). Furthermore, there was no significant association between changes in brain age and metabolic indicators such as total and HDL cholesterol. Lastly, no significant correlation was found between brain age and cognition.

Conclusion: In conclusion, our study showed a link between brain health (as assessed by brain age) and significant weight loss by anti-diabetic medication in patients with SCZ and comorbid obesity. These findings imply that large and extended weight loss, together with general improvements in cardiometabolic alterations, can prevent obesity-related abnormalities in brain health.

E.4: Investigating cell types responsible for increased seizure generation in a model of neurofibromatosis type 1

Student: Avery Cameron, Supervisor: Aylin Reid

Background: Neurofibromatosis type 1 (NF1) is a genetic neurocutaneous disorder with increased prevalence of seizures and epilepsy compared to the general population. Our lab previously showed increased seizure susceptibility and epileptogenesis in a mouse model of NF1 (*Nf1*$^{+/-}$ mice). As these mice do not have intracranial lesions, the increased seizure susceptibility appears to be related to the genetic mutation itself, a possibility also supported by reports from patients. However, the mechanism behind this remains unknown. NF1 is caused by a heterogeneous mutation of the *Nf1* tumor suppressor gene, resulting in decreased levels of the protein neurofibromin which plays a crucial role in regulating cellular growth, division, and migration. Loss of neurofibromin disrupts these processes through overactivity of the Ras-ERK and PI3K-mTOR pathways. Seizures are traditionally considered to be a result of an increase in neuronal excitation verses inhibition, though some studies have shown increased inhibitory activity can initiate seizure activity. *Nf1*$^{+/-}$ mice and patients with NF1 have increased GABAergic activity due to altered ERK signaling, which may play a role in increased seizure susceptibility. In this study, I will investigate the roles of excitatory verses inhibitory neurons in seizures and epilepsy in NF1 and determine whether drugs targeting the ERK and mTOR pathways can reverse increased seizure susceptibility.

Purpose: The mechanisms behind epilepsy in NF1 are unknown. ~75% of NF1 patients with epilepsy necessitate multiple anti-seizure medications, potentially leading to severe side effects. Even with multiple medications, up to 40% of NF1 patients with epilepsy continue to have seizures and are medically refractory. Understanding the mechanism of seizures in NF1 is crucial for targeted therapy development for this population and could also benefit the treatments of other populations with epilepsy.

Hypothesis: Decreased levels of neurofibromin in interneurons is sufficient to increase seizure susceptibility in mice. Normalization of mTOR or MEK signaling activity will reverse this increased susceptibility.

Objectives & Methodology: I generated mice with cell-specific deletions of *Nf1* by crossing *Nf1*$^{flox/flox}$ mice with mice expressing Cre recombinase under the control of the parvalbumin (PV; inhibitory neurons) or vesicular glutamate transporter (vGlut; excitatory neurons) promoter. Controls include wild-type (*Nf1*$^{+/+}$) and *Nf1*$^{+/-}$ (*Nf1* mutation present in all cell types) mice, crossed with PV-Cre or vGlut-Cre mice to control for the presence of Cre.

1) Determine which cell type is responsible for the increased seizure susceptibility observed in Nf1$^{+/-}$ *mouse:* Young adult male and female mice of the different genotypes are implanted with intracranial electrodes and undergo continuous video-EEG monitoring to detect spontaneous epileptiform abnormalities and seizures. Following this monitoring, they receive an i.p. injection of 10 mg/kg kainic acid, and a further 180 minutes of video and EEG signals are recorded. This data is being analyzed for latency to first epileptiform spike, first electrographic seizure activity, behavioral seizure severity, and total seizure duration.

2) Determine if MEK or mTOR inhibition will decrease seizure susceptibility: Pharmacologic rescue experiments will be performed in *WT* and *Nf1*$^{+/-}$ mice, plus groups demonstrating increased seizure susceptibility in the previous aim. Implanted mice will receive a daily dose of 5mg/kg s.c. U012669 (MEK inhibitor) or 1 mg/kg i.p. everolimus (mTOR inhibitor) for two weeks. Video-EEG monitoring and kainic acid-induced seizure susceptibility testing will be completed as previously described to determine the effects of pharmacological treatment.

Results to Date: My preliminary results show a decreased latency to first spike and first seizure and increased seizure duration in *Nf1*$^{flox/vGlut}$ mice versus *Nf1*$^{flox/PV}$ mice, suggesting that excitatory cells drive the altered seizure susceptibility we previously reported in *Nf1*$^{+/-}$ mice.

E.5: Individual differences in conditioned pain modulation are associated with functional connectivity between the sgACC and RVM

Student: Janet Li, Supervisor: Karen Davis

Background: Pain perception and coping ability is widely variable amongst people. These individual differences may in part be attributed to the balance of pathways within the dynamic pain connectome (DPC). Namely, the role of the descending antinociceptive pathway (Desc) is pivotal in pain inhibition. Conditioned pain modulation (CPM) is the effect of a painful conditioning stimulus applied to one body area on the pain evoked by a test stimulus applied to a different body area, a "pain inhibits pain" effect. It is thought to reflect the functioning of the endogenous opiate-mediated Desc. and can be used as a surrogate measure of endogenous pain modulation capacity. Functional connectivity (FC) is a measure of synchronous activity between brain areas and, as such, it can provide insight into the efficacy or strength of functionality within a brain pathway.

Purpose: A brain-behavior relationship was identified by the Davis lab for temporal summation of pain (TSP), with enhanced TSP associated with stronger FC within the ascending nociceptive pathway than within the opposing Desc. However, brain-behavior relationships for pain modulation, using CPM as a proxy, remain elusive. The goal of this study is to examine brain-behavior relationships between CPM capability and FC of key regions in the DPC. Specifically, the study objectives are to determine if individual variability in CPM is related to FC within the Desc and whether there are sex differences in this relationship.

Hypothesis: We tested the hypothesis that conditioned pain modulation (CPM), a change in stimulus-evoked pain applied to one region by a painful conditional stimulus applied to another region), reflects functional connectivity (FC) of the Desc.

Materials & Methods: A total of 151 healthy people (72 M, 79 F) were assessed for CPM using painful heat test and conditioning stimuli (TS, CS) applied to the volar forearms. Subjects also underwent resting-state functional MRI (rs-fMRI) and FC was determined between the subgenual anterior cingulate cortex (sgACC), periaqueductal gray (PAG), and rostroventral medulla (RVM).

Results: Participants exhibited one of three types of CPM responses: 1) the classic antinociceptive CPM effect whereby the CS attenuated TS pain (CPM/Antinociception group, 45% of subjects), 2) no significant change in TS pain during a CS (No-CPM group, 15% of subjects), and 3) increased TS pain during the CS (Pronociception group, 40% of subjects). There was no statistically significant correlations of % CPM effect with FC of the PAG with the RVM at a whole group level. However, within the CPM/Antinociception subgroup, FC between the left sgACC and RVM was correlated with the % CPM ($P < 0.05$), as was the FC between the right sgACC and RVM ($P < 0.05$). We also found that within the Pronociceptive subgroup, there was a higher ratio of women (65%) compared to men (35%).

Conclusion: Our main findings that links pain inhibition with FC between the sgACC and RVM highlights the importance of an individual's Desc system underlying their pain experience. Such brain-behavior relationships may provide predictive value to help guide personalized pain management approaches.x`

E.6: Cognitive flexibility in obsessive-compulsive disorder (OCD)

Student: Jenna Baer, Supervisor: Gwyneth Zai

Background: Obsessive-Compulsive Disorder (OCD) is characterized by obsessions with recurrent unwanted and intrusive thoughts, images, or urges, and/or compulsions, that can be physical or mental repetitive behaviours. Individuals with OCD have trouble with cognitive flexibility (CF) as obsessions and compulsions lead to rigid thought patterns. CF can be defined as one's ability to shift and re-focus their attention from a previously relevant task to a new one.

Purpose & Hypothesis: To identify whether there is a difference in CF between participants with OCD and healthy controls (HC) in a small independent sample (N=20, N=20), and in a meta-analysis (N=562, N=541) using a computerized cognitive task, CANTAB® intra-extra dimensional set shift (IED). We hypothesize that those with OCD will perform worse (i.e., having more total # of errors and extra-dimensional (ED) set shift errors) on the IED task when compared to HCs.

Methods: The CANTAB IED task was administered to 20 participants with OCD and 20 HCs. We performed an analysis of covariance (ANCOVA) using the SPSS software to compare the means of intra-dimensional (ID) errors, ED errors, and total errors between these groups. Meta-analyses was conducted using the random-effects model in the Stata program.

Results: In the small independent sample, after controlling for age, the adjusted model showed a significant difference in ED set shifting scores (p=0.004) and total errors (p=0.002), with the OCD group having higher mean errors for both. Meta-analysis results showed a significant difference in ED errors, with the OCD group having higher mean errors (p=0.0001).

Conclusions: Individuals with OCD experience cognitive inflexibility compared to HCs; therefore, the impact of CF must be considered when treating OCD.

E.7: Investigating the relationship between functional connectivity and emotional distress following acute concussion: an fMRI study

Student: Elizabeth R. Waye, Supervisor: Tom A. Schweizer

Purpose & Hypothesis: Sports-related concussions (SRCs) can cause significant emotional distress in the acute phase of injury, including elevated symptoms of anxiety and depression. It is unclear, however, to what extent these symptoms are a consequence of the brain injury itself and to what extent they represent a normal range of emotional distress in response to the injury event. This study uses blood-oxygen-level-dependent functional magnetic resonance imaging (BOLD fMRI) to compare functional connectivity changes related to emotional distress symptoms in concussed athletes and uninjured controls to investigate whether concussion has an abnormal relationship between distress symptoms and brain function.

Methods: A total of 182 varsity athletes, 55 acutely concussed and 127 non-concussed from a single university, underwent eye-closed resting-state fMRI scans and completed the Hospital Anxiety and Depression Scale (HADS). HADS depression and anxiety sub-scores were compared between the groups. Correlations between seed-based connectivity and HADS scores were also evaluated for both groups in regions relevant to depression and anxiety. Additionally, we tested for an interaction effect of HADS and concussion status on connectivity using multiple linear regressions.

Results: Behaviourally, the concussed group demonstrated significantly elevated symptoms of depression, but not anxiety, when compared to controls. Analyses of the amygdala showed an interaction effect of concussion and HADS anxiety score; higher anxiety for concussed athletes was associated with a more extreme increase in connectivity compared to controls. Similar results were observed when analyzing the HADS depression subscale, but to a lesser extent.

Conclusions: Our findings provide new insights into network-level changes that occur acutely following concussion. More specifically, symptoms of post-concussion emotional distress may have a distinct representation in the brain compared to uninjured controls. Translationally, these results may indicate a novel target for future post-concussion rehab interventions.

F: Neuroscience & Brain Health

F.1: Evaluating associations of individual level factors and features of the built environment with physical activity levels in youth with multiple sclerosis

Student: Sonika Kumari, Supervisor: Ann Yeh

Background: Higher levels of physical activity (PA) among youth with Multiple Sclerosis (MS) associate with lower levels of disease activity and better psychosocial outcomes However, youth with MS are inactive. Understanding environmental factors that associate with PA levels can help in designing exercise programs for this population.

Objective: To investigate 1) correlations between built-environment features and physical activity levels among youth with multiple sclerosis, 2) correlations between individual-level variables (self-efficacy and goal setting) and physical activity levels, and to determine 3) the role of individual level variables as a mediator between built-environment and physical activity.

Methods: Participants aged ≤18 years with a diagnosis of MS were included in this cross-sectional study. All participants completed the Godin Leisure-Time Exercise Questionnaire (GLTEQ) (score range 0-119), a health contribution score (HCS) is computed from the total score by adding the moderate and vigorous PA scores. The Canadian urban environmental health research consortium (CANUE) repository was used to access built-environment features using participant's postal codes. The variables were number of intersections, and growing season max, representing greenspace. The walk and transit score scale ranges from 0 to 100 and were extracted from walkability website. Correlational (Pearson and Spearman Rho) and regression analyses were performed. Ethics approval from the SickKids REB was granted and informed consent obtained.

Results: Median GLTEQ score was 43±44 METs/minutes/week. Most participants (n=59, median 15±3 years) were female (36, 61%). Green space at 500m positively correlated with total GLTEQ mets and HCS (rho= 0.3, p=0.012; rho=0.3, p=0.020) but no correlations were found with maximum greenspace value at the postal code. Number of intersections at 1km negatively correlated with total GLTEQ mets and HCS (r= -0.3, p=0.016; r= -0.3, p=0.036). There were no significant correlations of walk score and (r = -0.8, p=0.163) and transit score (rho= -0.1, p=0.306) with GLTEQ mets. As for individual variables, goal setting was positively correlated with total GLTEQ (r=0.4, p=0.003) and self-efficacy was not significantly correlated with GLTEQ (r =0.3, p=0.055). A multiple linear regression was run to predict total GLTEQ mets from green space at 500m, goal setting and the interaction between these two variables. This resulted in a significant model, $F(3, 37) = 4.362$, $p = 0.010$, $r^2=0.201$. The individual predictors and the interaction were not significant.

Conclusions: We found expected relationships between PA and access to greenspaces and an unexpected relationship between intersection density and PA. Reasons for this require further investigation. Additionally, excercise goal setting was significantly associated with PA levels. None of the variables were significant predictors of PA levels in a regression. Future studies will evaluate relationships between safety, park features, and park usage on these associations.

F.2: Repeated ketamine infusions for depression and anxiety: a retrospective naturalistic study

Student: Omer Syed, Supervisor: Peter Giacobbe

Background: Major Depressive Disorder (MDD) is a prevalent and profoundly incapacitating medical condition affecting more than 1 in 10 Canadians throughout their lifetimes. Despite its prevalence, conventional antidepressants can take several weeks to elicit a response, and many may discontinue them due to common side effects. As a consequence, a large proportion of the population experiences little to no relief from first-line treatments. Therefore, there is an urgent need for effective and rapid-acting treatments to address the gap in care for individuals with treatment-resistant depression (TRD).

Purpose & Hypothesis: Assess the real-world effectiveness of repeated ketamine treatments in alleviating depressive symptoms and anxiety. We hypothesized the majority of participants to experience a full- or partial-response in their depression and anxiety symptoms by the end of the 8-week treatment.

Methods: Patients with TRD (n=42) who received a minimum of eight doses of intravenous ketamine (0.5-1.0mg/kg) over the course of 4 weeks were included in the analysis. Psychometric assessments were completed before each treatment session. The primary outcome was self-reported depression symptoms as measured by the Patient Health Questionnaire (PHQ-9) and anxiety symptoms from the 7-item Generalized Anxiety Disorder (GAD-7) questionnaire. Clinical response was characterized by a reduction of ≥50% in these measures, partial response as a reduction of between 25-49%, and no response as a reduction of <25% in symptom scores. Remission was defined as a score of <5 on these questionnaires.

Results: The patient sample had moderately severe depression and severe anxiety at baseline (mean PHQ-9 score 19.2±5.0; mean GAD-7 score 15.9±5.3). There were large and significant antidepressant and anxiolytic effects observed following the ketamine treatments. After 8 ketamine treatments, the patient sample on average had mild depression and mild anxiety (mean PHQ-9 score 9.5±5.8; mean GAD-7 score 9.0±5.2). The one-way repeated measures ANOVA test showed a highly significant effect of the treatment sessions on PHQ-9 and GAD-7 scores ($p<0.0001$). By the end of the treatment, 71.4% of the patients experienced an antidepressant response, 9.6% a partial response, and 19% did not respond. Additionally, 14.3% of the patients achieved a remission for their depression. There were also significant reductions in anxiety symptoms: with 38% of patients achieving a full response, 31% partial response, and 31% did not respond. Moreover, 21.4% of the patients achieved remission for their anxiety. Lastly, patient response to ketamine after the fourth treatment session was indicative of their response at the conclusion of the 8 treatments.

Conclusion: Rapid antidepressant and anxiolytic effects were seen with intravenous ketamine in this cohort of patients with TRD. Additionally, early response to the ketamine treatments was predictive of end of study response.

F.3: Gut microbiome and metabolic dysfunction in antipsychotic naive patients

Student: Kristoffer Panganiban, Supervisor: Margaret Hahn

Background: Psychosis spectrum disorders (PSDs) comprise a debilitating series of mental disorders that have a prevalence rate of 3% and are observed to have a significantly higher risk of developing metabolic disorders. Antipsychotics (APs), the cornerstone of treatment for PSDs, are known to contribute to the risk of metabolic disorders. Although the mechanisms underlying PSD pathophysiology are largely unknown, the gut microbiome (GMB) may be a contributing factor as it has been observed to be dysregulated in other psychiatric illnesses.

Methods: The current study design is cross-sectional, case control and longitudinal. The cross-sectional, case-control aspect of the study involved 21 minimally treated AP patients (defined as AP usage of 3 weeks or less in the past 3 months) with PSDs and 19 healthy controls matched for age, sex, BMI and highest level of parental education. The longitudinal aspect included 14 patients that were followed for 3 months. Alpha and beta diversity measures were used to quantity GMB diversity.

Results: The results showed that there is no significant difference in alpha diversity between patients and controls. However, there is a significant difference in beta diversity, with controls showing higher diversity than patients. It was observed that controls had higher proportions of Prevotella and Bacteroides while having lower proportions of Ruminococcus and Bifidobacterium.

Discussion: The patients showing lower levels of beta diversity indicates that they have an unhealthier gut as more diverse GMBs are historically thought to be healthier. In addition, the proportions of the taxa seen in patients may contribute to psychopathology and metabolic dysfunction.

F.4: The effects of ketamine on suicidal ideation: results from the Canadian Rapid Treatment Centre of Excellence (CRTCE)

Student: David Chen-Li, Supervisor: Joshua Rosenblat

Background: Psychiatric disorders are strongly correlated with suicidality, with depression being one of the most relevant risk factors for completed suicide. The NDMA antagonist and dissociative anesthetic ketamine is an emerging intervention for unipolar and bipolar depression with demonstrated rapid and robust antisuicidal effects.

Purpose: To determine the effectiveness of intravenous (IV) ketamine on measures of suicidality in adults with treatment-resistant major depressive disorder in a community-based outpatient clinical and research centre for adults with treatment-resistant mood disorders.

Hypothesis: Ketamine may produce strong antisuicidal effects in a treatment-resistant, depressed population with existing suicidal ideation.

Methods: A retrospective chart analysis was conducted for patients at the Canadian Rapid Treatment Centre of Excellence (CRTCE). Patients received four infusions of ketamine hydrochloride over a period of 7-14 days at a dose of 0.5mg/kg during their first and second infusions. Patients who did not experience a clinically meaningful benefit were eligible for a dose increase to 0.75 mg/kg. Suicidal ideation was assessed with the Columbia-Suicide Severity Rating Scale (C-SSRS).

Results: C-SSRS data was available for 369 patients at baseline and 323 patients after four infusions. The mean C-SSRS score at baseline was 1.813 (SD = 1.673, n = 369) and 0.161 (SD = 0.599, n = 323) after four infusions, and the change in means was -1.652 (SD = 0.093).

Conclusions: IV ketamine produced rapid and robust antisuicidal effects in a population of patients receiving care in a community-based depression clinic.

F.5: Effects of extended cannabis abstinence on anhedonia in people with co-occurring cannabis use disorder and major depressive disorder

Student: Molly Zhang, Supervisor: Tony George

Background: Major depressive disorder (MDD) is a highly prevalent psychiatric disorder that is one of the most impactful contributors to the global burden of disease. Cannabis has harmful consequences on mental health outcomes, and individuals with cannabis use disorder (CUD) have been found to be at higher risk for MDD. Those with comorbid MDD and CUD demonstrate more severe clinical symptomatology, poorer treatment outcomes, and reduced life satisfaction than patients with just MDD. Anhedonia, which is defined as a loss of interest in or pleasure from previously rewarding activities, is both a core feature of MDD and plays a large role in reward processing and substance use disorders. No studies to date have analyzed CUD and MDD comorbidity with a rigorous study design in relation to anhedonia.

Purpose & Hypothesis: The objective of this study is to explore the effects of cannabis use on clinical symptomology and anhedonia in comorbid CUD and MDD using a controlled 28-day cannabis abstinence paradigm. This study will include both contingent reinforcement (CR) and non-contingent reinforcement (NCR) groups to explore the validity of CR for treatment of CUD,

Hypothesis: We hypothesize that a reduction in, or abstinence from cannabis will result in a greater reduction of subjective and objective anhedonia scores compared to participants who do not abstain. Further, we hypothesis that changes in depression scores will be positively correlated to changes in anhedonia scores.

Methods: Participants (N=13, recruitment ongoing) with co-morbid MDD and CUD underwent a 28-day cannabis abstinence paradigm, including weekly behavioural support sessions. Timeline Follow-Back for cannabis grams per day (GPD) and THC urine analysis was done weekly to assess cannabis abstinence. Anhedonia measures included the Snaith-Hamilton Pleasure Scale (SHAPS), the Dimensional Anhedonia Rating Scale (DARS), and the Motivation and Energy Inventory (MEI), administered weekly, and the Effort Expenditure for Rewards Task (EEfRT) administered at baseline and Day 28, to obtain subjective and objective measures to determine the effects of cannabis use on anhedonia.

Expected Results: We expect to see reductions in anhedonia as measured by the SHAPS, DARS, MEI, and EEfRT in abstainers, but not in non-abstainers. We also expect cannabis abstinence to be more likely from the CR group. A detailed analysis of nascent study data will be presented.

Conclusions: Our findings will hopefully provide insights into the effect of extended cannabis abstinence on anhedonia and other clinical symptoms in people with co-morbid CUD and MDD. Our results will have important implications for understanding and treating cannabis use in people with clinical depression.

F.6: Investigating the effects of nabilone on endocannabinoid metabolism in the human brain

Student: Raesham Mahmood, Supervisors: Isabelle Boileau, Stefan Kloiber

Background: Despite being the most popular recreationally used drug worldwide, little is known about the effects of tetrahydrocannabinol (THC), the main psychoactive component in cannabis, on the endocannabinoid system (ECS). More specifically, it is unknown whether brain levels of fatty acid amide hydrolase (FAAH), the enzyme responsible for the degradation of the endocannabinoid anandamide, is affected by THC exposure. We previously showed that FAAH was significantly reduced in individuals with cannabis use disorder.

Purpose & Hypothesis: The objective of this study is to test whether exposure to nabilone, a synthetic form of THC, is associated with reduced FAAH levels in the brain of healthy participants and whether changes in FAAH are related to self-reported measures of mood and drug-effects.

Methods: Thirty participants will complete two positron emission tomography (PET) scans with FAAH radiotracer [^{11}C]CURB and concurrent arterial blood sampling to generate the plasma radioactivity curve input function – one at baseline and one following one-week of nabilone exposure titrated up to 2 mg daily. Mood and drug effects will be measured with standard questionnaires. Participants will be genotyped for FAAH C385A (rs324420) which affects [^{11}C]CURB binding. MRI will be used to delineate brain regions.. A two-tissue compartment model with irreversible binding will be used to estimate [^{11}C]CURB binding. A repeated measures Analysis of Covariance will be used to analyze [^{11}C]CURB binding. Pearson and Spearman correlations will be done to investigate relationships between changes in [^{11}C]CURB binding and mood and drug effects between PET scans.

Conclusions: This study aims to understand the effects of THC on the ECS and whether self-reported measures of mood and drug effects related to THC can be in part explained by variability in FAAH. Findings from this study could inform the use of THC-based medicine for a broad range of conditions.

Disclosures: Caskey Francis Family Award (IB and SK), Canada Research Chair Tier 2 (IB), University of Toronto Centre for the Study of Pain (UTCSP) (RM) and Toronto Cannabis and Cannabinoid Research Consortium (TC3) (RM) are funding sources that have supported this work.

F.7: Antidepressant class and concurrent rtms outcomes in major depression: a systematic review and meta-analysis

Student: Alina Zaidi, Supervisor: Sean M. Nestor

Background: Repetitive transcranial magnetic stimulation (rTMS) is an established treatment for major depressive disorder (MDD). Evidence suggests that combining rTMS with antidepressant medication may improve treatment outcomes.

Purpose: The aim of this meta-analysis was to assess the clinical effects of antidepressant medication classes when initiated with adjunctive rTMS in MDD.

Hypothesis: We hypothesized that combining rTMS with medication would lead to significantly lower depression scores than medication alone and there would be no class specific effects.

Methods: Systematic searches were performed on MEDLINE, Embase, and PsycINFO from inception to March 2023 (PROSPERO registration: CRD42023418435). Randomized sham rTMS-controlled trials that initiated antidepressants and rTMS in MDD were included. A random-effects model with the DerSimonian and Laird method pooled standardized mean differences across studies. For dichotomous outcomes, the log Odds Ratio (OR) was pooled using the Mantel-Haenszel fixed-effects model. Cochran's Q test assessed heterogeneity. Analyses were conducted separately for antidepressant classes. The primary outcome measure assessed symptomatic improvement measured by formal depression scales. Secondary outcomes assessed response and remission in sham versus rTMS+SSRI/SNRI.

Results: Only SSRIs and SNRIs had sufficient studies to include in the meta-analysis. 3422 articles were screened, resulting in 12 studies, involving 848 participants. For selective serotonin reuptake inhibitors (SSRI): n=543, 63.5% female, mean [SD] age: 41.1[18.7] years, and for selective serotonin and norepinephrine reuptake inhibitors (SNRI): n=178, 69.6% female, 54.1[2.5] years. rTMS+SSRIs led to significantly lower depression scores (SMD=-0.65, p=0.0002), higher response rates (OR=0.87, p=0.0008) and remission rates (OR=0.84, p=0.0046), than the sham group. rTMS+SNRIs was no better than SNRI alone. Heterogeneity was non-significant across all outcomes.

Conclusions: The combination of rTMS with SSRIs led to superior antidepressant effects than SSRI alone, while there was no additive benefit for SNRIs.

F.8: Using speech-in-noise testing to assess hearing asymmetries in children using bilateral cochlear implants

Student: Lulia Snan, Supervisor: Karen Gordon

The objectives of this study were to: 1) use spatial separation of speech and noise to quantify asymmetric hearing in children and 2) to compare results from two different speech in noise tests.

Speech in noise testing can be used to identify speech perception challenges in children with hearing loss. The location of the noise relative to the speech is important; benefits of spatial separation for speech perception are expected due to binaural hearing. Our prior work showed that benefits can be different for noise presented to the left versus right side, highlighting asymmetric hearing abilities in children using bilateral cochlear implants (CIs). Moreover, there may be effects of the speech perception task and the type of noise used due to informational masking. The hypothesis was that effects of hearing asymmetries would be present regardless of the task or type of noise.

Speech perception in noise was measured in two ways: 1) speech recognition thresholds (SRTs) at 0-degree azimuth were measured amidst a 45 dB HL speech-weighted noise in three spatial positions: co-located with speech, at -90 degrees (left), and +90 degrees (right). 2) Accuracy repeating BKB sentences delivered at 52 dB SLP in three spatial positions (0, -45 and +45 degrees) amidst speech babble presented at two speakers located at -90 and +90 degrees. Speech babble was presented to deliver signal to noise ratios (SNRs) from +10 to -30 dB. Twelve children with bilateral CIs participated (9 males, 3 females). Age at testing was 14.51 ± 2.21 years (mean age ± SD); they received their first CI at 3.6 ± 2.19 years of age and had an inter-implant delay of 1.64 ± 4.86 years. Eight were simultaneously implanted and 4 sequentially implanted. A group of typically developing peers (7 males, 5 females, age at test 15.33 ± 1.18 years) were also recruited.

SRT testing revealed benefits of spatial separation between speech and noise in both groups (in both groups (NH: $t = 8.2$, $df = 11$, p-value = 4.9e-06; CI: $t = 2.5$, $df = 9$, p-value = 0.035). In the typically developing group, benefits were greater when noise was at -90 degrees, revealing a right ear advantage for this task. The sentence recognition test was consistent with this finding, demonstrating 75% accuracy for speech located on the right at lower SNR than speech located on the left (two-way interaction: $F(2, 33) = 4.94$, p-value = 0.013). The CI group showed more variable asymmetries with some children having a left ear advantage on the SRT test. Similar asymmetries were found on the speech sentence recognition test in the CI group and there was a significant correlation between these measures ($t = 2.2$, $df = 9$, p-value = 0.048).

The findings reveal that children with normal hearing consistently show a right ear advantage for perceiving speech in noise. By contrast, children with bilateral CIs can show an aural preference for either their right or left ears. Consistencies between speech in noise testing using different speech stimuli and measures and different noises suggest that these asymmetries are robust and can be detected using different speech in noise test paradigms.

G: Cancer

G.1: Meningioma molecular classification predicts response to surgical resection and adjuvant radiotherapy: an integrated clinicomolecular analysis

Student: Justin Wang, Supervisor: Gelareh Zadeh

Background: Meningiomas are the most common primary brain tumor and have heterogeneous outcomes, with a need to study response to surgery and adjuvant RT in the context of novel molecular classifications. The gold standard for treatment of symptomatic meningiomas has been surgical resection including complete removal of tumor and its surrounding dural margin, although the benefit of different degrees of marginal resection remains controversial. Adjuvant radiotherapy (RT) is generally reserved for aggressive or subtotally resected (STR) meningiomas. However, uncertainty exists regarding these tumors' highly variable response to RT which leads to uncertainty regarding optimal case selection for RT clinically.

Purpose & Hypothesis: In this study, we examine the effects of treatment (surgery/RT), molecular, and clinical factors on meningiomas in the context of molecular classifications using propensity score matching to mimic a randomized control trial.

Methods: We accrued a 20-year cohort of 2490 patients with meningiomas across 10 institutions. Molecular data (DNA methylation, RNA sequencing) was generated for 1645 cases. An additional 100 cases from the prospective RTOG-0539 phase II clinical trial was utilized as a validation cohort. Propensity score matching (PSM) was performed for key baseline covariates, including WHO grade and four different molecular classification systems. Progression-free survival (PFS), and overall survival (OS) were assessed based on extent of resection (EOR), Simpson grade, and receipt of adjuvant RT. Lastly, a DNA-methylation based predictor of response to RT was developed using meningiomas treated with adjuvant RT from the RTOG-0539 trial and validated in 2 independent cohorts.

Results: Gross total resection (GTR) was associated with improved PFS across all molecular groups (MG). The PFS benefit of GTR was lessened in molecularly-defined proliferative meningiomas (HR 0.69, 95%CI 0.50-0.95), but OS benefit was enhanced (HR 0.52, 95%CI 0.30-0.93). Surgically treating the dural margin (Simpson grade 1/2) was associated with improved PFS compared to removal of tumor alone (Simpson grade 3) specifically for immunogenic (HR 0.16, 95%CI 0.03-0.82) and NF2-wildtype meningiomas (HR 0.24, 95%CI 0.11-0.53). Adjuvant RT was associated with improved PFS across all molecular classifications. MG could reliably predict response to RT, including when controlling for WHO grade and EOR with immunogenic and NF2-wildtype meningiomas benefitting most while proliferative meningiomas were largely RT-resistant (HR 0.96, 95%CI 0.67-1.37). These results were validated using cases from the RTOG-0539 clinical trial. Moreover, the molecular predictive model developed using the prospective RTOG-0539 cases could predict individualized 3-year PFS after surgery and adjuvant RT more accurately than WHO grade in two additional, independent cohorts (average ΔAUC 0.13).

Interpretations: Molecular classification plays an important role in defining the prognostic benefit of surgical extent of resection and adjuvant RT in meningiomas and can better resolve the variability in treatment outcomes seen with traditional histopathological grading.

G.2: Multi-task machine learning of electronic medical records predicts future symptoms among cancer patients

Student: Baijiang Yuan, Supervisors: Geoffrey Liu & Robert Grant

Background: Cancer affects many people in Ontario, with about 550 out of every 100,000 individuals diagnosed. Chemotherapy is a common treatment that can vary in its side effects from person to person. For some, these effects are minimal, but they can be severe and distressing for others. Predicting which patients might experience worse symptoms during their treatment journey can help healthcare providers take proactive action to reduce these effects.

Purpose & Hypothesis: We aim to develop and integrate early warning systems using artificial intelligence (AI) for symptom deterioration into clinical care. We hypothesize that administrative data can be used to develop and evaluate early warning systems for undesirable events during systemic therapy. Longitudinal models that are guided by multiple targets would outperform models that only include a single target.

Methods: Patients treated for aerodigestive cancers at the Princess Margaret Cancer Centre were randomly divided into development and testing cohorts. For each treatment, machine learning was applied to preceding electronic medical record (EMR) data to predict patient-reported symptom deterioration, defined as at least a four-point worsening on the Edmonton Symptom Assessment Scale. Features included diagnostic and treatment characteristics, laboratory tests, and patient-reported symptoms. Single-task (e.g., LASSO and XGboost) and multi-task (e.g., temporal CNNs, LSTM, and Transformer) models were trained, tuned, and evaluated based on discrimination, calibration, and net benefit.

Results: The cohort consisted of 3,998 patients who underwent 45,904 treatment sessions, with data across 400 features. Among these patients, 1,547 (38.6%) were female; median age was 64.0 (interquartile range 13.0). The most common diagnoses were lung (1,505, 37.6%), head and neck (696, 17.4%), and pancreatic cancers (685, 17.1%). The best model, a multi-task transformer, predicted symptom deterioration with an AUROC range of 0.732-0.822, marking a 1.4-6.2% improvement over the best single-task model. At a 10% alert rate, treatments associated with alerts would be enriched 4-13 fold for symptom deterioration ($P<0.001$). The system was calibrated and would provide a net benefit across a wide range of threshold probabilities in decision curve analysis.

Conclusions: Longitudinal general-purpose multi-task machine learning systems trained using EMR data can accurately predict a wide range of symptoms. Based on these results, automated warning systems for symptoms should be implemented and evaluated in real-time clinical practice to guide preventative interventions.

G.3: Brain metastases among patients with gynecologic cancers: a single centre retrospective study of patients treated with brain radiotherapy

Student: Rania Chehade, Supervisors: Katarzyna Jerzak, Sunit Das

Objective: Brain metastases (BrM) among patients with gynecological cancers (GC) have historically been considered rare events. We aimed to characterize treatment patterns and outcomes of patients with GC and BrM.

Methods: We conducted a retrospective analysis of patients with gynecological cancers and BrM who were treated with whole brain radiotherapy (WBRT) or stereotactic radiation (SRS) to the brain at the Sunnybrook Odette Cancer Centre, Toronto between 2010 and 2022. Descriptive statistics and survival analyses were performed using R software. Median follow up from time of BrM development was 7.5 (range 2.9 - 15) months.

Results: We identified 94 patients with BrM who had primary gynecological cancers (GCs). Median age at time of BrM diagnosis was 66 (range 30-85) years and the median time from primary diagnosis of primary GC to development of BrM was 28.5 (range 0 - 218.1) months. Most patients with BrM presented with neurologic symptoms (95.7%, n=90) and multiple BrM (61.7%, n=58). All patients received radiotherapy; 63% (n=59) underwent SRS delivered in 1 to 5 fractions, 36% (n=34) received WBRT and one patient received WBRT plus an SRS boost. 40.4% (n=38) also had surgery for BrM.

Patients with Endometrial cancer (EC) accounted for 54.3% (n=51) of cases followed by ovarian cancer (OC) (25.5%, n=24) and cervical cancer (CC) (17%, n=16). Among patients with EC, 41% (n=21) had endometrioid histology, 24% (n=12) serous, 14% (n=7) carcinosarcoma, and 7.8% (n=4) sarcoma. Among patients with endometrioid EC, 69% (n=35) were high grade and 22% (n=11) were low grade; tumor grade was unknown in 5 cases. Where molecular status was known, BrM occurred in 33.3% (n=4/12) of patients with deficient mismatch repair protein (MMRd) ECs and 83.6% (10/12) of patients with protein 53 (TP53) overexpression. High grade serous (HGSC) was the most common subtype among patients with OC, representing 83.3% (n=20) of cases. Both squamous (44%, n=7) and adenosquamous (31%, n=5) histology were observed among patients with CC (n=16). Two patients with neuroendocrine CC developed BrM. In the overall cohort, the median overall survival (OS) from the time of BrM diagnosis was 10.6 months (0.1-143). The median OS among patients with OC and BrM (27.2 months) was longer than for those with EC (7.6 months) or CC (5.8 months), p=0.0034.

Conclusion: Among patients with GC and BrM in our cohort, the most common primary malignancy was EC and about two thirds of patients were treated with SRS. Patients with OC and BrM lived longer than those with other primary GC cancers. Investigation of molecular events that “drive” the development of BrM among patients with GCs is warranted.

G.4: Somatic tissue testing (STT) in prostate cancer using the Oncomine V3 panel: a single-institution retrospective analysis

Student: Lilian Hanna, Supervisor: Urban Emmenegger

Background: Approximately 20-30% of prostate cancer (PC) patients have a mutation in the homologous recombination repair or mismatch repair genes, 50% of which are somatic. These mutations have prognostic and predictive implications. Somatic tissue testing (STT) was implemented at Sunnybrook Health Sciences Centre using the Oncomine V3 panel, which includes 161 genes, but only four genes (BRCA1/2, ATM, PALB2) are reported due to funding restrictions. We describe our centre's testing experience and the utility of reporting beyond these four genes.

Methods: Sixty-two PC patients between May 1, 2021 - January 31, 2023 were included. Clinical characteristics were gathered via chart review. Somatic variants of genes of interest (GOI) within the Oncomine V3 panel were extracted by an annotation specialist.

Results: STT was ordered at diagnosis in 69.4% of cases (n = 42) vs. at progression in 27.4% (n = 17), 3 cases were external and lacked clinical context. Tests were ordered in the locoregional disease setting in 46.8% (n = 29), 37.1% (n = 23) in the metastatic castrate-sensitive setting, 8.1% (n = 5) in the metastatic castrate-resistant (CR) setting and 3.2% (n = 2) in the non-metastatic CR setting. Tests were ordered by Urology or Pathology (reflex testing) in 64.5% (n = 40), by Medical Oncology in 16% (n = 10), by Radiation Oncology in 13% (n = 8) and by others in 6.5% (n = 4). Fifty-one samples (82.2%) were of the prostate and 11 (17.7%) were of metastatic sites. The median sample age was 8 days (range 0-5781). The median test turn-around time was 28.5 days (range 5-57). There were 378 variants within the GOI and meeting the limit of detection (allele frequency of ≥5% and coverage ≥ 500), of which 63 (16.7%) were tier 1 or 2 and are listed by gene as follows: *Tier 1:* ATM(5), BRCA2(2), CDK12(5), FANCA(3), NBN(1); *Tier 2:* AKT1(2), BRAF(1), CTNNB1(3), GNAS(1), HRAS(1), MED12(1), MET(1), NBN(1), PALB2(1), PIK3CA(4), PIK3R1(7), PMS2(1), PTEN(1), RB1(1), RNF43(2), SETD2(1), SPOP(6), TP53(12). Eight of 63 variants (12.7%) were within the four funded genes whereas 55/63 (87.3%) were within unfunded genes of clinical significance. CDK12, FANCA, NBN (n = 7, 11.2%) are investigated in poly (ADP-ribose) polymerase inhibitor trials and PMS2 deficiency (n = 1, 1.6%) can select for immune checkpoint inhibitors. PIK3R1/PIK3CA/AKT1-mutated tumors (n = 11, 17.7%) have shown response to AKT-inhibitors. SPOP-mutated tumors (n = 6, 9.7%) are associated with prolonged response to androgen receptor blockade whereas PTEN/TP53/RB1-negative tumors (n = 12, 19.4%) are associated with anti-androgen resistance and worse prognosis.

Conclusions: The majority of STT variants identified were beyond the currently funded genes in Ontario, and many have important prognostic and predictive implications. This highlights that STT early in the disease course using expanded gene panels may help in delivering personalized PC care.

G.5: Identification of XPO1 cargo and modulation by SINE compounds in diffuse large B cell lymphoma

Student: Kyla Trkulja, Supervisors: Armand Keating, John Kuruvilla, Rob Laister

Background: Exportin 1 (XPO1) facilitates the export of proteins from the nucleus to the cytoplasm of eukaryotic cells and is overexpressed in almost all cancers. Selinexor, a selective inhibitor of nuclear export (SINE) inhibits XPO1 and is approved as a treatment for relapsed/refractory diffuse large B cell lymphoma (R/R DLBCL). However, critical knowledge gaps remain in understanding XPO1 biology and exported cargo, how they contribute to lymphoma, and the downstream consequences of XPO1 inhibition that lead to anti-cancer effects. These knowledge gaps are preventing selinexor from being used in effective combination therapies in the clinic.

Purpose & Hypothesis: This research is being conducted to identify what cargo proteins are exported by XPO1 in DLBCL and how selinexor is able to modulate these processes. Identification of XPO1 cargo relevant to DLBCL will facilitate an improved understanding of selinexor's mechanism of action in this disease to inspire effective combination therapies with the drug.

Methods: The initial steps for proximity-dependent biotinylation labelling (BioID) using the miniTurbo protein were performed on DLBCL cell lines. 16 DLBCL cell lines were screened for mRNA and protein expression of XPO1 and their sensitivity (IC50) to selinexor. A nuclear-cytoplasmic fractionation protocol was optimized in these cells and Western blot was used to determine optimal selinexor treatment conditions for effective XPO1 inhibition. Lastly, lentivirus for gene delivery of miniTurbo and a miniTurbo-XPO1 fusion was synthesized and used to infect DLBCL cells, which were then supplemented with 50µM biotin. Whole cells lysates were obtained and subject to Western blot to detect the presence of biotinylated proteins and verify activity of the system.

Results: Higher levels of XPO1 expression were associated with a decreased sensitivity to selinexor, in agreement with trends found in patient settings. Western blot following nuclear-cytoplasmic fractionation confirmed that treatment of DLBCL cells with 0.5µM selinexor for 24 hours was sufficient for XPO1 inhibition, as seen by nuclear accumulation of verified XPO1 cargo molecules. Detection of FLAG at the appropriate molecular weights in cells made to express both miniTurbo and XPO1-miniTurbo fusion proteins demonstrated successful gene expression, and these cells also showed a greater presence of biotinylated proteins when supplemented with biotin than non-infected cells, demonstrating activity of the system. Future work will complete the BioID assay by identifying these biotinylated proteins by mass spectrometry analysis.

Conclusions: Feasibility of expressing a proximity-dependent biotinylation system in DLBCL cells has been demonstrated. Optimal conditions for the assay have also been determined; these conditions will be replicated for the BioID experiment to identify XPO1 cargo that can be modulated by selinexor treatment in DLBCL to inform on potentially effective combination therapies that can be used in the clinic.

G.6: Variants in ATRIP are associated with breast cancer susceptibility in the polish population and UK Biobank

Student: Neda Zamani, Supervisor: Mohammad R. Akbari

Background: Breast cancer is the most frequent malignancy and the first leading cause of cancer-related mortalities in women globally. Inherited breast cancer cases account for ~5%-10% of all cases. Deleterious mutations in *BRCA1* and *BRCA2*, the two main breast cancer susceptibility genes, account for most hereditary breast cancer cases. Over the past decades, several other genes have been suggested as moderately or highly penetrant breast cancer susceptibility gene candidates. Based on two very large association studies, published in January 2021 in the *New England Journal of Medicine*, a core panel of ten genes including *ATM*, *BARD1*, *BRCA1*, *BRCA2*, *BRIP1*, *CHEK2*, *PALB2*, *RAD51C*, *RAD51D*, and *TP53* can be regarded as confirmed moderately to highly penetrant breast cancer susceptibility genes. However, the search for novel breast cancer susceptibility genes is ongoing, since mutations in these genes cannot explain a large proportion of familial cases.

Hypothesis: Almost half of familial breast cancer cases are negative for pathogenic mutations in the already known breast cancer susceptibility genes. Therefore, we hypothesize that a proportion of these cases is due to rare mutations in moderately to highly penetrant predisposition genes that are not identified yet.

Methods: To address this gap, we applied whole-exome sequencing (WES) on the germline DNA of a cohort of 510 highly familial breast cancer patients with unknown genetic etiology and 308 unaffected women from the founder population of Poland. The strategy for the discovery phase was to identify candidate genes that may be associated with an increased risk of breast cancer. Then, through a subsequent validation phase, we investigated the role of recurrent loss-of-function (LoF) variants in the identified candidate genes with breast cancer risk among a larger set of Polish women with breast cancer and healthy control subjects. Findings were also explored in predisposition to breast cancer among the UK Biobank study population.

Results: At the discovery phase, we identified a rare mutation in *ATRIP* (GenBank: NM_130384.3: c.1152_1155del [p.Gly385Ter]) in two women with breast cancer. At the validation phase, we found this variant in 42/16,085 unselected Polish breast cancer-affected individuals and in 11/9,285 control subjects (OR = 2.14, 95% CI = 1.13–4.28, p = 0.02). By analyzing the sequence data of the UK Biobank study participants (450,000 individuals), we identified *ATRIP* LoF variants among 13/15,643 breast cancer-affected individuals versus 40/157,943 control subjects (OR = 3.28, 95% CI = 1.76–6.14, $p < 0.001$). We showed that tumors of women with breast cancer who have a germline *ATRIP* mutation have loss of heterozygosity (LOH) at the site of *ATRIP* mutation and genomic homologous recombination deficiency (HRD).

Conclusion: ATRIP is a critical partner of ATR that binds to RPA coating single-stranded DNA at sites of stalled DNA replication forks. Proper activation of ATR-ATRIP elicits a DNA damage checkpoint crucial in regulating cellular responses to DNA replication stress. Based on our observations, we conclude *ATRIP* is a breast cancer susceptibility gene candidate linking DNA replication stress to breast cancer.

G.7: Evaluating the feasibility and acceptability of a tailored multicomponent rehabilitation program for adolescent and young adult (AYA) cancer survivors

Student: Lauren Corke, Supervisor: Jennifer Jones

Background: Due to advances in treatments, the large majority (>80%) of adolescents and young adults (AYAs; ages 15-39 years) diagnosed with cancer are expected to survive beyond 5 years. AYAs with cancer experience many unique challenges, including disruptions to major life milestones, formation of intimate relationships, financial independence, and family development. In addition, AYA cancer survivors are at increased risk of persistent and late adverse effects. As a result, there have been calls for the development of tailored interventions to minimize dysfunction and maximize well-being for this growing and distinct population.

Purpose: In response, we adapted a tailored multidimensional cancer rehabilitation to address the needs of AYA cancer survivors (CaRE-AYA). The current pilot study is being conducted to: 1) Assess the feasibility and acceptability of CaRE-AYA; and 2) Obtain preliminary estimates of variability in clinical outcomes including disability, physical and social functioning, mood, and quality of life.

Hypothesis: It is hypothesized that the CaRE-AYA program will be both feasible and acceptable for AYA participants.

Methods: Single-arm, explanatory sequential mixed-methods study conducted at Princess Margaret Cancer Centre, Toronto, Canada. CaRE-AYA is an 8-week tailored group program that includes individualized exercise prescribed by a registered Kinesiologist and weekly group-based exercise and self-management skills education addressing topics related to AYA cancer survivorship. Feasibility outcomes include participation and retention rates, and program adherence. Acceptability is being assessed through surveys (n=15) and qualitative interviews (n=7) with a subset of participants. Clinical outcome data is collected pre and post (immediate and 3-month) intervention through questionnaires and fitness assessments.

Results: To date, 15 participants (33.2 ± 5.5 years old) have been enrolled (Groups 1, 2, & 3), and 14 (93%) completed the program. Adherence was 76% to the exercise classes and 77% to the education classes. Feedback to date includes a later start time, more interactive components, and modifications to the educational content. Participants reported high satisfaction with the program; 100% rated the material as important and relevant to AYA survivors. One additional pilot group (n=11) is underway.

Conclusion: CaRE-AYA program appears to be feasible and acceptable. Updated data will be presented.

G.8: Targeting the anti-oxidant protein peroxiredoxin-4 in pancreatic ductal adenocarcinoma to initiate anti-tumour immune responses

Student: Vishal Pandya, Supervisor: Marianne Koritzinsky

Background: Although immunotherapy has proven successful in some aggressive cancers, pancreatic ductal adenocarcinoma (PDAC) remains resistant to various immune checkpoint inhibitors. To sensitize PDAC to immunotherapy, we aim to leverage a metabolic vulnerability. Aggressive PDAC reprogram metabolism and produce toxic levels of reactive oxygen species; PDAC tolerates this through expression of anti-oxidant proteins, like peroxiredoxin-4 (PRDX4). By interrupting the redox balance, cancer cells may suffer oxidative damage that can activate inflammatory signalling and subsequently the immune system. We propose targeting PRDX4 to sensitize PDAC to immunotherapies.

Hypothesis: We hypothesize that targeting PRDX4 in PDAC leads to inflammatory cytokine secretion from cancer cells, innate immune response via macrophages, and cytotoxic tumour immune infiltrate.

Methods: We measured changes in cytokine secretion after PRDX4 knockdown in PDAC cells using multiplexed enzyme-linked immunosorbent assay. To determine whether cytokine secretion can influence macrophage behaviour, we bathed macrophages in tumour conditioned media or co-cultured macrophages with PRDX4 knockdown PDAC cells. We then measured macrophage polarization using flow cytometry. Finally, we used tamoxifen-inducible PDAC mouse models (KPC-PRDX4$^{KO/Het/WT}$: KrasG12D; p53$^{fl/fl}$; Pdx1-CreER; PRDX4$^{KO/Het/WT}$) to determine whether targeting PRDX4 in-vivo can delay tumour growth and influence the tumour-immune microenvironment.

Results: We showed that targeting PRDX4 in PDAC cells leads to inflammatory cytokine secretion (CXCL10, CCL5, and CCL20). Our preliminary data show that tumour conditioned media from PRDX4 knockdown PDAC cells increases inflammatory cytokine production in macrophages. Our in-vivo model requires tamoxifen to induce tumourigenic mutations. We have shown that our mice tolerate 150mg/kg bodyweight tamoxifen treatment. Based on these results, we have administered tamoxifen and enrolled KPC-PRDX4$^{KO/het/WT}$ mice into survival and disease progression studies to asses the effect of PRDX4 levels on tumour growth and tumour immune infiltrate.

Significance: This project may contribute supporting evidence for targeting cancer metabolism, particularly antioxidant systems, as a mode for treating PDAC.

H: Cardiovascular, Respiratory, & Musculoskeletal

H.1: Comparison of machine learning (ML) and conventional statistical modeling for predicting readmission following acute heart failure hospitalization

Student: Karem Abdul-Samad, Supervisor: Douglas S. Lee

Background: Developing accurate models for predicting the risk of 30-day readmission for heart failure (HF) patients has been a major healthcare interest. Evidence suggests that models developed using machine learning (ML) may have better discrimination than conventional statistical models (CSM), but the calibration of such models is unclear.

Objectives: To compare models developed using CSM or ML to predict 30-day readmission for cardiovascular and non-cardiovascular causes in HF patients.

Methods: We studied 10,919 patients with HF (> 18 years) discharged alive from a hospital or emergency department (2004-2007) in Ontario, linked to administrative databases for hospitalization and vital status resulting in complete follow-up. The study sample was randomly divided into training and validation sets in a 2:1 ratio. CSMs to predict 30-day readmission were developed using Fine-Gray subdistribution hazards regression (treating death as a competing risk), and the ML algorithm employed random survival forests. Models were evaluated in the validation sample using discrimination and calibration metrics.

Results: In the validation sample of 3602 patients, Random Survival Forests (c-statistic = 0.620) showed similar discrimination to the Fine-Gray model (c-statistic = 0.621) for 30-day cardiovascular readmission. In contrast, for 30-day non-cardiovascular readmission, the Fine-Gray model (c-statistic = 0.641) slightly outperformed the random survival forests model (c-statistic = 0.632). For both outcomes, the Fine-Gray model displayed better calibration than random survival forests when plots of observed vs. predicted risks were compared.

Conclusions: In HF patients, time-to-event analysis of outcomes using Fine-Gray models had similar discrimination but superior calibration to ML models, highlighting the importance of reporting calibration metrics for ML-based prediction models.

H.2: Prevalence and persistence of CKD and hypertension at 36 months following cisplatin therapy in children

Student: Salma Abrahim, Supervisor: Michael Zappitelli

Background: Cisplatin is a nephrotoxin known to cause acute kidney injury (AKI) in children treated for cancer. This association requires further investigation to determine long-term kidney outcomes including chronic kidney disease (CKD) and elevated blood pressure (eBP) after cisplatin treatment.

Purpose & Hypothesis: In children treated for cancer, purpose is to: 1) Estimate rate of CKD and elevated BP or worse (≥eBP), 36 months post-cisplatin therapy; 2) Determine if cisplatin-AKI is associated with CKD and hypertension (HTN) at 36 months. Hypothesize that CKD and ≥eBP are prevalent at 36 months post cisplatin therapy, and cisplatin-AK during therapy is associated with these outcomes.

Methods: ABLE study was a 12-site Canadian prospective study that evaluated short- and long-term Cisplatin nephrotoxicity. Exclusion criteria: eGFR of <30 ml/min/1.73m2 pre-cisplatin treatment or pre-existing renal transplantation. Urine, blood, BP and lab data were collected at 3, 12, and 36-months post-cisplatin therapy. Main exposure: AKI throughout cancer therapy defined according to KDIGO guidelines. Primary outcomes: CKD was defined as eGFR <90ml/min/1.73m^2 or albuminuria (urine albumin to creatinine ratio (ACR) of ≥30 mg/g or ≥3 mg/mmol). eBP or worse was defined as per child HTN guidelines. Secondary outcomes: individual outcomes make the composite primary outcomes; composite of CKD or ≥eBP. Pearson's Chi-Squared test or Fisher's Exact test was performed to evaluate the association between AKI and 36-month CKD/HTN.

Results: Of 101 participants at 36 months, 32.3% had CKD; 29.5% had ≥eBP. Throughout cisplatin therapy, 42.6% developed AKI. Of patients who developed AKI during treatment, 58.3% developed CKD or elevated BP or HTN. Participants who had AKI throughout treatment had a significantly higher chance of having low eGFR or ≥eBP at 36 months.

Conclusions: Hypertension and CKD were common at 36 months post-cisplatin. Individuals who developed AKI throughout cisplatin therapy were more likely to have worse cardiovascular and kidney outcomes; interventions to improve these outcomes should be investigated.

H.3: High-flow nasal delivered through different cannulas: a bench study of their respective physiological effects

Student: Fernando Vieira, Supervisor: Laurent Brochard

Background: High-flow nasal cannula (HFNC) is a non-invasive therapy widely used clinically for acute respiratory failure. It has specific physiological effects based on the flow profile and pressure generated in the upper airways. We showed previously that this technique has beneficial effects through an increase in resistance. Different nasal cannula used to deliver HFNC may have different physiological effects, still largely unknown.

Purpose & Hypothesis: To compare a standard cannula (OPT944, Fisher&Paykel), with an asymmetrical cannula (OPT966), and an adapted single-nostril cannula in a simulated model of ventilation. We compare nasopharyngeal pressure (NP), inspiratory and expiratory resistance and upper airway washout of CO_2 with capnography.

Methods: A manikin equipped with airways was connected to an active breathing simulator (ASL5000), set to deliver a controlled tidal volume, at various respiratory rates (10 to 40 breaths/minute). Nasal cannulas were applied to the manikin's nostril. The high-flow varied up to 60 L/min. CO_2 was delivered to simulate a constant CO_2 production (300mL/min).

Results: The asymmetrical cannula generated a higher NP than standard and single-nostril cannulas; 5.5, 3.2, and 2.4cmH_2O at 60L/min, respectively. Inspiratory and expiratory resistances were higher with the asymmetrical cannula than with the other cannulas. The CO_2 washout from the upper airways was higher with the single nostril cannula, followed by the asymmetrical, and by the standard. Increasing the RR reduced the washout effect for all cannulas.

Conclusions: The asymmetrical cannula generated a higher pressure and better upper airways washout than the standard one. Single-nostril cannula generated low pressure and airway resistance with the best washout. A realistic upper airway model and a physiological study in healthy volunteers will be performed to further investigate these preliminary findings.

H.4: Diagnostic accuracy of community spine X-rays for adolescent idiopathic scoliosis

Student: Dorothy Kim, Supervisor: Andrew Howard

Background: Adolescent idiopathic scoliosis (AIS) is the most prevalent paediatric spine condition affecting 3-5% of children. Growth presents the greatest risk in this progressive spine deformity. Timely diagnosis allows for effective conservative management through brace treatment, potentially avoiding complex spine surgery. Alarmingly, approximately one third of patients are late referrals at initial presentation with curves at high risk of surgical intervention and less than 20% are brace candidates. The onus of AIS detection and specialist referral is on primary care physicians (PCPs). PCPs rely heavily on community radiology reports to confirm their clinical assessment prior to spine specialist referral. However, community spine x-rays may be of inadequate quality and inaccurately interpreted.

Purpose & Hypothesis: Study objectives were to determine 1) the diagnostic accuracy of community spine x-rays for brace candidates by Cobb angle, 2) the agreement of Cobb angle readings between community radiology and tertiary care clinicians and 3) if inaccurate measurements were associated with late referrals. We hypothesize that the accuracy of community spine radiology is suboptimal for AIS, impacting clinical decisions and timely care.

Methods: A retrospective review of AIS patients (n=281) seen for initial visit at a tertiary care paediatric hospital between January-September 2021 was conducted, excluding those seen for second opinion, missing index Cobb angle or with index imaging from the same institution (n=170). Index test was the community spine x-ray evaluated by community radiologist. Reference standard was the 3-foot standing spine x-ray evaluated by spine specialist. Diagnostic criterion for a brace candidate was dichotomized by Cobb angle (20-45°). Images were compared if obtained within 90 days (n=111). Available index x-rays (n=119) were remeasured by two blinded raters. Agreement statistics for Cobb angle and corresponding management between community radiology and tertiary care clinicians were calculated. Logistic regression was used to estimate odds of late referrals from the discrepancy in Cobb angle readings between index test and gold standard when images were within 90 days. Discrepancies were defined as differences in Cobb angle measurements > 5°.

Results: Accuracy of the index test to detect a brace candidate was 65.8% (95% CI 56.2-74.5). Sensitivity was 65.4% with a false negative rate of 34.6%. Specificity was 66.1% with a false positive rate of 33.9%. Of true brace candidates (n=52), 32.7% were missed because of underestimation (95% CI 21.5-46.2). Comparing community radiologist and spine specialist, agreement in Cobb angle and management on index x-ray was moderate (ICC=0.78 95% CI 0.66-0.86, SEM=6.14°, κ=0.58). On the same image, agreement between community radiologist and paediatric radiologist remained moderate (ICC=0.74 95% CI 0.65-0.81, SEM=6.73°, κ=0.65). Comparatively, agreement between spine specialist and paediatric radiologist was excellent (ICC=0.96, 95% CI 0.89-0.98, SEM=2.57°, κ=0.73). The odds of late referral increased with inaccuracies in index test (OR=3.52 95% CI 1.90-6.53), after adjusting for confounders.

Conclusion: Inaccuracies in community radiology impact timely referrals, contributing to an increased number of avoidable spine surgeries due to missed opportunities for conservative treatment.

H.5: Predicting major adverse carotid cerebrovascular events in patients with carotid artery stenosis: integrating a panel of plasma protein biomarkers and clinical features

Hamzah Khan, Supervisor: Mohammad Qadura

Background: Carotid artery stenosis (CAS) is an atherosclerotic disease of the carotid artery and can lead to devastating cardiovascular outcomes such as stroke, disability, and death. The current available treatment for CAS is medical management through risk reduction, including control of hypertension, diabetes, and/or hypercholesterolemia. Surgical interventions are currently suggested for patients with symptomatic disease with stenosis >50% where patients have suffered from a carotid related event such as a cerebrovascular accident, or asymptomatic disease with stenosis >60%, if the long-term risk of death is <3%.

Purpose: To reduce CAS related adverse events, cardiovascular risk optimisation is typically initiated once CAS diagnosis is established. However, there is a lack of current plasma protein biomarkers available to predict patients at risk of such adverse events.

Hypothesis: A panel of plasma biomarkers, when modeled using machine learning may predict adverse outcomes such as stroke, need for surgery, and death in patients with CAS.

Methods: In this study, we investigated several growth factors and biomarkers of inflammation as potential biomarkers for adverse CAS events such as stroke, need for surgical intervention, myocardial infarction and cardiovascular related death. Machine learning models including decision trees, random forest, and support vector machine were used to construct a model using plasma biomarkers and clinical features.

Results: Using support vector machine, random forest models, and the following four significantly elevated biomarkers; C-X-C Motif Chemokine Ligand 6 (CXCL6), Interleukin-2 (IL-2), Galectin-9 and angiopoietin-like protein (ANGPTL4), a model was created that predicted carotid cerebrovascular events with an area under the curve (AUC) of >0.8, demonstrating strong prognostic capability.

Conclusions: The combination of biomarkers suggested in this study demonstrate a strong capability to predict adverse outcomes in patient with CAS.

H.6: A scoping review of the use of magnetic resonance imaging in spinal cord ischemia

Student: Angela Lee, Supervisor: Thomas Lindsay

Background: Spinal cord ischemia (SCI) presenting as paresis and paraplegia are a devastating complication seen after thoracoabdominal aortic aneurysm (TAAA) repairs. SCI is diagnosed by neurological deficits on physical exam. However, imaging modalities, such as magnetic resonance imaging (MRI), help confirm the sequalae of SCI on the spinal cord and possible SCI etiologies. Currently, there are no vascular surgery guidelines on the use and timing of MRIs in SCI.

Purpose & Hypothesis: We aim to identify different practices of MRI spine use in the evaluation of spinal cord ischemia post-aortic surgery within the literature.

Methods: A scoping review of the literature was conducted following the Preferred Reporting Items for Systematic review and Meta-Analyses extension for Scoping Reviews (PRISMA-ScR) guidelines. The databases of EMBASE, PubMed, and the Cochrane Library were searched. All publications prior to August 1, 2023 were considered. There was no restriction in study method or design. The review included studies examining patients ages 18 years or older who had SCI post-aortic surgery, that underwent MRI-spine. Studies examining solely traumatic spinal cord injury or SCI due to hematoma were excluded. A title, abstract and full-text screen was conducted by two independent reviewers. The study design, sample size, surgical strategy, onset of neurological deficits, timing of MRI, sequencing of imaging, and signal abnormalities were extracted from accepted manuscripts.

Results: Following the removal of duplicates, 254 titles were screened and 46 abstracts and full-texts were assessed for eligibility. Twenty-four studies were included in the review. Sixteen studies were single-patient case reports, one study was a multi-patient case report, six studies were retrospective reviews and one study was a prospective observational study. A total of 129 patients with MRI-spine for SCI post-aortic surgery were included in the review. All studies used T2 sequencing, however, only four of 24 studies used diffusion-weighted imaging sequencing (DWI). The timing of MRI varied from immediately upon the discovery of neurological deficits to 3 months following neurological deficits. Thirteen of the studies had at least one patient with a MRI-spine conducted within 24 hours of neurological deficits.

Conclusions: The practices regarding the use of MRI to evaluate SCI post-SCI is quite variable within the literature. Variability in imaging timing is seen between institutions, but also within the same institution. All studies used T2 sequence, however the vast majority of studies omitted the DWI sequence, which is more sensitive to acute changes compared to T1 and T2 sequences.

H.7: Machine learning in pediatric Brugada syndrome: can electrocardiographic parameters diagnostically discriminate in children?

Student: Meena Fatah, Supervisors: Robert M. Hamilton, Mike Seed

Asymptomatic children of individuals with Brugada Syndrome (BrS) often present with normal electrocardiograms (ECG) that cannot be grossly distinguished from asymptomatic BrS or healthy children, contributing to their challenging clinical management. Using machine learning (ML), we identified ECG parameters that discriminate between these three groups.

The study included ECGs from children with BrS (n=22, 59% male), asymptomatic children with BrS family history (n=73, 59% male, a priori risk of ≤ 50%), and healthy children (n=161, 53% male). From standard 12-lead ECGs, 192 measures were obtained using GE's MUSE measurement-matrix (MM). ML was used to create prediction models and a principal component analysis was conducted to stratify and determine correlations of the top measures.

The S-wave durations in leads V4-V6, I, II, R-wave in aVR, ST level at the J-point in V5-V6, aVR, and at the S-wave end in V2 discriminated BrS from controls (ROC AUC 0.96; 95% CI: 0.58, 0.99). ST segment at J point in V1, V4-V6, III, aVR, S-wave mid and end in V1, and S-wave duration in V5-V6 discriminated referred children from controls (ROC AUC: 0.86; 95% CI: 0.75, 0.94). Using the ML model BrS vs Control, 33% of asymptomatic children were reclassified to BrS.

This study is the first to use ML to define novel ECG parameters that sensitively and specifically discriminate between children with BrS and controls and identify a range of intervening measurements in asymptomatic children referred for assessment. Discriminatory ECG parameters could be used to diagnostically stratify children at risk for BrS.

I: Endocrine & Gastroenterology

I.1: An assessment of adaptation and fidelity in the implementation of an audit and feedback-based intervention to improve transition to adult type 1 diabetes care in Ontario, Canada

Student: Syed Zain Ahmad, Supervisor: Rayzel Shulman

Background: The fit between an intervention and its local context may affect its implementation and effectiveness. Researchers have stated that both fidelity (the degree to which an intervention is delivered, enacted, and received as intended) and adaptation to the local context are necessary for high-quality implementation. This study describes the implementation of an audit and feedback (AF)-based intervention to improve transition to type 1 diabetes adult care, at five sites, in terms of adaptation and fidelity.

Methods: An audit and feedback (AF)-based intervention for healthcare teams to improve transition to adult care for patients with type 1 diabetes was studied at five paediatric sites. The Framework for Reporting Adaptations and Modifications to Evidence-based Implementation Strategies (FRAME-IS) was used to document the adaptations made during the study. Fidelity was determined on three different levels: delivery, enactment, and receipt.

Results: Fidelity of delivery, receipt, and enactment were preserved during the implementation of the intervention. Of the five sites, three changed their chosen quality improvement initiative – however, within the parameters of the study protocol, therefore, fidelity was preserved whilst still enabling participants to adapt accordingly.

Conclusions: We describe implementing a multi-center AF-based intervention across five sites in Ontario to improve the transition from paediatric to adult diabetes care for youth with type 1 diabetes. This intervention adopted a balanced approach considering both adaptation and fidelity to foster a community of practice to facilitate implementing quality improvement initiatives for improving transition to adult diabetes care. This approach may be adapted for improving transition care for youth with other chronic conditions and to other complex AF-based interventions.

I.2: Feeding regulatory mechanism of leptin action in the nucleus of the solitary tract and area postrema

Student: Kyla Bruce, Supervisor: Tony Lam

Background: Obesity is associated with brain leptin resistance. Leptin action in the hypothalamus activates STAT3 to lower feeding and weight. However, it is not fully understood how regions outside the hypothalamus with leptin receptors, such as the nucleus of the solitary tract (NTS) and area postrema (AP) of the hindbrain, contribute to leptin's effect and resistance. While peripheral leptin administration of leptin activates STAT3 in both the hindbrain and hypothalamus, the direct impact of leptin on the NTS and AP in controlling feeding and weight management remains unclear.

Hypothesis: Leptin action and resistance in the NTS and/or AP alter feeding.

Purpose: The outcomes of these experiments hold the promise of elucidating the roles of the NTS and AP in energy balance, possibly identifying these areas as novel sites of leptin resistance. Importantly, this research will enhance our understanding of whole brain leptin action and resistance, which is necessary if we are to develop more effective approaches for treating obesity.

Methods: Sprague-Dawley male rats (300-320g) were anaesthetized and received stereotaxic surgery targeting the NTS or AP. Recovered rats were placed on chow or 3d HF (Lard oil enriched) diet to induce hyperphagia. For feeding studies, food was be removed at 10am and given back at 4pm after NTS/AP infusions. Leptin (0.5ug) or chemical STAT3 peptide inhibitor were infused at 0.04 uL/min for 5 min. Western blots were performed on NTS or AP lysates using phospho-STAT3 specific antibodies.

Results: Leptin vs vehicle infusion into the NTS (0.5µg; 0.2µL for 5min) increased STAT3 phosphorylation [lep: 1.7±0.1 vs. veh: 1.0±0.1 pSTAT3/total STAT3 intensity; $p<0.01$; n=6,6] and lowered food intake [lep: 77±5 vs. veh: 100±4 kCal; $p<0.01$; n=11,10] within 24h of refeeding in chow rats, while leptin infusion into the AP failed to lower feeding [lep: 93±6 vs. veh: 92±5 kCal; n=10,6]. When leptin was co-infused with STAT3 cell-permeable peptide (9.8ng dose that blocks the enzyme's phosphorylation) into the NTS, leptin failed to exert effects on STAT3 [lep + inhibitor: 0.9±0.1 vs. lep: 1.7±0.1 pSTAT3/total STAT3 intensity; $p<0.01$; n=5,6] and food intake [lep + inhibitor: 96±3 vs. lep: 77±5 kCal; $p<0.05$; n=13,11]. In 3d HF-induced hyperphagic [HF: 275±2 vs chow: 225±7 kCal; $p<0.05$; n=16,34] rats, NTS leptin vs vehicle failed to activate STAT3 [lep: 0.8±0.2 vs veh: 1±0.3 pSTAT3/total STAT3 intensity; n=5,5] or inhibit feeding [lep: 110±4 vs. veh: 113±3 kCal; n=10,6] in association with a reduction in leptin receptor expression in the NTS [HF: 0.68±0.02 vs. chow: 1±0.08; $p<0.01$; n=7,10].

Conclusion: In summary, we discover that 1) leptin administration into the NTS, but not the AP, lowers feeding and the NTS effect is depended on STAT3 activation, 2) 3d of HF feeding induces leptin resistance in the NTS to activate STAT3 and lower feeding in parallel to a reduction in leptin receptor expression. Thus, we unveil NTS as a site for leptin-STAT3 signaling to lower feeding and HF-induced leptin resistance. In contrast, AP appears do not sense leptin to regulate feeding.

I.3: Unraveling the role of the adaptor protein stimulator of interferon genes (STING) in the development of kidney disease

Student: Emily S.H. Yeung, Supervisor: Andrew Advani

Background: Chronic kidney disease (CKD) affects over 1 in 14 Canadians, and despite advances in guideline-based treatments, new therapeutic approaches are still urgently needed. Inflammation and fibrosis play major roles in the progression of kidney function decline, yet are not currently directly targeted by any drugs approved for kidney outcomes. Recently, the STING pathway has emerged as a primary driver of kidney inflammation and fibrosis. In my research to date, I discovered that STING is expressed by injured kidney tubule cells and by infiltrating myeloid cells in diseased mouse and human kidneys. What is presently unknown is whether the effects of STING in kidney disease are primarily mediated by its actions in damaged tubule cells, infiltrating myeloid cells, or both. This is important to answer to inform future kidney-targeted drug therapies.

Hypothesis: Knocking out STING globally or specifically from either kidney tubule cells, or from myeloid cells will attenuate experimental kidney inflammation and fibrosis.

Methods: STING expression and localization were assessed in the kidneys of wildtype sham mice (n=12) and wildtype mice that had UUO surgery (n=12), UUO being a well-established model of kidney inflammation and fibrosis, through 1) RNAscope in situ hybridization; 2) qPCR; 3) immunohistochemistry; and 4) immunoblotting. To study the role that STING plays globally, in proximal tubule cells and myeloid cells, we generated $STING^{KO}$, Pax8Cre+Stingfl/fl mice ($STING^{TubKO}$) that lack STING specifically from tubule cells, and LysMCre+Stingfl/fl mice ($STING^{MyeloidKO}$) that lack STING from myeloid cells. Confirmation of STING knockout was determined by immunoblotting and immunohistochemistry.

Results: RNAscope in situ hybridization and immunohistochemistry demonstrated STING expression (gene name *Tmem173*) and STING protein upregulation in UUO mice tubule and myeloid cells. Immunoblotting revealed increased protein levels of both STING and its upstream activator cGAS in UUO kidneys. Immunoblotting confirmed absence of STING protein in the kidneys of global $STING^{KO}$ mice. Additionally, immunoblotting confirmed absence of STING protein in bone marrow derived macrophages isolated from $STING^{MyeloidKO}$ mice. Immunohistochemistry confirmed absence of STING protein in kidney tubules of $STING^{TubKO}$ mice subjected to ischemia reperfusion injury (IRI) surgery (bilateral renal pedicle clamping for 30 minutes, with kidney harvesting 24h later).

Conclusion & Significance: Findings suggest that STING is localized to kidney tubule cells and infiltrating myeloid cells in obstructed kidneys. STING protein was elevated in UUO mice. Following confirmation of knockout, $STING^{KO}$, $STING^{MyeloidKO}$, and $STING^{TubKO}$ mice have been subjected to sham or UUO surgery and followed for 14 days to determine the pathogenic role of STING in specific cell types in CKD. Future experiments will involve molecular biological and histological comparison of the different groups to determine whether STING mediates its proinflammatory and profibrotic effects through its actions in kidney tubule cells, infiltrating myeloid cells or both. Cell-specific targeting of STING could circumvent the adverse effects that may be associated with systemic antagonism and untargeted immunosuppression.

I.4: The adipocyte-specific role of caspase-8 signaling in adiposity

Student: Carmen K. Chan, Supervisor: Cynthia T. Luk

Background: Fat cell (adipocyte) death occurs in prolonged overnutrition and obesity. This is hypothesized to be a potent inducer of fat (adipose) tissue inflammation, which is associated with insulin resistance, a hallmark of type 2 diabetes. Caspase-8 is a key effector in the apoptotic pathway and possesses additional non-apoptotic roles, including activation of pro-inflammatory nuclear factor kappa B (NF-κB) signaling. Previously, we demonstrated that caspase-8 expression is increased in adipose tissue of mice and humans with obesity and insulin resistance, highlighting a critical role of caspase-8 in these settings. Additionally, we demonstrated that adipocyte-specific disruption of caspase-8 reduced weight gain on high fat diet (HFD), improved insulin resistance, and diminished adipose tissue inflammation in obese mice. The molecular mechanisms involved in this phenotype have yet to be elucidated.

Purpose: To further this work, the purpose of this study was to investigate the role of adipocyte caspase-8 in the regulation of adipose tissue homeostasis.

Hypothesis: We hypothesized that in addition to its role in apoptosis, adipocyte caspase-8 plays non-apoptotic roles, such as regulation of adiposity and adipose tissue inflammation, under a diet-induced obese setting.

Methods: Mice with adipocyte-specific disruption of caspase-8 were generated using the adiponectin-Cre*LoxP* recombination system ($Cre^{+}Casp8^{fl/fl}$). Littermates were used as controls ($Cre^{-}Casp8^{fl/fl}$). Mice were placed on up to 20 weeks of 60% HFD as a model of diet-induced obesity. Changes in adiposity were assessed by measuring fat pad weight. Changes in adipocyte function were assessed by real-time PCR (qPCR). To study the role of adipocyte caspase-8 in adipose tissue inflammation, 3T3-L1 adipocytes were treated with or without the caspase-8 inhibitor, Z-IETD-FMK, and stimulated with a potent inducer of cell death, TNFα. Changes in inflammatory gene expression were assessed by qPCR.

Results: Consistent with previous findings, caspase-8 protein levels were increased in adipose tissue of diet-induced obese control mice fed HFD compared to lean controls fed normal chow diet. Mice with adipocyte-specific disruption of caspase-8 exhibited decreased white adiposity and increased interscapular (brown) fat pad weight compared to littermate controls. Consequently, *Lep* gene expression was decreased in perigonadal fat of these mice. In parallel, mice with adipocyte-specific disruption of caspase-8 had smaller perigonadal adipocytes, which was associated with decreased expression of genes involved in lipid accumulation and increased thermogenic gene expression. Adipocyte caspase-8 was also demonstrated to regulate non-canonical NF-κB signaling and inflammatory gene expression *in vitro*.

Conclusions: Using both *in vitro* and *in vivo* approaches, findings from this study demonstrate an adipocyte-specific role of caspase-8 in regulating white adiposity and provide a potential mechanism of adipose tissue inflammation. Disruption of caspase-8 in adipocytes delineate an important role of caspase-8 in diet-induced obesity. Therefore, disruption of adipocyte caspase-8 may be an interesting therapeutic target for obesity or type 2 diabetes, both of which are major healthcare issues.

I.5: Glucagon-like peptide 1 analogue use in hospital: a multicentre cohort study

Student: Prachi Ray, Supervisors: Michael Fralick, Bruce Perkins

Introduction: Glucagon-like peptide 1 (GLP-1) analogues are one of the most effective classes of medications for type 2 diabetes mellitus (T2DM) and obesity. Despite their benefits, the uptake of GLP-1 analogues among patients most likely to benefit from these drugs has been slow.

Objective: To identify the prevalence of GLP-1 analogue use among adults hospitalized in Ontario and the patient-level characteristics associated with their use.

Methods: We conducted a multicentre cohort study of adults hospitalized at 19 hospitals in Ontario, Canada between 2015 and 2022. We included adults over the age of 18 years who received a GLP-1 analogue during their hospitalization. Our primary objective was to determine the frequency of GLP-1 analogue use among adults hospitalized in Ontario and to identify characteristics associated with GLP-1 analogue use. To contextualize these results, we also report the frequency with which other diabetes medications (e.g., sulfonylureas). Our secondary objective was to identify the number of patients who stood to benefit from a GLP-1 analogue by identifying all those with T2DM or obesity who were admitted during this time period.

Results: We identified 1,617,993 hospitalizations and approximately one-third of the patients had T2DM (n=498,655) and one-quarter had obesity (n=380,000). Among all hospitalizations, 0.08% (n=1,274) received a GLP-1 analogue, 9% (n=143,914) received metformin, 15% (n=236,612) received insulin, and 3% received a sulfonylurea (n=54,885). Patients who received a GLP-1 analogue were typically younger (66 years vs. 70 years), more likely to be male (58%), and more likely to have diabetes (94% vs. 31%) or obesity (15.8% vs. 2.4%) compared to those who did not receive a GLP-1 analogue.

Conclusion: Over half a million patients in our dataset had T2DM or obesity but only 1,274 received a GLP-1 analogue in hospital. In contrast, nearly 250,000 patients received drugs known to cause weight gain such as insulin or sulfonylurea. Our data indicate that knowledge translation initiatives are needed to increase the uptake of GLP-1 analogues among patients with T2DM or obesity.

I.6: Remote ischemic conditioning in necrotizing enterocolitis: an international phase II feasibility randomized controlled trial

Student: Niloofar Ganji, Supervisor: Agostino Pierro

Background: Necrotizing enterocolitis (NEC) is a devastating gastrointestinal emergency in neonates, in dire need of novel treatments. Remote ischemic conditioning (RIC) is a simple maneuver involving brief cycles of ischemia and reperfusion in the neonate's limb. Our preclinical models have shown that in early-stage of NEC, RIC counteracted intestinal injury and prolonged survival by promoting intestinal blood flow. A phase I trial demonstrated that RIC is safe in neonates with NEC.

Purpose: To evaluate the feasibility and safety of RIC in infants with NEC in a phase II multicenter trial which will inform the design of a future efficacy phase III randomized controlled trial (RCT).

Hypothesis: It is feasible to conduct a multicenter RCT to evaluate RIC during the management of neonates with medical NEC.

Methods: The RIC-NEC trial is a prospective, masked, international, phase II feasibility RCT. Neonates with medical NEC are randomized to RIC (intervention) or no-RIC (control) and continue to receive standard-of-care. RIC involves 4 cycles of limb ischemia (5-min blood pressure cuff inflation) followed by reperfusion (5-min cuff deflation), repeated on two consecutive days. The primary endpoint is feasibility of recruiting and randomizing neonates within 24-hours of NEC diagnosis and delivering masked intervention.

Results: Between February-2023 and February-2024, in 4 out of 12 centers, 61 patients have been screened, 25 met the eligibility criteria, 11 did not give consent, and 14 were enrolled, with a total enrollment goal of 78 patients. The preliminary data suggests a 56% recruitment rate is achievable (There is a 95% Bayesian probability that the recruitment rate exceeds 40%). The intervention has been feasible in all patients with no changes in safety outcomes and no occurrence of adverse events. Secondary clinical outcomes at 1-month post-randomization are being assessed including duration of total parenteral nutrition (RIC: mean 21.4d; no-RIC: mean 24.4d), abdominal operations performed for failed medical treatment (RIC: 0/5; no-RIC: 3/5), and development of chronic lung disorder (RIC: 2/5; no-RIC: 2/5).

Conclusions: Our international NEC consortium has defined the feasibility parameters for the introduction of RIC as a novel treatment for NEC. The ongoing RCT will inform a future phase III RCT to evaluate the efficacy of RIC in the early management of medical NEC.

I.7: The effect of TRPML1 inihbition by helicobactor pylori vacuolating cytotoxin on mitochondrial dynamics and function

Student: Julia Novielli, Supervisor: Nicola Jones

Background: *Helicobacter pylori* inhabits the stomachs of ~50% of the world's population and is the leading cause of gastric cancer. *H. pylori* damage the gastric epithelia by releasing virulence factors, including the vacuolating cytotoxin A (VacA) which has a strong association with severe disease. Secretion of VacA allows *H. pylori* to establish chronic infection and disrupts host organelle function, particularly lysosomes and mitochondria. The intertwined roles of mitochondria and lysosomes are crucial for maintaining cellular homeostasis, as lysosomal function regulates mitochondrial dynamics. VacA interferes with lysosomes by inhibiting Transient Receptor Potential Membrane Channel 1 (TRPML1), leading to formation of non-degradative lysosomes that serve as intracellular survival niches for bacteria.

Purpose: VacA is implicated in altering mitochondrial dynamics, with the prevailing belief that it induces mitochondrial fragmentation, ultimately causing apoptosis. However, only a fraction of cells exposed to VacA experience such changes. Given the chronic nature of *H. pylori* infection, it is imperative to further investigate the long-term impact of VacA on mitochondria in the population of cells that do not undergo cell death.

Hypothesis: Based on preliminary data, we predict that long-term exposure to VacA increases mitochondrial elongation, and that this response is dependent on VacA's inhibition of TRPML1.

Methods: Gastric adenocarcinoma (AGS) cells were treated for 24 hours with toxigenic (VacA+) *H. pylori* or an isogeneic VacA- mutant strain or the TRPML1 agonist ML-SA1. To assess mitochondrial and lysosomal morphology, cells were immunolabelled. To determine whether elongation occurs due to increased fusion or decreased fission, key mitochondrial dynamics proteins were assessed by immunoblotting. Mitochondrial function in cells treated with VacA was evaluated by measuring mitochondrial membrane potential using a tetramethyl rhodamine (TMRM) dye assay.

Results: Immunofluorescent imaging revealed longer mitochondria in cells treated with VacA+ compared to VacA- or untreated cells. Increasing TRPML1 activity elevation using an agonist prevented VacA+-induced mitochondrial elongation, suggesting a dependence on TRPML1 activity. Immunoblot data indicated that elongation is independent of Dynamin-related Protein 1 but could involve mitochondrial fusion protein Optic Atrophy 1. Prolonged VacA exposure causes mitochondrial dysfunction, evidenced by reduced mitochondrial membrane potential. Notably, treatment with ML-SA1 was ineffective in preventing mitochondrial dysfunction in this context.

Conclusions: While investigation into the mechanism is ongoing, we have shown that VacA-induced mitochondrial elongation is not due to a reduction in mitochondrial fission, but likely an increase in fusion. These findings have important implications for further understanding chronic *H. pylori* infection and the role of VacA in the development of gastric cancer.

J: Cancer

J.1: The prevalence and predictors of bone health complications in childhood cancer survivors

Student: Yeasmin Sultana Begum, Supervisors: Sumit Gupta, Paul Nathan

Childhood cancer survivors face increased risks of bone health complications due to treatment-related factors. This study aimed to assess the prevalence and predictors of bone health complications between Childhood Cancer Survivors (CCSs) and healthy counterparts within the OHIP-eligible population of Ontario, Canada, as well as between CCSs. Based on data from the Institute for Clinical Evaluative Sciences (ICES) databases, including POGONIS and IMPACT, a matched cohort study design was employed. The primary objective was to evaluate the risk of fractures, orthopedic surgical intervention, osteoporosis, and avascular necrosis between CCSs and their healthy matched counterparts. Secondary objectives included identifying high-risk groups within CCSs. Survival analysis models, such as Cox proportional hazards regression, are to be utilized to analyze the associations between childhood cancer survivorship, cancer types, and bone health outcomes, adjusting for potential confounders such as age, gender, and income quintile. Results revealing the significance of the associations between cancer diagnosis as well as specific cancer types, and bone health complications are expected to be elucidated. Furthermore, age, gender, and income quintile are expected to impact the likelihood of bone health complications varyingly and significantly among childhood cancer survivors. These potential findings will help to illuminate the importance of tailored interventions and surveillance strategies for mitigating bone health risks in childhood cancer survivors, particularly among high-risk groups. In conclusion, childhood cancer survivors are expected to be at elevated risk of bone health complications, highlighting the need for personalized preventive measures and long-term monitoring in survivorship care programs.

J.2: Investigating CD59 as a novel therapeutic target against TP53-mutated acute myeloid leukemia (AML)

Student: Abdula Maher, Supervisor: Steven M.C. Chan

Background: Acute myeloid leukemia (AML) is a hematologic malignancy with a highly variable prognosis depending on the genetic makeup of leukemic cells. Mutations in the tumor suppressor gene, *TP53*, which are present in about 10% of newly diagnosed AML, are associated with a lower likelihood of response to frontline treatment, higher probability of relapse, and a 2-year overall survival rate of less than 10%. The lack of effective treatment options for *TP53*-mutated AML presents an unmet need for the development of novel therapies.

Methods: We sought to identify therapeutic targets against *TP53*-mutated AML by analyzing the expression of cell surface proteins and their impact on survival in the BeatAML2 and TCGA AML datasets. We identified that the gene, *CD59*, was more significantly expressed in *TP53*-mutated AML versus *TP53*-wildtype (WT) disease ($log_2FC > 1$ and adjusted P value < 0.01). In addition, *CD59* expression correlated with worse outcomes, an adverse-risk ELN disease classification, and refractory disease in the BeatAML2 patient cohort.

Hypothesis: CD59 is a cell surface glycoprotein that prevents complement-mediated cell lysis by inhibiting formation of the membrane attack complex, however, it also has a less well characterized role in modulating intracellular signal transduction pathways. Given that conventional AML therapies do not activate the complement system, we hypothesized that CD59 confers a survival advantage to AML cells in a complement-independent manner.

Results: To study the role of CD59 in AML, we transduced 3 *TP53*-WT (OCI-AML-3, EOL-1, MOLM-13) and 3 *TP53*-mutated (SKM-1, HL-60, NB-4) AML cell lines with short-hairpin RNAs (shRNAs) against *CD59* or a non-targeting (NT) control sequence. DNA cell cycle and EdU staining reveals growth arrest of sh*CD59*-expressing cells at the G1/G0 stage which was accompanied by increased levels of cell death. Importantly, the effect of *CD59* knockdown was not rescued by heat-inactivation of fetal bovine serum (a procedure that inactivates the complement system) in cell culture medium, confirming a complement-independent role of CD59. In addition, NOD/SCID//IL2Rγ-null (NSG) mice transplanted with SKM-1 and HL-60 cells expressing sh*CD59* prevents the expansion of leukemic burden in comparison to shNT-expressing cells. To decipher the mechanism of CD59 signaling, we performed RNA-sequencing on sh*CD59* and shNT-expressing SKM-1 and HL-60 cells. Differential gene expression and pathway enrichment analyses revealed a downregulation of E2F target, G2M checkpoint, and MYC target genes upon *CD59* knockdown. Moreover, we performed mass spectrometry-based phospho-proteomic analysis in HL-60 cells expressing sh*CD59* or shNT. Our analysis predicted a decrease in extracellular signal-regulated kinases (ERK) activity in sh*CD59*-expressing cells. ERKs are downstream effectors of the Ras/Raf/MEK signaling cascade and their activation has been shown to drive G1 to S phase transition in AML. We probed for activating ERK phosphorylation via western blot and confirmed reduced ERK activation in sh*CD59*-expressing cells in comparison to shNT-expressing cells. These findings indicate that *CD59* silencing causes cell cycle arrest by inhibiting key nodes of the signaling pathway upstream of ERK.

Conclusion: Our results demonstrate that CD59 expression is essential for AML cell growth and cell survival, and as such, we identify it as a novel therapeutic target. The survival outlook for *TP53*-mutated AML patients has remained unchanged for the past 25 years. Our findings offer critical insights that can significantly alter the current treatment landscape for this deadly subtype of AML.

J.3: Assessing the efficacy of fine-tuned open-source large language models in triaging for cancer care

Student: Daniel Mau, Supervisor: Robert Grant

Background: Efficient triage is an important aspect of cancer care that ensures patients receive appropriate responses to their concerns between visits. However, manual triage is labor-intensive, and quality varies. Recent advancements in large language models (LLMs) may assist in automating some aspects of the triage processes, such as directing cancer patients to the emergency department (ED). Due to regulations and privacy concerns of using proprietary LLMs, open-source LLMs can be a viable alternative. However, open-source LLMs have different training datasets and methodologies, raising concerns about their effectiveness in the clinical setting. Therefore, we want to investigate whether fine-tuned open-source LLMs can generate ED referral recommendations equivalent in quality to those generated by GPT-4.

Purpose: The objective of this study was to assess and compare the performance of selected open-source large language models in making ED referral recommendations for cancer patients. These recommendations would be compared with those generated by GPT-4, which was one of the leading proprietary LLMs.

Hypothesis: Fine-tuned open-source LLMs would demonstrate significant variations in ED referral recommendations. It was hypothesized that newer foundational models would be more aligned with GPT-4 in terms of ED recommendation.

Methods: The study would fine-tune two open-source LLMs, Mistral-7b and Asclepius-7b. The primary outcome measure was the frequency at which each model advised ED referral. Contingency tables and Fisher's Exact Test were used to determine the differences in ED recommendations between the models.

Results: There was a statistically significant ($p = 0.004404$) difference between fine-tuned Mistral-7b and GPT-4 in recommending ED visits. The odds of a fine-tuned Mistral-7b recommending an ED visit were 9.6 times higher when compared with the odds of GPT-4 recommending an ED visit. Additionally, there was a statistically significant difference in the frequency of ED visit recommendation between GPT-4 and fine-tuned Asclepius-7B ($p = 0.0264$). The odds of GPT-4 recommending an ED visit were almost 7 times higher than the odds of Asclepius-7B recommending an ED visit.

Conclusions: The findings revealed substantial variations in the performance of different open-source LLMs, particularly when these models were fine-tuned using smaller datasets. Both models exhibited significant differences in ED recommendation when compared to GPT-4. This variability highlighted the necessity for further research between LLM-generated outputs and outputs generated by clinicians.

J.4: Cellular impact of local sub-ablative radiotherapy on pleural mesothelioma

Student: Sara Shariati, Supervisor: Marc de Perrot

Background: Pleural mesothelioma (PM) is a rare–but, aggressive–cancer that primarily affects the lining of the lungs, known as the pleura. PM is strongly linked to asbestos exposure and has a latency period of 30 to 40 years. The prognosis for PM is generally poor, as it is often diagnosed at an advanced stage, and this poor prognosis highlights the importance of searching for more effective treatment options.

Purpose: Clinical trials have demonstrated that oligo-fractioned sub-ablative radiotherapy can help improve outcomes of surgery. Sub-ablative radiotherapy is thought to work through induction of an immune response, rather than initiation of cell death. We aim to study the cellular and immune impact of sub-ablative radiotherapy in PM.

Hypothesis: Based on literature, we hypothesize that lymphocytes and macrophages play an important role in induction of post-radiotherapy immune response.

Methods: A subcutaneous mouse model of PM was used, in which AE17-OVA cells (a murine PM cell line) were injected into wild-type mice, and the resulting tumor was subjected to 3 doses of radiation (3 × 5 Gy) over three consecutive days in the treatment group. Size of the tumor was measured at the following time points: before radiation, 1 day after radiation, and 8 days after radiation. Tumor samples were then collected and analyzed through flow cytometry.

Results: No statistically significant change in tumor size was observed in the treatment group, demonstrating that radiotherapy halts PM tumor growth for at least 8 days in mice. Flow cytometry analysis showed that both $CD4^+$ and $CD8^+$ T cells were upregulated in the treatment group 8 days after treatment. In analyzing the macrophage population, Ly6C proved to be a useful marker. Analysis of $F4/80^+$ $Ly6C^{high}$ and $F4/80^+$ $Ly6C^{low}$ cells in tumor tissue demonstrated an upregulation of $Ly6C^{high}$ macrophages at both one-day and eight-days post-radiotherapy.

Conclusion: Sub-ablative radiotherapy leads to an upregulation of pro-inflammatory cells in the tumor microenvironment, such as $CD8^+$ T cells and $Ly6C^{high}$ macrophages. This suggests that radiotherapy can be used in mesothelioma treatment to boost the efficacy of surgery or chemotherapy. In future, we intend to investigate gene expression changes in the tumor microenvironment immune cell population through single-cell RNA-sequencing to better understand the cellular and biochemical pathways involved in the response to radiotherapy.

J.5: Elucidating the mechanistic and pre-clinical implications of DNA damage response inhibitors in combination with chemotherapy to target homologous recombination deficiency in leiomyosarcoma

Student: Christina Plahouras, Supervisor: Rebecca Gladdy

Background: Leiomyosarcoma (LMS) is the most common type of soft tissue sarcoma in adults. Currently, surgical resection is the primary curative method, however it poses significant challenges due to its aggressive nature and potential for metastasis. To date, doxorubicin and gemcitabine-based therapies are the mainstay approach for treatment of metastatic disease and beyond first line chemotherapy there are limited treatment options. The chemotherapy response rate is 20-30%, highlighting the need for improved systemic therapy for patients with advanced disease. To address this, the Gladdy lab in collaboration with the Schlien lab has previously conducted whole genome sequencing and transcriptome sequencing, identifying the presence of homologous recombination deficiency (HRD) signatures SBS3 and ID6 in 64% of LMS samples. This novel discovery highlights the pivotal role of DNA repair defects in LMS and opens up the possibility of using DNA damage response inhibitors (DDRi's) to induce synthetic lethality in patients that present with HRD.

Purpose: To test and define which combination therapies may be successful in LMS and validate the HRD phenotype.

Hypothesis: Combination therapy using DDRi's and chemotherapy will have a synergistic effect leading to tumour growth inhibition and increased cell death in LMS cells that are HRD.

Methods: Hit validation using half-maximal inhibitory concentration curves (IC_{50}) confirmed that LMS cell lines are sensitive to various DDRI's and chemotherapies. Bliss analysis was performed to analyze if DDRi's could be successfully combined with doxorubicin, the current standard of care chemotherapy, and demonstrated high rates of synergy *in vitro*. To assess and validate the HRD phenotype in LMS, immunofluorescence (IF) analysis of γH2AX and RAD51 will be conducted. Analysis of DNA damage biomarkers, γH2AX and RAD51 will highlight the impact of selected drug combination therapies on LMS DNA repair mechanisms.

Results: To establish if LMS cells lines were sensitive to DDRi's and if this correlated with the presence of HRD substitution signatures, the response of LMS cell lines was characterized to a panel of DDRi's using 36-point dose concentration viability assays (0.0026 µM-10 µM). LMS cell lines derived from primary and metastatic tumours were used and demonstrated striking IC_{50}'s (<1µM). Following individual drug screens, the Bliss independence model was used to screen for candidate synergistic drug combinations. To validate the HRD phenotype in LMS, γH2AX and RAD51 IF will be employed, using the selected candidate drug combinations. An increase in γH2AX foci is expected as it is correlated with increased DNA damage, which may reflect the inefficiency of HR repair mechanisms in LMS. A reduction in RAD51 foci is expected as it suggests a deficiency in the HR repair pathway, indicating HRD.

Conclusion: The identified sensitivity of LMS cell lines to DDRi's and chemotherapies, validated through comprehensive hit validation and Bliss analysis, emphasizes the potential for synergistic therapeutic strategies. Further investigation employing IF to assess DNA damage biomarkers will validate the HRD phenotype and provide insights into the critical role of DNA repair defects in LMS, highlighting potential vulnerabilities that can be targeted for therapeutic intervention. This research represents an important step towards advancing the development of improved treatment modalities for LMS patients and the development of innovative clinical trials.

J.6: Depression and decision making in advanced cancer patients considering medical assistance in dying (MAiD)

Student: Stefan Aguiar, Supervisor: Madeline Li

Consistent with international literature, over 65% of patients request medical assistance in dying (MAiD) on the basis of cancer. Many of these patients who request and receive MAiD have been found to have high levels of depressive symptoms. Depression can result in cognitive and emotional biases which affect the appreciation component of capacity. It has long been proposed that such psychological states impair "affective forecasting," resulting in poor predictions of the future. Currently there are no medico-legally defined criteria of decision-making capacity for (MAiD). Studying decision making capacity for MAiD in the presence of co-morbid depression may be very informative for the anticipated expansion of MAiD for mental disorders in Canada.

The goal of the study was to determine the forms, facilitators, and mitigators of psychological suffering among depressed and non-depressed advanced cancer patients at different levels of MAiD engagement. Qualitative interviews were conducted on advanced stage cancer patients that explored different aspects of psychological suffering across a range of attitudes towards MAiD and MAiD requests. Open-ended questions focused on MAiD thinking and the decision-making process, facilitators, and types of psychological suffering. Interview transcripts were independently coded by two researchers and a grounded theory approach was used to analyze the data. 26 interviews were conducted among patients with and without clinically depressive symptoms, out of which 11 have requested MAiD. Patients described decision making styles during their medical journey and around MAiD, coping strategies, support systems, burdens, and types of psychological suffering.

J.7: Spatial and single-cell proteomic landscaping of the hypoxic tumour microenvironment in glioblastoma

Student: Shreya Gandhi, Supervisor: Gelareh Zadeh

Background: Glioblastoma (GBM) is a fatal adult solid tumour of the central nervous system with a median overall survival of 18-20 months post-diagnosis; contributing factors to therapeutic inefficacy include acquisition of genomic alterations post-therapy, immune evasion, deregulated hypervascularization, and complex tumor microenvironmental factors (TME) driven by hypoxia (low O_2 partial pressure ($1<pO_2<10$ mm Hg). Cellular adaptation to hypoxia involves stabilization of hypoxia inducible transcription factors (HIFs), which regulate the expression of several hundred downstream genes, including those involved in cell survival, angiogenesis, and invasion/metastasis. Combined with extensive inter- and intra-tumoral heterogeneity at the bulk and single-cell level, hypoxia contributes to a gradient of molecular alterations that are specific to different cell populations that make up the tumour bulk and reside in specific niches of the TME. Associated with this phenotype is the secretion of immunosuppressive cytokines by infiltrating immune cells, a predominantly exhausted t-cell functional state, and an M2 (protumorigenic) polarization shift in tumour-associated macrophages (TAMs).

Purpose & Hypothesis: Hitherto, high-dimensional histopathologic analyses of hypoxic regions within the GBM TME have not been performed. This led to among the first in human trials of PIMO for GBM, where in addition to hypothesizing PIMO's utility as a candidate marker of hypoxia relative to traditional HIF-target genes, we used spatial and single-cell proteomic analyses to characterize the cellular heterogeneity of the hypoxic TME.

Methods: We took a combined spatial and single-cell proteomic profiling approach to investigate the histopathologic features of hypoxia by leveraging a unique clinical study wherein the exogenous hypoxia marker, Pimonidazole (PIMO), is administered to GBM-patients 16-20 hours preoperatively. Tissue specimens were subjected to imaging mass cytometry, cytometry by time-of-flight, and serial immunohistochemistry using a panel of markers associated with cellular hallmarks of hypoxia, metabolism, proliferation, stemness, angiogenesis, and immune cell types.

Results: Our findings showed that PIMO staining is associated with histopathologic features of hypoxia and demonstrate inter and intra-patient heterogeneity in primary and recurrent GBMs. PIMO can indeed label cells under varying levels of hypoxic stress, and can label hypoxic immune and non-immune cell subpopulations within the TME. We identified a great proportion of PIMO+ T-cell infiltrate (albeit in a predominately exhausted functional state), an elevated proportion of $PIMO^+$ cytotoxic T-cells, but a reduced proportion of $PIMO^+$ helper T-cells. These cell populations likely further propagate the antitumor immune response via secretion of immunosuppressive cytokines and lack thereof proinflammatory cytokines. As expected, we identified an increased proportion of $PIMO^+$ protumorigenic M2-like TAMs relative to $PIMO^+$ proinflammatory M1-like TAMs which are known to contribute to chemoresistance, angiogenesis, and facilitate cancer progression in GBM. Interestingly, however, we see a significantly greater proportion of $PIMO^+$ eosinophils, which are otherwise generally associated with improved survivorship in GBM patients. This novel finding provides untapped translational value in understanding how specific immune cell subsets may be harnessed to improve therapeutic outcome.

Conclusions: Our study is among the first to report use of PIMO to interrogate spatial, single-cell, and phenotypic architecture associated with tissue hypoxia and altered expression of biomarkers associated with hypoxia, immune infiltration, proliferation, and stemness. Identification of targetable biomarkers and mediators of hypoxia-driven habitats in GBM may provide direction for future immunotherapeutic research.

K: Infection & Immunology

K.1: Evaluation of hepatitis C virus transmitted/founder variants obtained from observed HCV infection through lung transplantation from HCV-infected donors to uninfected recipients

Student: Nahla Fadlelmawla, Supervisor: Jordan Feld

Background: In contrast to the large diversity seen in chronic hepatitis C virus (HCV) infection, samples taken from acute HCV infection have shown only a small number of viral lineages, suggesting the presence of a limited number of Transmitted/Founder (T/F) variants that are able to efficiently expand and establish infection in a new host. Characterization of T/F variants in HCV infection has been limited due to the difficulty of obtaining samples from early HCV infection and in identification of the donor virus. Using samples from lung transplant recipients who received organs from HCV-infected donors, we evaluated the presence of T/F variants in observed HCV infection.

Purpose & Hypothesis: To compare viral diversity in donors and recipients and evaluate sequence-dependent functional characteristics of founders compared to non-founder populations

Methods: The HCV genomes of our donor and recipient pairs were amplified. Using Illumina MiSeq, 250bp reads we obtained the sequences of donors and their respective recipients from day 7 post-transplant. ShoRAH, a bioinformatics tool used for haplotype reconstruction, was then used to identify haplotypes in the Core-E2 region and estimate their frequencies. To analyze diversity, we subsampled the reads and counted haplotypes for each subsample to identify the point of saturation. We used maximum likelihood phylogenetic trees (PhyML) to identify founders and non-founders and used Highlighter plot to compare silent and non-silent mutations in founders and non-founders.

Results: Our preliminary data show that at the time of transmission, there exists a decrease in population diversity and selection of founder variants for their enhanced infectivity and absence of non-founder variants that may be less fit at establishing infection, as evidenced by the transmission of HVR1 variant with basic residues at position 3 and the absence of variants with acidic residues in our lung transplant RA. More analysis is in progress to track founder evolution in longitudinal recipient samples and assess functional characteristics in vitro.

K.2: Interplay of microbes and metabolites: correlation of plasma metabolites with microbiome health and disease

Student: Taylor Kain, Supervisor: Bryan Coburn

Background: Gut epithelial integrity links microbiome composition/function and immune system tone and function and has been implicated in infectious and non-infectious diseases. Some plasma analytes are uniquely produced by gut microbes and translocate into the systemic circulation. Gut obligate anaerobes convert primary bile acids to secondary bile acids (SBAs), produce microbial short-chain fatty acids (SCFAs), and are critical to nitric oxide synthesis pathways. SBAs have been shown to impede pathogen colonization and proliferation, while SCFAs serve as an energy source for gut epithelial cells and promote epithelial barrier integrity. Nitric oxide is important for endothelial function and stability. These analytes are candidate plasma indicators of gut microbiome composition which have the potential advantages of ready availability, rapid turnaround time and technical ease. Moreover, circulating microbial metabolites may be more directly associated with extra-intestinal disease pathogenesis, and therefore a preferable biomarker of microbiome composition and function.

Hypotheses:

1) Plasma primary bile acid concentrations will negatively correlate with gut microbial diversity and positively with pathogen dominance, while plasma SBAs and SCFAs will positively correlate with gut microbial diversity and negatively correlate with pathogen dominance.

2) SBA and SCFA levels will be able to predict onset and recurrence of *C. difficile* infection (CDI).

Methods: We performed a *de novo* analysis of published paired stool microbiome/plasma metabolome samples from 68 patients with acute myeloid leukemia (AML) and assessed correlations between stool Shannon diversity, Berger-Parker dominance, and OTU relative abundance to 945 different plasma microbial metabolites. We also assessed candidate microbial metabolites in a cohort of heart transplant recipients before and after CDI as an example of a well-established human model of microbiome perturbation.

Results: In the AML cohort, multiple plasma metabolites correlated with stool microbiome compositional indices. The SBAs deoxycholic acid 12-sulfate ($r^2 = 0.52$, $p<0.0001$), lithocholate sulfate ($r^2 = 0.50$, $p<0.0001$), and glycolithocholate-sulfate ($r^2 = 0.43$, $p<0.0001$), positively correlated with Shannon diversity index (SDI), while the PBA glycochenodeoxycholate-3-sulfate ($r^2 = -0.30$, $p=0.001$) negatively correlated with diversity. The SBA, deoxycholic acid 12-sulfate also correlated with relative abundance of *Lachnospiraceae*, a key family of obligate anaerobes associated with healthy microbiomes($r^2 = 0.49$, $p<0.0001$). The PBA cholate positively correlated with the relative abundance of Enterococcus, a potential pathogen ($r^2 = 0.36$, $p=0.0001$). A logistic regression model using concentration of the SBA deoxycholic acid was able to predict low vs normal SDI (cut off 3.59) with 82.4% accuracy in this population. Plasma secondary bile acids were lower in CDI cases than controls and there were significant decreases in the same SBAs after CDI onset (deoxycholic acid pre 0.358µM vs post 0.137µM, $p=0.01$; glycodeoxycholic acid pre 0.621µM vs post 0.356µM, $p=0.012$). PBAs and SCFAs did not show significant changes between samples before and after diagnosis of CDI.

Discussion: Plasma microbial metabolites correlated with microbial composition/perturbation in two cohorts of patients, demonstrating the potential utility of plasma as an indirect marker of gut microbiome composition. Future steps include a formal systematic review and summary of all published literature linking microbial metabolites and microbiome composition to assess the reproducibility of these relationships in different cohorts.

K.3: Mechanisms of club cell apoptosis in chronic lung allograft dysfunction

Student: David Sebben, Supervisor: Tereza Martinu

Background: While lung transplantation (LTx) is the only available treatment for many end-stage lung diseases, it has one of the worst outcomes of all solid organ transplants. Different insults, including infection, rejection, and aspiration, accumulate and lead to chronic lung allograft dysfunction (CLAD), the main cause of post-LTx death. Airway epithelial cell (AEC) injury is crucial in CLAD development. Our preliminary data show increased club cell apoptosis in CLAD lungs compared to control donor lungs. However, the mechanisms driving club cell loss in human CLAD have not been elucidated. Our pilot single-cell RNA sequencing data shows club cell-specific upregulation for tumour necrosis factor-related apoptosis-inducing ligand (TRAIL), signaling through TRAIL-receptor 1 (TRAIL-R1), compared to other cells.

Purpose & Hypothesis: We aim to investigate whether primary human club cells undergo preferential apoptosis mediated by TRAIL/TRAIL-R1 in CLAD. We hypothesize that CLAD develops as a direct consequence of TRAIL/TRAIL-R1 mediated club cell apoptosis.

Methods: The experimental aims are to determine whether, 1) TRAIL causes preferential club cell apoptosis of primary human AEC *in vitro*, 2) blocking TRAIL-R1 reduces TRAIL-mediated club cell apoptosis *in vitro*, and 3) TRAIL-R1 is preferentially expressed on club cells in human CLAD lung tissue. A cohort of CLAD and non-CLAD patients will be identified at various time points post LTx, and distal AECs will be obtained from transbronchial brushings during clinical bronchoscopies. Brushes will be processed using enzymatic digestion to preserve epithelial cells and cells will be grown in submerged cultures. For aim 1, AEC cultures will be grown and then incubated with recombinant TRAIL. Cells will be analyzed utilizing flow cytometry to detect co-localization of club cells expressing TRAIL/TRAIL-R1 with associated death markers (active-caspase 3, annexin V). Aim 2 will be carried out similarly, with the addition of TRAIL-R1 antibody before incubation with TRAIL. Aim 3 will employ immunofluorescence staining of human CLAD lung tissue obtained during re-transplantation and control donor lungs. TRAIL- and TRAIL-R1-positive club cells and other epithelial cells will be quantified.

Results: Samples from 16 subjects have been collected and processed. Samples have successfully expanded in T25 flasks, with subsequent freezing and storage of cells for later analysis. Pilot immunofluorescence staining shows club cell secretory protein expression in bronchial brush samples after being grown in submerged culture for about 10 days.

Conclusion: This preliminary data promises that primary human club cells can be studied *in vitro* with these samples. Findings will lead to a better understanding of CLAD pathogenesis, specifically with regards to mechanisms driving club cell loss in CLAD. This research may translate to guiding diagnostic and therapeutic approaches, with the goal of improving long-term outcomes post LTx. Specifically, blockade of TRAIL signaling may be feasible in LTx patients.

K.4: Impacts of penile-vaginal sex on the microbiome and immunology of the penile urethra

Student: Marcio Gabriel Amancio de Carvalho, Supervisor: Rupert Kaul

Background: The penile urethra is a key HIV acquisition site in heterosexual men. In the penile coronal sulcus and the vagina, inflammation and microbiome composition play an important role in HIV exposure outcome, and are affected by sexual intercourse. However, direct impacts of penile-vaginal sex on those factors remain understudied in the penile urethra.

Purpose: To investigate the impacts of penile-vaginal sex on the microbiome and immune milieu of the penile urethra.

Hypothesis: Penile-vaginal sex causes a transient influx of vaginal bacteria into the penile urethra, accompanied by an increase in inflammation.

Methods: The Sex, Couples and Science (SECS) study was designed to examine the impacts of penile-vaginal sex on the immune milieu and microbiomes of established couples. First-void urine was collected to characterise the penile urethra microbiome (through 16S sequencing and qPCR) and immune parameters (through a multiplex chemiluminescent immunoassay) before and after sex (immediately, 1, 7 and 72 hours after).

Results: Following sex, the urethra was enriched for up to 72 hours with *Lactobacillus crispatus* and *jensenii*, common vaginal species associated with HIV protection in the vagina. Species associated with higher HIV risk in the vagina, including *L. iners* and *Gardnerella vaginalis*, were common in the urethra at baseline and remained unaffected by sex. Most inflammatory markers increased immediately after sex for up to 1 hour. However, this did not reflect baseline vaginal or semen levels of the same markers, and were not tied to changes in the microbiome.

Conclusions: Penile-vaginal sex caused transient increases in inflammation, as well as microbiome changes in the penile urethra which reflect a transfer of vaginal *Lactobacillus* species. Additionally, bacteria associated with higher HIV risk in the vagina were commonly found in the urethra. These findings highlight the urethra as a potential reservoir for key vaginal bacteria.

K.5: The Rho GAP MYO9B regulates Rho activity associated with endomembrane remodeling and cargo traffic in macrophages

Student: Kaitlin Eh Lees, Supervisor: Lisa Robinson

To achieve dynamic surveillance of tissues, innate immune cells constantly remodel their surface membrane, executed by specialized forms of endocytosis that are tightly coupled to membrane recycling. These processes are orchestrated by the submembrane cytoskeleton which is itself constantly remodeled. Actin polymerization, as mediated by the Rho family of GTPases, and depolymerization is critical to maintain the immune surveillance programs of macrophages. This is emphasized in phagocytosis whereby large particles are bound and internalized into sealed phagosomes which then undergo stepwise maturation to become terminal degradative phagolysosomes. While the role of Rho GTPases, including their activation by Guanine Exchange Factors (GEFs) and inactivation by GTPase Activating Proteins (GAPs), in phagocytosis are firmly established, their localization and activity during phagosome maturation is largely unknown.

This project investigates a Rho specific GAP, MYO9B, which has been shown to regulate innate immune cell behavior, including cell migration and phagocytosis, in various contexts. The precise role of MYO9B, however, is poorly understood. By analyzing cells from MYO9B knockout (-/-) mice using Rho activity assays and high-resolution microscopy, we have determined that macrophages from these mice showed highly upregulated RhoA/B/C activity. Curiously, these macrophages did not show any defects in phagocytosis, cell morphology, membrane ruffling, or regulation of F-actin in the cortex. Instead, we found that adhesion structures (called podosomes) which are highly dependent on integrins were demonstrably attenuated on extracellular matrices. This phenotype, together with the seemingly normal cortex of the macrophages, more closely resembles defects in the regulation of RhoB rather than RhoA activity. Accordingly, preliminary results show that RhoB is highly enriched in the endocytic pathway of macrophages and that increasing the activity of RhoB caused extensive actin polymerization that caged nascent phagosomes. Further investigations into the role of MYO9B in the recycling of integrins from phagosomes in these contexts is ongoing.

Taken together, this project reveals mechanisms of F-actin remodeling on endomembranes that support traffic and surveillance functions of innate immune cells.

K.6: Evaluation of the hepatitis C virus immune exhaustion profile following administration of direct-acting antivirals during acute infection

Student: Ayoub Mahassine, Supervisor: Jordan Feld

Background: T-cell exhaustion results from continuous antigen stimulation and develops in a progressive fashion after infection. Exhausted T-cells display distinct phenotypic and functional properties from memory and effector T-cells with distinct transcriptional and genetic profiles. Exhausted T-cells display weak antiviral effector functions and impaired proliferation, leading to restricted ability to eliminate infected cells. Previous studies investigated whether exhausted T-cells could recover normal function with removal of the antigen, specifically after cure of chronic HCV infection. Recent data show that a majority of exhausted T-cells died without antigen exposure, however, although surviving cells recovered some gene expression patterns found in memory-type T-cells, upon antigen stimulation they returned to a terminally exhausted state. Further evaluation showed retention of an epigenetic phenotype similar to that of exhausted T-cells, suggesting formation of an 'immunological scar' that likely limits their ability to support effective immune responses upon reinfection. While these data clearly show that protective immunity is unlikely to develop in patients with chronic infection treated with antiviral therapy, it is unknown whether this is also true of acute/recent HCV infection.

Hypothesis: We hypothesize that HCV treatment during acute infection prior to the onset of T-cell exhaustion will prevent development of the 'immunological scar' seen after treatment of chronic HCV, leading to a response similar to that of spontaneous clearers and possibly providing some protection against reinfection.

Experimental Design: We will evaluate the presence of an 'immunological scar' in three populations: Patients treated with direct-acting antivirals (DAA) during acute infection, during chronic infection (positive control), and spontaneous resolvers who recovered from acute HCV (negative control). Pre- and post-treatment samples are available from clinical trials of acute and chronic HCV infection treated with DAAs. *Aim 1: Characterization of HCV-specific T-cells in acute HCV infection:* The frequency of HCV-specific T-cells before and after treatment will be measured by enrichment using MHC-class-1 pentamers and phenotype will be evaluated by flow-cytometry using markers of T-cell memory, activation and exhaustion.

Results: In current conduction/processing

Significance: If patients treated early in infection before exhaustion arises do not develop the immunological scar, this could have important implications for understanding development of immune exhaustion and for practical management of patients, as it would make a strong case for recognition and treatment of acute/recent infection. It would also be relevant to vaccine development, as potential future vaccines may be less effective in patients with prior chronic HCV if the 'immunological scar' persists.

K.7: CRIg expression confers phagocytic function and immunomodulating activity to Kupffer cells in the liver

Student: Fiorelle Aguilar, Supervisors: Blayne Sayed, Sergio Grinstein

Background: Kupffer cells (KCs) are liver-resident macrophages vital for liver homeostasis, defense against pathogens, and inflammation resolution. They express Complement Receptor of the Immunoglobulin superfamily (CRIg), crucial for clearance of infectious bacteria. CRIg recognizes complement-associated opsonin C3b during phagocytosis, exerting an anti-inflammatory effect unique among complement receptors. Loss of CRIg leads to impaired bacterial clearance, chronic liver inflammation, and fibrosis. Additionally, CRIg promotes T cell tolerance, inhibits complement pathway amplification, and suppresses NLRP3 and IL-1β transcription in response to inflammation, such as hepatic ischemia-reperfusion injury (IRI). KCs, by expressing CRIg, play a crucial immunoregulatory role in maintaining liver health and combating inflammation. Key questions regarding CRIg function in KC biology persist, including the elucidation of receptor mechanisms and downstream signaling pathways in phagocytosis, the independence of CRIg from complement ligands, and the linkage between its non-inflammatory, immunomodulatory role and its phagocytic function.

Hypothesis: We hypothesize that CRIg expression is sufficient to confer phagocytic activity to heterologous cell systems and KCs, and that immunoregulatory signaling pathways are augmented by CRIg's phagocytic function.

Purpose: To assess the role of CRIg in mediating phagocytosis – Our primary aim is to delineate the phagocytosis function of CRIg by assessing its ability to bind and internalize fluorescent microbeads coated with C3b, iC3b, or lipoteichoic acid, a component of gram-positive bacterial cell wall, in stably overexpressed CRIg RAW-ASC cells, elucidating the involved signaling pathways. Additionally, we will investigate how CRIg expression and ligand binding influence NLRP3 function and IL-1β release in these cells expressing ASC, contrasting with untransfected cells.

Results: Via qPCR and blotting, RAW-ASC cells appear to not endogenously express CRIg. When the receptor is transiently overexpressed, CRIg has a largely intracellular distribution, with approximately 10% of the protein being on the cell surface, as seen by confocal fluorescent imaging. When CRIg interacts with ligand-coated beads, an intracellular re-distribution of the receptor to the cell surface occurs.

Conclusion: KCs, a pivotal tissue-resident macrophages population of the body, crucially maintain liver homeostasis, defend against pathogens, and orchestrate inflammation resolution by expressing the unique CRIg receptor, which is essential for pathogen clearance and immunoregulation. This KC marker highlights the critical role of resident macrophages in liver health and inflammation, as in IRI and liver fibrosis. Phagocytic RAW-ASCs can interact with complement-coated beads via exogenous CRIg receptor. Our project aims to unravel the critical yet poorly defined cascades of CRIg in phagocytosis and immunomodulation, offering insights into how it confers its protective effects against inflammation, which may pave the way for therapeutic interventions in liver transplantation.

K.8: The immunobiology of multisystem inflammatory syndrome in children

Student: Elsa Salvant, Supervisor: Rae Yeung

Background: Multisystem Inflammatory Syndrome in Children (MIS-C) is a novel post-infectious hyperinflammatory syndrome that occurs 2-6 weeks after acute COVID-19. This illness resembles Kawasaki disease (KD), a hyperinflammatory vasculitis syndrome, with overlapping symptoms including prolonged fever, multiorgan dysfunction, laboratory evidence of hyperinflammation, and severe cardiovascular involvement. However, MIS-C is more severe with increased cardiac complications, gastrointestinal involvement, and evidence of coagulopathy.

Purpose: Previous groups have set out to characterize the immune response of MIS-C in patients with conflicting results given the small patient numbers and often post-treatment samples. A reproducible and comprehensive characterization of the immune response in patients with MIS-C is lacking, as well as an understanding of why only a fraction of kids develop MIS-C after having acute COVID-19. Preliminary results demonstrate that clinical, biological, and genomic factors suggest distinct clinical and biological phenotypes of MIS-C (Dr. Paul Tsoukas, Sophie Sun, Henry Lu) and that immune cell subsets and other inflammatory markers can be used as a measure for stratifying disease severity in MIS-C patients.

Hypothesis: Children who develop MIS-C have distinct endotypes with unique immune signatures that differ from children who do not develop MIS-C post-COVID infection.

Methods & Objectives: Peripheral blood mononuclear cells (PBMCs) banked from MIS-C Positive and Acute COVID-19 (MIS-C negative) patients before any immunosuppressive treatment will be used. These PBMCs will be subject to both unstimulated and stimulated conditions using Toll-Like Receptor agonists, CD3-CD28 antibodies (Dynabeads), and SARS-CoV-2 Spike protein peptide pools to (1) Characterize the unique immune signatures in patients with MIS-C and (2) Characterize the SARS-CoV-2 mediated immune response of MIS-C and acute COVID-19 patients. Distinct immune signatures identified from these objectives can inform establishing different clinical and biological phenotypes of MIS-C and their respective prognosis. All baseline and functional immunophenotyping will be done through CyTOF (Cytometry by Time of Flight) mass cytometry using 56 surface and intracellular markers to phenotype over 37 immune cell populations to identify the unique immune profiles.

Results: Optimal stimulation conditions for standard immune cell stimuli LTA, R848 and CD3-CD28 Dynabeads were determined to be 0.125 ug/mL, 0.500 ug/mL, and 1 bead:2 cells respectively when stimulating $1x10^6$ cells/well for 6 hours. Preliminary standardization of immunophenotyping with CyTOF using control PBMCs shows that monocytes and T-cells produce greater intracellular cytokines, including TNF-a and IL-2, upon stimulation with CD3-CD28 Dynabeads, LTA and R848 compared to baseline. Optimizations are still ongoing to determine the ideal stimulation conditions of the SARS-CoV-2 Spike Protein peptide pool of the same seeding density of PBMCs.

Conclusion: The findings of this study can be utilized to better understand and perhaps identify individuals who advance to MIS-C following acute COVID-19 disease, as well as discriminate between the various MIS-C endotypes and potentially prescribe the best course of treatment.

L: Cardiovascular, Respiratory, & Musculoskeletal

L.1: The impact of sensory protection and glial derived neurotrophic factor on fibro-adipogenic progenitors

Student: Christina Doherty, Supervisor: Jane Batt

Background: Peripheral nerve trauma causes atrophy and fibro-fatty infiltration (FFI) of skeletal muscle. Duration of denervation determines whether re-innervation can reverse these muscle sequelae[1]. Fibro-adipogenic progenitor cells (FAPs) are muscle resident stem cells that differentiate to fibroblasts and adipocytes, which mediate FFI[2]. Sensory protection (SP), whereby a sensory nerve is anastomosed to denervated muscle, partially mitigates FFI and correlates with a decrease in muscle Glial Derived Neurotrophic factor (GDNF), suggesting that GDNF may regulate denervation-mediated FAPs recruitment and differentiation[3].

Purpose: Determine the role of sensory protection and GDNF on FAPs recruitment and differentiation.

Hypothesis: I hypothesize that neurotrophic support provided to a denervated muscle by a surgically anastomosed sensory nerve mitigates fibro-fatty degradation by regulating FAPs recruitment and phenotypic switch from a pro-regenerative to pro-fibrotic/adipogenic phenotype, and that GDNF acts a key factor that promotes the persistence and differentiation of FAPs to adipocytes, and possibly fibroblasts, in denervated muscle.

Methods: Rats underwent tibial nerve transection of the right hindlimb to denervate the gastrocnemius followed by no nerve repair, sensory protection of the denervated muscle (with surgical transposition of the sural nerve) or immediate repair of the tibial nerve, and were assessed at 5 & 12-weeks post denervation. The left limb served as an internal control. Flow cytometry/FACS assessed gastrocnemius FAPs expression. To assess the impact of GDNF on FAPs proliferation and differentiation, healthy sorted FAPs were treated with GDNF (15-100ng/mL*) in vitro* and assessed for markers of proliferation as well as fibrogenic and adipogenic differentiation.

Results: FAPs content increased in 5 & 12-week denervated gastrocnemius muscle relative to control. The FAPs increase was mitigated by sensory protection. Muscle receiving immediate nerve repair showed minimal FAPs increase. FAPs demonstrated a dose dependent response to GDNF. PCNA expression increased with increasing concentrations of GDNF demonstrating increased proliferation. Increasing concentrations of GDNF induced increasing Perilipin-1 expression (adipogenic differentiation) as well as collagen 1a1 and SMA expression (fibrogenic differentiation), with nearly an 8-fold, 2-fold and 3-fold increase seen respectively ($P < 0.05$). Perilipin-1 protein expression was increased in FAPs subjected to 100ng/mL of GDNF, with a 10% increase seen relative to controls ($P < 0.05$).

Conclusion: Sensory protection decreases GDNF expression, FAPs recruitment and mitigates fibro-fatty degradation of denervated muscle. GDNF is a novel mediator of FAPs adipogenic and Fibrogenic differentiation, inducing proliferation, as well as fibrogenic and adipogenic differentiation in a dose dependent manner. Together these data suggest that sensory protection of denervated muscle results, in part, from a decrease in GDNF-mediated stimulation of FAPs adipogenic differentiation.

L.2: Multi-omics data integration for psoriatic disease

Student: Sreemoyee Ghosh, Supervisor: Vinod Chandran

Background: Psoriasis (Pso) is a chronic immune mediated inflammatory skin disease. Approximately 24% of Pso patients develop psoriatic arthritis (PsA). PsA is a systemic, clinically heterogeneous inflammatory disease. It is characterized by multiple clinical manifestations including peripheral arthritis, axial arthritis, enthesitis, dactylitis, uveitis, nail lesions and skin psoriasis. PsA is associated with progressive joint damage, if left untreated it can lead to worsening of symptoms and permanent disability. Pathophysiology of PsA is complex and not completely understood. Recent advances have been made in targeted therapeutics for both Pso and PsA. However, at least 40% of PsA patients exhibit no response or only a partial response. Therefore, there is an urgent unmet need for alternative therapeutic strategies for PsA. Identification of new pathways relevant to PsA pathogenesis may point towards potential novel therapeutic targets. Multi-omics integration is a promising strategy that involves combining multiple 'omic' or biological layers ,as described in the central dogma, systematically to obtain a holistic view of the complex pathways underlying a disease.

Purpose & Hypothesis: The purpose of this study is to identify additional pathways associated with PsA that might provide potential therapeutic targets for PsA. I hypothesize that integrating multiple single omics datasets from publicly available omic studies comparing PsA and cutaneous only Pso patients using network analysis can identify novel and relevant pathways for PsA.

Methods: A scoping review was conducted to curate all publicly available omic studies in the field of psoriatic disease from 3 databases-Ovid Medline, Embase and Cochrane. Inclusion criteria included all English language studies related to Pso and PsA investigating biomarkers/molecular signatures in human subjects using non-targeted high throughput experiments. Lists of differentially expressed markers, study and clinical information were extracted from all papers that passed the eligibility criteria, to develop a multi-omics data integration portal. Next, to identify PsA specific pathways in serum, lists of differentially expressed proteins, miRNAs and metabolites from 3 independent single omic studies comparing serum samples of PsA and cutaneous only Pso were collected. All differentially expressed markers were then integrated with the help of the following network analysis tools-MirDIP 5.2 (miRNA-target gene interactions), IID (protein-protein interactions), STITCH5 (metabolite-gene interactions), pathDIP5 (integrated pathway enrichment analysis), NAViGaTOR (network analysis, visualization of biological networks). Single omic markers-proteins, metabolites and miRNAs were connected via a network of biological interactions and overlapping pathways.

Results: 5 miRNAs, 34 proteins and 18 metabolites differentially expressed between PsA and PsC were derived from the 3 independent studies selected. 71 target genes of the 5 miRNAs were found to be connected with 10 proteins and 19 gene interactors of 3 metabolites via protein-protein interactions and 71 statistically significant pathways were found to be common among them. Of these 71 pathways, TNF/stress related signaling, RANKL pathway, angiogenesis, NOTCH signaling were found to be important to PsA pathogenesis from literature review and a significant number of pathways such as PI3-Akt, JAK/STAT, IL-7 signaling pathways were found to be specifically important to Pso pathogenesis.

Conclusion: Multi-omics integration of independent single omic datasets may identify complex disease specific pathways.

L.3: Association of preoperative right ventricular function with short-term outcomes after cardiac surgery

Student: Neeki Alavi, Supervisors: Wilton van Klei, Justyna Bartoszko

Background: The assessment of right ventricular (RV) function using quantitative parameters such as RV fractional area change (RVFAC) and tricuspid annular plane systolic excursion (TAPSE) is recommended as part of a comprehensive echocardiography exam before and after cardiac surgery. Despite the growing recognition of the impact of RV function on outcomes in many patient populations, the connection between RV function and outcomes in cardiac surgical patients is not well-defined.

Purpose & Hypothesis: The purpose of this study was to investigate the association between echocardiographic RV function parameters and postoperative outcomes to improve evaluation of high-risk patients undergoing cardiac surgery. It was hypothesized that patients with impaired TAPSE or RVFAC experience an increased risk of adverse events.

Methods: This retrospective cohort study included adult patients (>18 years) who underwent cardiac surgery at Toronto General Hospital between January 2016 and December 2021 with a preoperative echocardiogram report within 365 days before surgery. The primary outcome was 30-day in-hospital mortality, and secondary outcomes were hospital length of stay (HLOS) and acute kidney injury (AKI). AKI was defined as an increase in postoperative serum creatinine ≥1.5 times preoperative serum creatinine within the first 5 days after surgery. Unadjusted and adjusted logistic regression and negative binomial regression models were conducted to estimate the association between the RV function parameters and outcomes. Confounding variables were determined *a priori* and adjusted for in multivariable models (age, gender, hypertension, chronic lung disease, congestive heart failure, procedure length, pre-operative creatinine, and procedure type). Results are presented as risk ratios (RR) or odds ratios (OR) with 95% confidence intervals (CI).

Results: A total of 6,070 patients were included. Preoperative TAPSE was reported in 2,148 patients (62±14 years old, 30% female, BMI 28±10 kg/m^2). The incidence of 30-day mortality in this patient group was 2.7% (n=59), median HLOS was 9 days [IQR: 6,14], and incidence of AKI was 10.9% (n=235). Preoperative RVFAC was reported in 1,309 patients (60±15 years old, 32% female, BMI 28±13 kg/m^2). The incidence of 30-day mortality in this patient group was 3.2% (n=42), median HLOS was 8 days [IQR: 6,12], and incidence of AKI was 10.2% (n=134). After adjusting for confounders, TAPSE was significantly associated with mortality (OR per 1 mm increase: 0.92; 95% CI: 0.87-0.98) and HLOS (RR: 0.99; 95% CI: 0.99-0.99); however, not with AKI (OR: 0.99; 95% CI: 0.96-1.02). RVFAC was also significantly associated with mortality (OR per 1% increase: 0.96; 95% CI: 0.93-0.99) and HLOS (RR: 0.99; 95% CI: 0.99-0.99), and not with AKI (OR: 0.99; 95% CI: 0.97-1.01). In daily care, clinicians often evaluate TAPSE with a cut-off value of <17 mm. At this value, TAPSE was significantly associated with mortality and HLOS.

Conclusion: Our findings provide evidence that RV function as measured with echocardiographic parameters such as TAPSE and RVFAC is associated with 30-day in-hospital mortality and HLOS, but not with early postoperative AKI.

L.4: Alcohol consumption in axial spondyloarthritis: preliminary results highlighting sex differences in the impact of alcohol on radiographic progression

Student: Evelyne Gendron, Supervisor: Nigil Haroon

Background: Axial spondyloarthritis (axSpA) is a chronic inflammatory arthritis, mainly affecting the sacroiliac joints and the spine. Some patients with this condition develop new bone formation in the spine (syndesmophytes/bridging syndesmophytes), leading to significant functional impairment. Most of the risk factors contributing to radiographic progression are non-modifiable. Consequently, identifying other modifiable lifestyle factors of disease progression in axSpA would enable to implement an individualized intervention strategy in the management of axSpA.

Purpose & Hypothesis: We evaluated the impact of alcohol consumption (AC) on spinal radiographic progression in axSpA as measured by the modified Stoke Ankylosing Spondylitis Spinal Score (mSASSS). Progression was defined as an increase of ≥2 mSASSS units in 2 years. The underlying hypothesis guiding this study is that alcohol consumption leads to increased radiographic progression. AC has the potential to induce chronic inflammation by both increasing pro-inflammatory cytokines and triggering alcohol-induced intestinal dysbiosis.

Methods: We included AxSpA patients from the University Health Network (UHN)- Spondyloarthritis cohort, where they are prospectively followed annually with a standardized protocol. We used data collected from March 3, 2008 to May 17, 2023. Among individuals who had sacroiliitis based on the modified New York (mNY) criteria, univariable and multivariable logistic regression analyses were conducted to determine the association between baseline AC and mSASSS progression. The covariates included were syndesmophytes at baseline, ASDAS-CRP at baseline, disease duration, ever-smoking, current biologic use, sex and HLA-B27 status. We used a stepwise regression technique for the multivariable analysis. Sensitivity analyses were performed to assess the impact of sex, types of alcohol (wine/beer/spirits), patterns of AC (social drinking/daily drinking), and quantities of alcohol consumed per week. Patients were categorized as having low AC if their intake was fewer than 2 units per week and as moderate-heavy AC if their intake was equal to or higher than 3 units per week.

Results: The study included 398 patients with axSpA (134 in the drinker group and 264 in the non-drinker group). The mean age was 38.8 years old (SD = 13.7). 68% were male participants and 74% were positive for HLA-B27. 89% had sacroiliitis satisfying mNY criteria. 60% of participants were treated with biologic therapy. Among drinkers, the mean quantity of AC at baseline was 5.1 units per week (±6.4). Drinkers had more frequently a significant mSASSS progression than non-drinkers (27% (35/132) vs. 17% (43/253), p=0.03). In contrast to univariable analysis results, alcohol consumption was not a significant predictor of mSASSS progression in the multivariable regression (1.84, 95%CI 0.87, 3.89). ASDAS-CRP, age, male sex, syndesmophyte at baseline were significant predictors of mSASSS progression. Sensitivity analyses showed no association between mSASSS progression and different types of alcohol, patterns of AC, and quantities of alcohol consumed per week. However, our analysis stratified by sex revealed a trend towards increased odds of mSASSS progression only among men who consumed alcohol (2.19, 95%CI 1.00, 4.79).

Conclusions: We found a trend towards increased odds of 2-year mSASSS progression only in men. Our findings suggest a potential sex difference related to alcohol's effect. Furthermore, ASDAS-CRP, male sex, and syndesmophyte at baseline were identified as predictors of spinal radiographic progression, which is consistent with the current literature.

L.5: Developing a digital lung: a deep learning approach to simulating donor lung function during ex vivo lung perfusion

Student: Xuanzi Zhou, Supervisors: Shaf Keshavjee, Andrew Sage

Background: Ex vivo lung perfusion (EVLP) is an established repair and reconditioning platform that generates multi-modal physiological data from isolated human lungs and is well-suited for machine learning. *Digital twins* – computer simulations of physical objects, are an emerging concept in medicine, but their potential in transplant medicine remains largely unexplored.

Purpose & Hypothesis: We aim to develop a digital twin model of the ex vivo lungs that can be used to simulate critical lung functional parameters. We hypothesize that a digital twin of an ex vivo lung will accurately simulate donor lung function during EVLP and can be used as a robust control for clinical research.

Methods: Flow and pressure data were recorded at a resolution of 100Hz using the Servo-i ventilator (Maquet, USA) during n=50 clinical EVLP cases (n = $1.2x10^6$ data points/case). Neural Network (NN) models were trained to predict lung function for the subsequent evaluation period (i.e., 2^{nd} hour) based on the previous lung assessment (i.e., 1^{st} hour) for physiological parameters such as: lung compliance, peak and mean airway pressures, and stress index. To demonstrate the utility of the predicted lung data, a long-short term memory (LSTM) model was trained to predict transplant suitability using either the observed or forecasted EVLP lung function data.

Results: The NN-based model accurately forecasted future lung function. Mean absolute error (MAE) and mean absolute percentage error (MAPE) were reported for each parameter: dynamic compliance (MAE=4.9 mL/cmH_2O; MAPE=6.0%); stress index (MAE=0.07; MAPE=6.7%); mean airway pressure (MAE=0.25 cmH_2O; MAPE=3.1%); and peak airway pressure (MAE=0.8 cmH_2O; MAPE=4.5%). Importantly, we found no difference in the predicted EVLP outcome using the actual or simulated ventilation data as input data to the LSTM model (p=0.69).

Conclusion: This study demonstrates an innovative deep learning method to enable precise simulation of lung function. This approach lays the foundation for ongoing efforts to develop a digital model of ex vivo lungs that has the potential to replace the control arm of clinical donor lung studies in the future.

L.6: Leveraging a transgenic mouse model to characterize extracellular vesicle-based cell-cell communication in atherosclerosis

Student: Kunze (Mandy) Guo, Supervisors: Kathryn Howe, Jason Fish

Introduction: Atherosclerosis is a multi-cellular disease characterized by unresolved inflammation and the deposition of lipoproteins and immune cells in the arterial vessel wall. It occurs in response to smoking, increased cholesterol, diabetes, and high blood pressure, with clinical manifestations such as myocardial infarction and/or stroke occurring as a result of plaque build-up and rupture. An early marker of atherosclerosis is endothelial cell (EC) dysfunction. ECs are uniquely positioned as a boundary layer of the vessel wall capable of communicating with the circulating blood and the vessel wall. Extracellular vesicles (EVs), nanoparticles that carry cargo capable of governing cellular function and/or signalling disease have emerged as a novel communication paradigm. While *in vitro* models have shown EC-EV crosstalk with monocytes and vascular smooth muscle cells, animal models interrogating EC-EVs in atherosclerosis are lacking. We therefore created a model that allowed us to detect, quantify and visualize fluorescent EC-EVs in a murine model of atherosclerosis.

Methods: I have generated a novel transgenic EC-EV tracking mouse model, in which an inducible endothelial-specific Cre is used to activate the expression of an emerald GFP-tagged CD63 protein, a key component of EVs. Because this is on an *Ldlr*$^{-/-}$ background, this model can be used to study the role of endothelial derived EVs in the initiation and progression of atherosclerosis. Nine-week-old mice (LCE^{Gr}; Ldlr$^{-/-}$:Cdh5Cre^{ERT2}:CD63-emGFP$^{l/s/l}$ and LE^{Gr}; Ldlr$^{-/-}$:CD63-emGFP$^{l/s/l}$) were injected with tamoxifen (3 mg i.p., 3 doses, male & female) to induce green fluorescent EC-EVs. Mice received high-cholesterol diet (HCD, 12 wks) to promote atherosclerotic lesion formation or normal chow. EVs were enriched by size exclusion chromatography from plasma, quantified, and characterized by nanoparticle tracking analysis (NTA). Flow cytometry was performed to detect EC-EVs in cells isolated from whole blood. EC-EVs were visualized within atherosclerotic plaques using confocal microscopy.

Hypothesis: ECs from atheroprone regions will secrete EVs that are detectable in circulation and transferred to neighbouring vascular cells. The quantity of EC-derived EVs will increase in circulation and within vascular cells with progression of atherosclerosis, and their contents will be enriched for pro-atherogenic mediators.

Results: As anticipated, HCD-fed mice developed atherosclerotic plaques compared to mice fed normal chow (n=3/grp). Fluorescent (GFP+) EVs were visualized in plasma of tamoxifen treated LCE^{Gr} mice (NTA, 488nm laser; EVs pooled from 3 mice) and detected in blood cells of tamoxifen treated LCE^{Gr} mice, but not in LE^{Gr} mice (flow cytometry, n=2-4/grp). NTA demonstrated total plasma EVs were increased in HCD-fed mice compared to mice on chow diet ($2.21x10^{12}$ vs $2.40x10^{11}$ particles/ml; $p<0.0001$; n=3/grp). Using confocal imaging we observed tissue-based EC communication in atherosclerotic plaques, where EC-EVs colocalize within leukocytes (GFP+CD68+; n=3/grp).

Conclusions & Significance: This is the first *in vivo* approach to track the fate of EC-EVs in an intact vessel - enabled detection of fluorescent (GFP+) EC-EVs in the circulating blood and visualization in leukocytes within atherosclerotic plaques. Significantly, my research will determine the role of EC-EV communication in atherosclerosis and help define novel targets that can be explored in pre-clinical studies using an animal model that has been unavailable to date.

L.7: Evaluating the impact of extended warm ischemic time using ex-vivo heart perfusion in juvenile porcine models of circulatory death

Student: Mimi Deng, Supervisors: Osami Honjo, Fillio Billia

Background: In heart transplant (HT), the donor pool is insufficient to meet the demand. This issue is particularly severe in the pediatric population, where the waitlist mortality or withdrawal from clinical deterioration is 25-50%. Donation after circulatory death (DCD) is one strategy to expanding the donor pool and shown to increase HT rate by 15-30% in countries that perform DCD. However, DCD hearts are considered high-risk allografts due to the injury sustained from the warm ischemic time (WIT) between withdrawal of life-sustaining therapy and graft procurement. Hearts with a WIT >30 minutes are abandoned. This threshold is particularly limiting for pediatric hearts that take longer to arrest, compared to adult hearts. In recent years, ex-vivo heart perfusion (EVHP) has emerged as a technique to rehabilitate and assess marginal cardiac allografts prior to transplant.

Purpose & Hypothesis: The aim of the study is to apply EVHP to recover and appraise DCD hearts subjected to WIT of 30, 45, and 60min. We hypothesis a correlation between longer WIT and decline in cardiac function, with the potential for EVHP to improve hearts with 45min WIT such that they are comparable to 30min WIT control.

Methods: Juvenile porcine DCD hearts subjected to WIT of 30, 45, and 60min will be reperfused for 2hr using the validated SickKids EVHP protocol, then reanimated using the EVHP's working mode for 2hr functional assessment using pressure-volume relationship analysis and epicardial echocardiography. Serial serum will be collected for assessment of myocardial damage (e.g. lactate, myocardial oxygen consumption, troponin) and inflammatory response (porcine cytokine/chemokine array). Tissue at the end of the experiment will be analyzed for oxidative stress (e.g. myocardial 8-isoprostane, troponin I, CK-MB, LDH, mitochondrial cytochrome C, myeloperoxidase by ELISA assay; 8-hydroxy-2'-deoxyguanosin, and malondialdehyde, phosphorylated and total protein kinase B and endothelial nitric oxide synthase by qRT-PCR), apoptotic markers (e.g. hematoxylin & eosin staining, activated capase-3 TUNEL assay), and metabolic function (e.g. untargeted metabolic analysis by Metabolon Inc., Na+/K+ ATPase activity).

Results: DCD hearts subjected to 30min WIT (n = 3/6) are able to achieve 120% cardiac output (CO) in working mode, while targeting physiological left atrial pressure of 3-7mmHg. 60min WIT hearts (n = 1/6) show reduced CO of ~20%. Clarifying the acceptable pediatric WIT threshold and establishing the role of EVHP will facilitate the advent of DCD HT in Canada, for the pediatric population that fares worst on the transplant waitlist and has the most life-years to gain.

M: Cardiovascular, Respiratory, & Musculoskeletal

M.1: Healthcare professional identified priorities for a new musculoskeletal examination scoring system for people with hemophilia – an international consensus

Student: Lizabeth Teshler, Supervisor: Brian Feldman

Background: Joint bleeds, the main clinical manifestation in hemophilia, can cause physical damage that may eventually lead to lasting physical impairments. This may impact health-related quality of life, school and work performance, activities and interpersonal connections for people living with hemophilia. The accurate examination of joint health is essential as part of best practice care of hemophilia patients and must be consistently monitored in clinic. The International Prophylaxis Study Group (IPSG) Musculoskeletal Expert Working Group has identified that developing a shorter and efficient physical joint examination tool, for clinic use, is a priority.

Purpose: To conduct an international consensus process to identify the priority needs for healthcare professionals when examining the musculoskeletal health of hemophilia patients in clinic.

Methods: Priorities were identified and consensus was reached through a modified online Delphi process and an expert steering committee meeting. Three surveys were distributed to healthcare professionals worldwide that are participants of the World Federation of Haemophilia (WFH) Musculoskeletal (MSK) Committee meetings.

Results: The three surveys achieved response rates of 58%, 54% and 57%. The top two priorities as to why a new MSK scoring system should be developed were 1) Current tools cannot detect the earliest and subtle changes of hemophilia, and 2) Current tools are too time consuming. The top two attributes and purposes of a new MSK scoring system were 1) To measure the earliest changes of hemophilia, and 2) To measure joint health changes over the course of time. The majority of the respondents believed that a new MSK scoring system should be used by physiotherapists in clinic.

Conclusion: The Delphi process successfully identified which future priorities should be considered in order to develop an improved MSK scoring system in clinic.

M.2: Dynamic profile of endothelin-1 levels during ex vivo lung perfusion varies across donation type and is associated with post-transplant outcomes

Student: Abby McCaig, Supervisor: Mingyao Liu

Background: Lung transplantation is the primary intervention for end-stage lung diseases. However, limited donor organs are accepted for transplantation due to the concern of poor patient outcomes. The ex vivo lung perfusion (EVLP) system was developed to extend the donor assessment period by mimicking the lung's physiological conditions via mechanical ventilation and perfusate circulation. EVLP allows for a comprehensive assessment of biological, physiological, and biochemical measures to evaluate marginal donor organs. Research shows that measuring various biomarkers (e.g., cell death markers and cytokines) circulating in the perfusate can be useful in determining organ suitability. Endothelin-1 (ET-1), a chemokine that promotes vasoconstriction, has been proposed as a biomarker that determines endothelial function. We have found ET-1 to have a unique profile during EVLP characterized by an initial spike in levels followed by either sustained levels or a significant decrease. This unique fluctuation pattern sets ET-1 apart from all other biomarkers studied in perfusate.

Purpose & Hypothesis: The purpose of this study is to investigate the kinetic profile of ET-1 during EVLP and understand the biological mechanism behind this unique fluctuation pattern. We hypothesize that ET-1 levels can be used to predict donor lung outcomes.

Methods: Perfusate samples were collected at 15-minute intervals from the start of perfusion during clinical EVLP at the Toronto Lung Transplant Program from 2017 to 2019. ET-1 levels in perfusate were quantified by ELISA (Protein Simple, San Jose, CA, USA). A total of 19 donation after brain death (DBD) cases (12 transplanted and 7 rejected), and 16 donation after circulatory death (DCD) cases (10 transplanted and 6 rejected) were included in this study.

Results: Our results show a unique and dynamic profile of ET-1 during EVLP. Interestingly, multiple DCD lungs showed a pronounced pattern of rapid ET-1 accumulation and then clearance during the first 90 minutes of EVLP. Accordingly, ET-1 levels were higher over the first 90 minutes of EVLP in injured lungs in DCD donors (4.00±2.09 pg/mL) compared to DBD donors (1.96±1.50 pg/mL) ($p=0.038$). The ET-1 levels in DCD lungs over the first 90 min of EVLP for recipients extubated within 72h (0.86±0.38 pg/mL) were significantly lower than those extubated after 72h (4.47±2.44 pg/mL) ($p=0.0019$), and significantly lower than the lungs rejected after EVLP (3.76±2.10 pg/ml) ($p=0.021$).

Conclusion: Here, we are the first to show a dynamic profile of ET-1 not seen in any other biomarkers. We observed a unique and dynamic profile of ET-1 during the first 90 minutes of EVLP in DCD lungs, mainly in transplant recipients extubated after 72h and rejected lungs. This may reflect endothelial injury in donor lungs and recovery during EVLP. The results obtained from this study will provide valuable information to clinicians regarding the endothelin function of donor lungs to make informed decisions regarding transplant suitability, leading to improved recipient outcomes.

M.3: Sex differences in serum proteomic profiles in psoriatic arthritis

Student: Steven Dang, Supervisors: Vinod Chandran, Lihi Eder

Background: Psoriatic arthritis (PsA) is an immune-mediated inflammatory skin and joint disease affecting males and females equally. However, they experience different disease courses, clinical presentations, and responses to therapy. The mechanisms underlying the sex differences remain unclear.

Purpose & Hypothesis: We aim to identify sex-specific serum proteins and biological pathways influencing PsA disease course and presentation. We hypothesize that sex dimorphisms exist in the immune and inflammatory pathways in PsA and that these profiles correlate with the clinical features in male and female patients.

Methods: We analyzed 100 active age-matched PsA patients (1:1 sex ratio) and 50 age- and sex-matched healthy controls (1:1 sex ratio). Serum samples were sent for proteomic analysis using the multiplex, aptamer-based SomaScan 7K assay. Differentially expressed proteins (DEPs) were identified as false discovery rate-adjusted p-value < 0.05 between PsA and healthy controls overall and by sex. Upregulated and downregulated DEPs in males and females were independently assessed in a pathway enrichment analysis using pathDIP version 5. Lastly, machine learning classifiers were used to identify the best-performing protein signatures for disease status (overall and sex-specific) from the DEPs.

Results: The differential expression analysis revealed 1272 upregulated and 386 downregulated DEPs in all PsA patients vs. healthy controls. When disaggregated by sex, the number of DEPs for PsA males was substantially higher than for PsA females (male PsA vs. controls: 1002 upregulated, 209 downregulated; female PsA vs. controls: 188 upregulated, 98 downregulated).

Conclusions: The preliminary findings suggest unique sex differences in serum immune and inflammatory proteins in PsA. Our study highlights the need to address sex in future PsA work for the development of sex-specific tools for the diagnosis, management and treatment of the disease.

M.4: Interleukins in peripheral arterial disease

Student: Niousha Djahanpour, Supervisor: Mohammad Qadura

Background: Peripheral Arterial Disease (PAD) is a cardiovascular disease of the arteries of the lower limb(s) leading to reduced blood flow, resulting in complications ranging from exertional muscle pain to critical limb ischemia, and in severe cases amputations. This inflammatory condition is characterized by systemic atherosclerosis that often results in major cardiovascular events such as lower extremity amputation, myocardial infarction, cerebrovascular accidents, and cardiovascular mortality. Recent studies have highlighted the role of interleukins (ILs) in the development of atherosclerosis and PAD. ILs are cell signaling proteins that orchestrate the immune response to acute and chronic inflammation along the Inflammasome-Interleukin axis, a pathway critical to the pathophysiology of PAD. Understanding the role of ILs can be instrumental in the development of targeted therapeutics and in risk-stratifying patients for the progression of PAD, enhancing the management of this disease.

Purpose: The study aimed to examine the prognostic capabilities of interleukins as biomarkers for the progression of PAD, major adverse limb, and cardiovascular events.

Hypothesis: The study hypothesized that the elevated expression of interleukins can distinguish patients with PAD from those without the disease. The study also aimed to assess the ability of Interleukins to serve as predictive biomarkers for adverse clinical outcomes in high-risk PAD patients; specifically Major Adverse Limb Events (MALE) – composite of a decrease in Ankle Brachial Index (ABI) greater than 0.15, the need for vascular revascularisation, and major amputation – and Major Adverse Cardiovascular Events (MACE), encompassing myocardial infarction, cerebrovascular accident, and cardiovascular-related death.

Methods: A cohort of 441 subjects was recruited from the Vascular Surgery Clinic at St. Michael's Hospital, comprising 148 non-PAD and 293 PAD patients. Multiplex assays were used to analyze plasma samples to quantify 25 protein levels.

Results: A panel of ILs was analyzed, revealing elevated levels of IL-1Ra, IL-2, IL-6, IL-8, IL-15, IL-16, IL-18, and notably IL-28A in PAD patients. Among these, Interleukin-28A (IL-28A) emerged as a critical biomarker with its levels significantly higher in PAD patients [median (IQR) 516.76 (345.45 – 654.63) pg/ml] in comparison to non-PAD patients [median (IQR) 410.38 (273.95 – 561.97) pg/ml] (p-value <0.001). Multivariate cox analysis demonstrated IL-28A to be an independent predictor for the need for vascular revascularization [HR = 1.42 (1.09 – 1.84); p-value 0.009], and major amputation [HR = 2.28 (1.11 – 4.67); p-value = 0.024]. Additionally, a cumulative survival analysis demonstrated a direct correlation between high IL-28A levels and the need for vascular revascularization (p-value <0.004), major amputation (p-value <0.016), and MALE outcomes (p-value<0.005) during the follow up period.

Conclusions: Our findings reveal eight interleukins to be elevated in patients with PAD, and an association between elevated levels of IL-28A and adverse limb outcomes in PAD patients, suggesting IL-28A as a potential therapeutic target, or as a biomarker for risk stratification of PAD patients.

M.5: The clinical relevance of necroptosis in ischemia reperfusion injury in human lung transplants

Student: Kimberly Main, Supervisor: Mingyao Liu

Background: Currently, lung transplantation (LTx) exists as the only treatment option for patients suffering from end-stage lung disease. However, lung transplantation is severely limited due to the low utilization rates of donor lungs. These low utilization rates result from the frequent rejection of marginal donor lungs by surgical teams, due to the concern of poor patient outcomes post-transplant. Of particular concern, is the high incidence of primary graft dysfunction (PGD), the major cause of patient morbidity and mortality post-transplant. PGD primarily results from the inevitable ischemia-reperfusion injury (IRI) that occurs during the transplant process. As such, new therapeutic avenues to prevent IRI are needed to improve patient outcome post-transplantation and increase the utilization of donor lungs. Increasing evidence has implicated necroptosis, a form of regulated necrosis, as a driver of IRI in experimental lung transplant models. Inhibitors targeting the necroptosis pathway in cell culture and animal models have shown promise in attenuating IRI and improving PGD outcomes. However, the evaluation of necroptosis in IRI in human lung tissue has not been done.

Purpose & Hypothesis: The purpose of this study is (1) to identify the activation of necroptosis during ischemia-reperfusion injury in human lung transplants and (2) to assess the relationship between necroptosis activation and the development of poor patient outcomes post-transplant. We hypothesize that necroptosis plays a critical role in the pathogenesis of IRI in human lung transplantation and may influence the clinical outcome of the patient post-transplantation.

Methods: Paired human lung tissue biopsies (n=10) taken at the end of cold static preservation (CIT) and 2h post-reperfusion (2h REP) were collected from the Toronto Lung Transplant Program BioBank. Immunohistochemistry (IHC) was performed to identify the presence of necroptosis related proteins (i.e., RIPK1, pRIPK1, RIPK3, pRIPK3, MLKL, pMLKL). Staining of these proteins were quantified using the image analysis platform HALO and analyzed using GraphPad Prism. All transplants associated with the paired biopsies were assessed and received a PGD score (using the 2005 ISHLT PGD Guidelines) at 72 hours after transplant. A secondary cohort of human lung tissue biopsies were collected from 12 patients at CIT and 2h REP after bilateral lung transplantation. Six patients were intubated for over 72 hours post-transplant, and six patients were intubated for 72 hours or less post-transplant. Levels of necroptosis related proteins will be measured and quantified. The relationship between levels of necroptosis related proteins and patient outcome will then be analyzed.

Results: Levels of RIPK3 were greatly increased in the 2h REP samples compared to the CIT samples and trended towards significance (P=0.06). The increase in RIPK3 after 2h REP was most profound in the samples scored as PGD 2 and 3. Results from additional antibodies and Western blotting have not yet been obtained.

Conclusion: Current results have highlighted the presence of necroptosis related proteins in human lung tissue during ischemia-reperfusion injury, with a potential relationship to PGD. Further results will continue to uncover the relationship between necroptosis and patient outcome in the human LTx setting. Understanding this relationship will promote the next-generation of therapeutics targeting necroptosis to attenuate IRI, ultimately improving patient outcomes post-transplant.

M.6: Poor outcomes in pregnancies with concurrent maternal and fetal heart disease

Student: Beatriz Aldara Fernandez Campos, Supervisor: Candice Silversides

Background: Pregnancies in women with heart disease are associated with increased risk of adverse fetal and neonatal outcomes including transmission of their heart disease to offspring. However, little is known about outcomes when both the mother and the baby have heart disease.

Purpose: The aim of this study was to examine adverse fetal/neonatal and maternal obstetric events in pregnancies with *both* maternal and fetal heart disease.

Hypothesis: We hypothesized that there may be a "two-hit" mechanism whereby pregnancies complicated by both maternal and fetal heart disease (MF-HD) would have a higher rates of adverse fetal/neonatal and maternal obstetric events compared to pregnancies with maternal heart disease (M-HD) only.

Methods: Singleton pregnancies (> 24 weeks gestation) in women with structural heart disease followed in the University of Toronto Pregnancy and Heart Disease Program were included. All pregnancies had fetal and/or neonatal echocardiographic assessment for heart disease. Rates of adverse events in pregnancies with maternal heart disease (MHD) were compared to pregnancies with maternal and fetal heart disease (MF-HD). Fetal/neonatal adverse events were a composite of fetal or neonatal death, pre-term birth (<37 weeks gestation), and small for gestational age (<10^{th} percentile) babies. Maternal obstetric adverse events included preeclampsia or eclampsia. Differences between groups were examined using Chi square, Fisher´s exact test, Students t-test or Mann Whitney U test as appropriate. For the total cohort, univariable logistic regression analysis was used the identify determinants of adverse fetal/neonatal events. Spearman's Rho and Pearson's were used to test for collinearity when appropriate. Univariable predictors with a P value ≤0.1 were included in a multi-variable logistic regression model. A P value <0.05 (2-sided) was considered significant.

Results: From 1104 patients, 1011 pregnancies had MHD and 93 had MF-HD. Patients in MF-HD were younger (31 ± 5.59 vs 29 ± 6.22, p=0.023) and more likely to have a congenital cardiac fetal abnormality in prior pregnancies (9.7% vs 3.0%) than women in the MF-HD. There was no difference between other baseline characteristics between groups. Fetal events occurred in 26% of the entire cohort. Fetal/neonatal events (38.7% vs 25.3%, p=0.006) and spontaneous pre-term birth (10.8% vs 4.9%, p=0.021) were more frequent in MF-HD compared to MHD. Respiratory distress syndrome and neonatal intensive care unit admission were more frequent in MF-HD than in MHD group. The overall incidence of obstetric events was 4.6%, with no difference in the rates between groups. Univariable determinants of adverse fetal events such as smoking, prior history of diabetes, previous maternal cardiac intervention, worsening functional class, medications, and nulliparity were included in the multivariable model. MF-HD remained as a significant determinant of adverse fetal/neonatal events after adjustment (OR: 1.883; 95% CI:1.182–3.000; p=0.008).

Conclusions: Pregnancies with MF-HD are at increased risk of adverse fetal/neonatal events when compared to pregnancies with M-HD only. Pregnancies where maternal and fetal heart disease co-exist benefit from close surveillance and interventions to allow for a term-delivery to improve neonatal outcomes. Further studies are needed to elucidate associations between MF-HD and risk of developing preeclampsia.

M.7: Intraoperative blood management: identification and mitigation of inappropriate transfusions in cardiac surgery

Student: Jad Sibai, Supervisor: Keyvan Karkouti

Background: Alongside a myriad of health risks, inappropriate transfusions contribute to the depletion of limited, and expensive, healthcare resources. A recent multicenter Ontario audit revealed that 22% of red blood cell transfusions were inappropriate. This pattern was amplified in a 12-week audit of platelet transfusions across 57 hospitals, wherein 41.5% of adult orders were deemed inappropriate. Incorrectly gauging the necessity for transfusion is therefore a major concern – and one that is particularly pronounced in the cardiac surgical context. With the added effect of limited standardization, the incidence of inappropriate intraoperative transfusions remains alarmingly high, displaying wide inter-hospital variability.

Purpose: 1) To statistically navigate the prevalence of inappropriate platelet and fibrinogen transfusions in cardiac surgery while considering bleeding severity and relevant variables in defining and predicting inappropriateness, and 2) to qualitatively explore provider perceptions on transfusion decision-making factors and associated guidelines.

Hypothesis: We hypothesize a high prevalence of inappropriate transfusions, exacerbated by bleeding severity, procedural type, pump time, and neglected behavioural, environmental, and social factors.

Methods: *Quantitative study*: Employed a retrospective cohort design to analyze data from 872 consecutive patients who underwent cardiac surgery between July 5, 2021, and July 4, 2022. Statistical patterns with a focus on platelet and fibrinogen transfusions were investigated using R. Bleeding severity was stratified using Dyke et al.'s Universal Bleeding definition. *Qualitative study:* Semi-structured interviews with 15 cardiac surgery-centered healthcare professionals. Interviews are transcribed using a speech-to-text software. Coding and thematic analysis are performed by 2 independent coders using MAXQDA software.

Results: A majority of the 268 platelet transfusions were deemed inappropriate (60.82%) according to the AABB guideline trigger of 100000 total platelets/microliter. By contrast, only 11.24% of the 267 fibrinogen transfusions were inappropriate according to a 2g/dL threshold. Complex surgery and total CPB time displayed a statistically significant relationship with platelet transfusions via logistic regression ($p>0.05$). Both fibrinogens and platelets saw increased rates of inappropriate transfusion at moderate-to-severe bleeding levels. Qualitative transfusion decision-making influences included communication roadblocks, institutional culture, limited transfusion training, poor guideline dissemination and patient characteristics (incl. transfusion preferences).

Conclusions: The high prevalence of inappropriate platelet transfusions reinforces concerns over pervasive guideline non-adherence – the determinants of which are made clearer in considering often disregarded qualitative factors. Under this framework, the reductive assumption that inappropriate transfusions are driven by physician negligence is challenged. Instead, the integration of quantitative and qualitative predictors lays the groundwork for more holistic targeted interventions.

N: Population Health & Education

N.1: Designing a healthbot to help people take their varenicline with the use of patient perspectives

Student: Sowsan Hafuth, Supervisor: Nadia Minian

Background: More than 8 million people die yearly due to tobacco use. Despite major efforts worldwide to aid in the cessation of tobacco use, smoking remains the main cause of preventable death and disease. Varenicline, an α4β2 nicotinic receptor agonist, is the most successful approved smoking cessation medication available. Providing individualized behavioral support helps improve adherence to smoking cessation medication. Being able to provide tailored care and connect with patients through web-based programs has become more popular due to the wide transition to various online platforms over the last decade. Additionally, incorporating patient perspectives in the development of treatments makes patients more excited about the implementation and dissemination of research. Highlighting diverse perspectives can also make the treatment more acceptable in different communities.

Objectives: To design a patient-centered, theory informed, evidence-based healthbot that helps people adhere to their varenicline prescriptions.

Methods: Using the COM-B model and Behavior Change Wheel as a guide, we conducted 20 interviews with people who were either recent or current varenicline users trying to quit smoking. We asked participants about their varenicline adherence habits and what they wanted in terms of support and advice given through a healthbot. We then conducted Wizard of Oz testing where 40 participants interacted with the preliminary version of the healthbot to understand what kind of questions were being asked to the healthbot, so we could generate a bank of answers.

Results: Both the interviews and Wizard of Oz testing had almost equal numbers of men and women, and most participants were above the age of 41. The most common reported barrier to adhering to varenicline was people's lack of preparedness regarding side effects (95%, n=19). The most common reported facilitator for adhering to varenicline was receiving social support from friends, family, or their healthcare provider (95%, n=19). The most common features participants wanted implemented into the healthbot were being able to ask general questions (95%, n=19), receiving positive feedback messages (80%, n=16), and being reminded to take their medication (80%, n=16). The most common questions asked to the healthbot revolved around side effects, the mechanism of action of varenicline, and setting a quit date.

Conclusion: We learned how patients want the healthbot to act as a form of accountability and support during their varenicline prescription. Patients also want updates on their progress and have an extra set of reminders to take their medication. We plan to use these data to build and improve our evidence-based and individualized healthbot that will aid in the cessation of smoking and encouragement of varenicline adherence.

N.2: A survey of knowledge, attitudes, and behaviors of Canadian family physicians in response to the Canadian guidelines on prescribing opioids for chronic non-cancer pain: preliminary results

Student: Lillian Saberian, Supervisor: Andrea Furlan

Background: We are currently conducting the 3rd Canadian survey of Family Physicians' (FPs) practices and knowledge in prescribing opioids for chronic non-cancer pain (CNCP).

Purpose & Hypothesis: We aim to assess changes from the previous survey conducted in 2018. We hypothesize that there are changes in FPs' knowledge, attitudes, and behaviors in prescribing opioids for CNCP related to the Canadian Guidelines.

Methods: We invite FPs from all Canadian provinces and territories to participate in this cross-sectional online survey in English and French on SurveyMonkey from July 2023 to March 2024. The survey contains five screening questions for eligibility, twelve demographic characteristics, fourteen knowledge about opioids and CNCP, thirty behaviors, and thirty-eight attitudes.

Results: By February 2024, there were 367 responses, with a 43% completion rate; most respondents were from Ontario (57%). The percentage of physicians not prescribing opioids for CNCP did not have a statistically significant difference from 2018 (17% vs. 11%, P-value: 0.058). Concerns about long-term adverse effects primarily influenced their decision (96% vs. 88%). The percentage of respondents who are "Very Confident" in prescribing opioids for CNCP did not have a statistically significant difference from 2018, which was 16% (P-Value = 0.2, diff = -0.04). Canadian FPs' adherence to guidelines remained unchanged compared with 2018; however, urine drug screening dropped by 16% before initiating and 18% during monitoring of opioid therapy in 2023 compared to 2018.

Conclusions: The decision of FPs to refrain from prescribing opioids for CNCP and their reduced confidence in such prescriptions may be due to the ongoing opioid crisis. Strategies like targeted education may enhance guideline adherence, emphasizing the need for ongoing assessment to optimize patient care.

N.3: Feasibility and effectiveness of extended-release buprenorphine (XR-BUP) among correctional populations: a systematic review

Student: Cayley Russell, Supervisors: Jürgen Rehm, Shannon Lange

Background: Medications for opioid use disorder (MOUD) reduce risks for overdose among correctional populations. Among other barriers, daily MOUD dosing requirements hinder treatment continuity post-release. Extended-release buprenorphine (XR-BUP) may therefore be beneficial. Due to its novelty, limited evidence exists on XR-BUP's feasibility and effectiveness among correctional populations.

Purpose: The current systematic review aimed to assess the feasibility and effectiveness of XR-BUP among correctional populations.

Hypothesis: Due to its extended-release formulation that reduces the reliance on daily dosing, XR-BUP will be a feasible and effective alternative MOUD formulation for correctional populations as it can support treatment continuity and engagement during the high-risk transition period between incarceration and community release.

Methods: Searches were carried out in Pubmed, Embase, and PsychINFO in October 2023, using relevant search terms. Studies were included if they reported on feasibility or effectiveness of XR-BUP. Ten studies were included, representing n=819 total individuals (81.6% male). Data were extracted and results were narratively reported under the following main outcomes: 1) Feasibility; 2) Effectiveness; and 3) Barriers and Facilitators.

Results: Studies were heterogeneous. Available data indicate that providing XR-BUP to correctional populations is viable, safe, and cost-effective, and incarcerated individuals have an interest in this formulation. Results also highlighted XR-BUP's effectiveness in reducing drug use, overdoses, and healthcare utilization, as well as in increasing treatment retention and improving community reintegration. Barriers and facilitators to receiving XR-BUP were also identified.

Conclusions: XR-BUP appears to be a feasible and potentially effective alternative treatment option for correctional populations with OUD. XR-BUP may reduce many community release-related risks, as well as barriers to treatment retention. Efforts to expand access to and uptake of XR-BUP among correctional populations are warranted.

N.4: Social cognition and dysregulation jointly contribute to social behavior across neurodiverse young people

Student: Iciar Iturmendi-Sabater, Supervisors: Meng-Chuan Lai, Hsiang-Yuan Lin

Background: Differences in social behavior when interacting with others or responding to social cues are common (i.e., transdiagnostic) across young people with neurodevelopmental conditions (NDCs). Recent findings challenge the hypothesis that difficulties understanding others' mental states (i.e., social cognition) explain social behavior differences across this population, also showing that social cognition differences between NDC and typically developing (TD) groups are not consistently pronounced.

Purpose: Here, we elaborate the social cognition theory by examining how affective, cognitive, or behavioural regulation challenges to adapt to the environment (i.e., dysregulation), together with social cognition, transdiagnostically explain social behaviour across neurotypical and neurodivergent children and adolescents.

Hypothesis: We hypothesized that self-dysregulation would, over and above social cognition, influence social adaptive behaviors; high self-dysregulation would dampen one's capacity to leverage social cognitive abilities to achieve social adaptive behaviors.

Methods: We conducted cross-sectional, secondary data analyses across 646 6-to-18-year-old TD participants and participants with NDCs (i.e., autism, attention-deficit/hyperactivity disorder, obsessive-compulsive disorder, or other NDCss) from the Province of Ontario Neurodevelopmental Network. Caregivers reported social behaviors (Adaptive Behavior Assessment System Social domain) and dysregulation (Child Behavior Checklist Dysregulation Profile). Participants completed the Neuropsychological Assessment Affect-Recognition and Theory-of-Mind subscales, the Reading the Mind in the Eyes, and Sandbox False-Belief social cognition tasks. We split the sample into training (n=324) and test (n=322) sets. After normative modelling of all metrics, we used principal component and hierarchical regressions to investigate how social cognition and dysregulation explained social behavior in the training-set. We tested social cognition-dysregulation interactions, and whether dysregulation mediated the social cognition-social behavior association. Model fits were assessed in the test-set.

Results: Two social cognition principal components adequately explained social behavior variance (13.88%) across the training-set. Beyond better social cognition, lower dysregulation explained better social behavior ($p<2e-16$). The social cognition-dysregulation interaction was non-significant ($p=0.95$). Dysregulation partially mediated social cognition-social behaviour associations in the training ($p<2e-16$) and test ($p<2e-16$) sets.

Conclusions: Replicable findings suggest that dysregulation, beyond social cognition, substantially explains social behaviour across neurotypical and neurodivergent children and adolescents.

N.5: Effective digital solutions for problem drinking in treatment-seeking smokers to reduce the risk of cancer

Student: Anuijan Chandran, Supervisors: Nadia Minian, Peter Selby

Background: Clinical guidelines recommend addressing alcohol and tobacco at the same time, but few primary care providers in Ontario offer brief alcohol interventions routinely, and tobacco and alcohol are often treated separately. Digital interventions could help overcome some barriers. While several interventions have aimed to address dual use, there remains a gap in identifying behaviour change techniques (BCTs) designed to change or modify causal processes controlling behaviour.

Purpose: Identify effective BCTs that reduce dual use of alcohol and tobacco.

Methods: Following Cochrane recommendations, a rapid review to identify effective BCTs for reducing dual tobacco and alcohol use was conducted. Eligibility was determined using the following PICOD criteria: *Population:* adults who consume both tobacco and alcohol. *Intervention:* Patient-level behavioural interventions for dual use of alcohol and tobacco. *Comparator:* any control group. *Outcome:* objective and/or self-report measures of tobacco and alcohol use. *Design:* quantitative designs. We searched academic databases for relevant empirical studies and used the Behavioural Change Taxonomy V1 tool to identify effective BCTs.

Results: 2040 articles were initially screened, the full texts of 91 articles were screened, and 38 articles were included in this review. Goal setting, action planning, and pharmacological support were the most common BCTs identified. 68.4% of studies were of moderate bias.

Conclusion: Further analyses of the BCTs outcomes will be completed to identify the most effective components to use in our practice. Overall, these results aim to optimize, reproduce, and spread effective digital solutions for problem drinking in treatment-seeking smokers.

N.6: Bridging the gap in pediatric pharmacotherapy: a web-based application for enhancing drug safety and prescribing practices

Student: Suhani Patel, Supervisor: Christopher Parshuram

Background: Informed pharmacotherapy is cornerstone of safe and effective use of medications, particularly in children where off-label prescriptions are common, and the risk of drug-related harms is more. Pediatricians frequently consult published medical literature as a key source of information. However, translating this drug safety knowledge from research findings to clinical decision-making demands meticulous review and adaptation. Given the complexity and the barriers inherent in this process, there is an urgent need for innovative solutions to ensure accurate and practical application in pediatric settings.

Objective: This pilot project aims to bridge the knowledge gap in pediatric pharmacotherapy by facilitating transfer of drug safety information from a broad corpus of medical literature to the clinical decision-making arena via a web-based application.

Methods: Systematic literature reviews were conducted to compile a database with drugs- Bumetanide, Hydrochlorothiazide, and Pantoprazole. This included studies of various designs, unrestricted by drug dose, indication, or exposure duration. Data on adverse event occurrences and types, classified according to MedDRA categories, were analyzed and augmented by subgroup analyses considering age, gender, dosage, and other factors.

Results: We developed a web application designed to provide intuitive access and visualization of data from our database. This platform enables users to navigate and filter information seamlessly, conduct precise subgroup analyses, and interpret results via interactive graphs and tables. By doing so, it offers insights that support informed prescribing decisions.

Conclusion: The deployment of this tailored web application is a significant advancement in pediatric pharmacotherapy, promoting rigorous, data-driven clinical decision-making. Prospective enhancements to this platform are expected to further solidify pediatric patients' medication safety and optimize therapeutic outcomes.

N.7: Impact of primary care physicians on early cancer symptom investigation and specialty referral in Saudi Arabia compared to the International Cancer Benchmarking Partnership

Student: Daniya Abdouh, Supervisors: Eva Grunfeld, Paul Krueger, Rahim Moineddin

Background: Cancer survival rates vary significantly between countries with similar health systems, and the factors contributing to these differences are complex and not well understood. The International Cancer Benchmarking Partnership (ICBP) was established to examine international variations in cancer outcomes and identify potential causes. Module 3 of the ICBP hypothesized an association between the readiness of Primary Care Physicians (PCPs) to investigate cancer symptoms and cancer survival rates, identifying potential differences in primary care systems, structure, or clinical practice that could contribute to variations in cancer outcomes. Correlations were found between the readiness of PCPs to investigate cancer symptoms and survival rates, offering a possible explanation for the variation in cancer survival observed among ICBP countries. Quantitative studies from Saudi Arabia have reported a higher number of cancer cases presenting at cancer centers with advanced stages compared to Western countries. However, the reasons behind this phenomenon have not been sufficiently explored.

Purpose & Hypothesis: This study aims to determine the awareness of Saudi Arabian Primary Care Physicians (PCPs) regarding early investigation and referral to specialty services for early cancer diagnosis. Our hypothesis is that there is an association exists between the readiness of PCPs to investigate cancer in its early stages and the survival of that cancer (lung, colorectal and ovarian).

Methods: A cross-sectional study that will include family medicine consultants working in National Guard Hospitals in Jeddah and Riyadh. Data will be collected via an online survey administered using Qualtrics. Descriptive statistics will be used to analyze demographic factors and primary care structure. The cumulative proportion of PCPs opting to investigate or refer at each phase for each vignette will be calculated using relevant cancer's percentage 1-year survival rates using SPSS.

Results: With a sample size of 21 participants, 45.5% of physicians strongly agree that if they have a patient with high suspicion of cancer it is easy for them to arrange faster access to investigations. The average waiting time between a referral for suspected cancer being made and their first appointment in secondary/specialist care is from 2 to 7 days for 54.6% of the patients. Two-sided p-value was 0.13 which shows a statistically significant correlation between the more agreement from the physician to investigate early results in a shorter time (days) between the referral and the patients first appointment.

Conclusions: There is a correlation between the physician's attitude to arrange faster access to investigations and therefore have a shorter waiting time between the referral and the patient's first appointment to see a specialist and starting the diagnosis prosses and having a better survival.

O: Population Health & Education

O.1: Development and evaluation of a standardized tool to evaluate the quality of interaction between AI and clinicians: development of iCARE framework

Student: Argyrios Perivolaris, Supervisor: James J. Jung

Background: Artificial intelligence (AI) has the potential to improve healthcare quality when thoughtfully integrated into clinical practice. However, many clinicians remain hesitant towards its adoption. Current evaluations of AI solutions tend to focus solely on model performance.

Purpose: There is a critical knowledge gap in the assessment of AI-clinician interactions. Our project aims to develop a standardized framework to measure the quality of clinician's interaction with AI-enabled systems in healthcare.

Methods: We performed a systematic review of published studies to June 2022 that reported elements of interactions that impacted the relationship between clinicians and AI-enabled clinical decision support systems. We conducted semi-structured interviews to supplement the results obtained from the systematic review. All review and interview results were utilized to develop a draft of the novel framework. This draft would undergo a consensus meeting with a panel of experts, pilot testing with recruited clinicians, and iterative refinements.

Results: We identified 90 unique interaction traits from the review and interviews with the most common interaction traits including usefulness, ease of use, trust, satisfaction, willingness to use, and usability. These traits were subjectively categorized into seven categories, each with various subcategories. After the consensus meeting and pilot testing, the framework was finalized into eight categories: usability, workflow impact, system performance and technical integration, trust and acceptance, impact on patient outcomes and care delivery, clinician engagement, communication and collaboration, and ethical and professional concerns.

Conclusion: We identified eight categories of interaction traits between clinicians and AI systems. To our knowledge, this proposed framework serves as the first of its kind to assess the quality of AI-clinician interactions within clinical practice settings.

O.2: Utilization of eye-care providers by Ontario residents in 2019: a population-based study

Student: Kiko Zi Yi Huang, Supervisor: Ya-Ping Jin

Background: Eye-care services are provided by ophthalmologists, optometrists, primary care providers (PCPs, including family physicians, pediatricians, and nurse practitioners), and emergency physicians. Ophthalmologists are medically trained physicians and surgeons specializing in diseases of the eye. Optometrists are non-physician eye-care providers who oversee general eye health by providing primary eye-care services, including prescriptions for eyeglasses and medications. It is unknown how frequently each type of eye-care provider is used. Such information is needed for improved planning of the healthcare workforce supply.

Purpose: To understand the utilization patterns of eye-care providers in Ontario in 2019.

Hypothesis: The utilization of eye-care providers varies by urban/rural residency, age, and emergency and non-emergency visit and that rural residents may not receive the same level of eye-care compared to urban residents, as the clinics of ophthalmologists (who received the highest level of eye-care training) are concentrated in large cities.

Methods: Using the Ontario Health Insurance Plan (OHIP) billing data in 2019 and eye-related diagnostic codes, we calculated the number of eye-visits (including revisits) and distinct eye patients (excluding revisits) per 100 population in 2019. Results were stratified by urban/rural residency, age, physician-specialty, and visit-type (emergency/non-emergency, determined by whether the visit occurred in an emergency room).

Results: In 2019, the number of eye-visits per 100 population was 76.9 for non-emergency cases and 0.9 for emergency cases. On average, each patient had 3.1 visits for non-emergency cases and 1.2 visits for emergency cases. Excluding revisits, the number of distinct eye patients per 100 population was 25.1 for non-emergency and 0.8 for emergency cases. Among non-emergency eye visits, 57% of the care was provided by ophthalmologists (6.6 million visits or 14,133 visits per ophthalmologist), 35% by optometrists (4.0 million visits or 1,525 visits per optometrist) and 8% by PCPs. Among emergency eye visits, 74.7% of the care was provided by PCPs with training in emergency medicine, 16.6% by emergency-physicians, and 7.7% by ophthalmologists. In non-emergency cases, rural patients (55.3%) received slightly fewer ophthalmologist services than urban patients (57.4%). For emergency cases, ophthalmologist care received by rural patients (4.7%) was nearly half of urban patients (8.3%). For children aged 0-4 and 5-19, optometrists were the primary providers for non-emergency visits (48% and 80%, respectively). In adults, ophthalmologists were the primary provider (45%-74% for age groups 20-39, 40-64, 65-79 and 80+).

Conclusions: In 2019, about 1 in 4 individuals received non-emergency eye-care in Ontario's public healthcare system, while 1 in 100 obtained emergency eye-care. Over half of non-emergency care was provided by ophthalmologists. Emergency eye-care was predominantly provided by PCPs and emergency-physicians, particularly in rural areas. Further research is needed to find solutions for the observed eye-care inequity between rural and urban residents.

O.3: Assessing the characteristics, housing needs, and preferences for forensic patients designated ALC

Student: Vanessa Ip, Supervisors: Vicky Stergiopouls, Sandy Simpson

For individuals found Not Criminally Responsible on account of a Mental Disorder (NCRMD), appropriate housing is necessary for reducing recidivism and facilitating independent living. Forensic patients are at greater risk of housing vulnerability, homelessness, and substance-use. The literature on supportive housing for forensic populations is scant and appropriate housing in short supply.

This thesis describes a multi-methods study including a clinical chart review of forensic patients awaiting for housing (ALC) at CAMH in the last 5 years (2018-2023), qualitative interviews with X forensic patients awaiting for housing; and focus groups with CAMH clinicians. The study further aims to identify the characteristics of forensic patients designated ALC, their housing needs and preferences, and healthcare provider experiences in accessing and providing housing supports for this population. The results will inform the development of housing solutions for forensic patients.

O.4: Identifying strategies that can be implemented to promote use of question prompt lists: a multiple methods study in the context of preventing premature cardiovascular disease after hypertensive pregnancy

Student: Sara Sino, Supervisor: Anna Gagliardi

Background: 37,000+ Canadian women annually develop hypertensive disorders of pregnancy (HDP), otherwise known as high blood pressure during pregnancy. This includes several conditions, affecting ~10% of all pregnancies. This can lead to a 2-5x higher risk of premature cardiovascular disease (CVD) compared to normotensive pregnancies. In Canada, despite HDP guidelines, 50% of family physicians, cardiologists, and obstetricians surveyed in 2007 and 2018 were unaware of HDP-related CVD risk and prevention.

Introduction: Women and clinicians said that a question prompt list (QPL) about HDP could improve awareness of premature cardiovascular disease (CVD) risk. A QPL is a pre-formed list of questions aimed at improving patient-clinician communication. In this study, we will identify strategies that can support the awareness and use of a QPL for HDP by affected women and clinicians.

Methods: This study will employ a multiple methods research design: *(1)* A scoping review exploring strategies that can improve healthcare help-seeking, *(2)* Secondary analysis of qualitative interview data from diverse women and clinicians to identify additional ways to increase use of a QPL for HDP, and *(3)* Qualitative interviews with various healthcare professionals about their role in brokering use of a QPL for HDP by women. We will use various theoretical models and frameworks to guide data collection, analysis, and interpretation.

Conclusions: Wide dissemination and uptake of the QPL for women with HDP and clinicians across Canada will improve patient-centred communication, thereby improving the heart health and quality of life among thousands of Canadian women with HDP annually.

O.5: A profile of pregnant women with alcohol-related diagnoses who utilized health care and their children's outcomes

Student: Danijela Dozet, Supervisor: Svetlana Popova

Background: Alcohol is a teratogen and, if consumed during pregnancy, can have a direct toxic effect on the developing fetus and, in turn, can cause Fetal Alcohol Spectrum Disorder (FASD) and other detrimental health outcomes in the child. Despite public health efforts, about 10% of pregnancies in Canada are alcohol-exposed.

Purpose: This population-based descriptive cohort study was the first to use administrative health care data from Ontario, Canada (2003-2017) to examine women with alcohol-related diagnoses during pregnancy and their children's health outcomes as well as their health care utilization. The study examined maternal and neonatal characteristics at birth, and children's healthcare utilization, mental health diagnoses, and diagnoses of P04.3 (Newborn affected by maternal use of alcohol) and Q86.0 (Fetal Alcohol Syndrome) during follow-up.

Hypotheses: Women with alcohol-related diagnoses during pregnancy will display high rate of adverse maternal, mental health and substance use problems. Children diagnosed with P04.3 and/or Q86.0 will display higher rates of adverse neonatal/ child outcomes, as well as healthcare utilization, compared to the remaining children with prenatal alcohol exposure (PAE).

Methods: Linked maternal-child records housed at ICES and the Office of the Registrar General-Death Vital Statistics were utilized. Pregnant women with alcohol-related ICD-10 and Ontario Health Insurance diagnoses recorded during hospitalizations (including for delivery) or emergency department visits were included in the cohort (2003 – 2017). Children from the resulting pregnancies were allocated to the following groups based on their alcohol-related diagnoses recorded at any point during follow-up: 1) Q86.0 and P04.3; 2) P04.3 only; and 3) children with PAE, and were compared on health outcomes, health care service utilization as well as characteristics of their mothers at birth.

Results: The study identified 1,804 mothers with alcohol-related diagnoses who delivered 1,957 children from 2003 to 2017. Mothers of children with P04.3 and/or Q86.0 had higher rates of spontaneous abortion ($p<0.001$), were more likely to receive care for known/suspected fetal abnormality/damage ($p<0.0001$) and suspected damage to fetus from alcohol ($p=0.0004$); were more likely to have schizophrenia ($p<0.0001$) and mood disorders ($p=0.0253$); and more likely to have higher rates of mental and behavioural disorders due to use of alcohol (ICD-10 code F10) ($p<0.0001$). Children with Q86.0 and/or P04.3 (n=31) and children with P04.3 only (n=31) had a significantly lower mean birthweight, higher rates of respiratory, cardiovascular, transitory endocrine and metabolic disorders, and congenital malformations and chromosomal abnormalities as compared to children with PAE (n=1,882). Also, children with P04.3 and/or Q86.0 were more likely to utilize urgent inpatient care, inpatient psychiatric care, same-day surgeries, ambulatory clinic visits, and home/community care services as compared to the remaining children with PAE. Across the entire cohort of children, 15.8% had developmental delays, 13.6% behavioural disorders and 1.2% died during the study period.

Conclusions: Alcohol poses a significant risk to maternal health and pregnancy outcomes, emphasizing the importance of universal screening for alcohol use during pregnancy and FASD prevention initiatives.

O.6: How shall we measure participation: patient preferences

Student: Benjamin Traubici, Supervisor: Brian Feldman

Background: "Participation" is defined as "involvement in life situations" by the World Health Organization, in their international Classification of Functioning, Disability and Health (ICF). An issue with the ICF is that the category of *participation* is not clearly distinguished from *activities*. A framework called the family of participation related constructs (fPRC) has been proposed to characterize participation. The fPRC defines participation with two constructs: attendance and involvement. Attendance is the frequency or range of activities in which an individual takes part. Involvement is the experience of participation while attending.

Purpose: This study will seek to determine patient preference of participation questionnaires and whether a patient-centred or patient-reported approach is preferred.

Hypothesis: Patients will prefer a patient-centred approach to participation questionnaires rather than the standard patient-reported approach.

Methods: A new, patient-centred participation questionnaire was designed and administered to participants, along with three existing participation questionnaires. The four questionnaires administered to participants were two PROMIS (Patient Reported Outcomes Measurement Information System) bank measures (Ability to Participate in Social Roles and Activities and Satisfaction with Social Roles and Activities), Participation Scale – Kids, and the Patient-Centred Questionnaire, which was designed in this study. After completion of the questionnaires in randomized order, participants scored each questionnaire based on how satisfied they were with how it allowed them to express their participation.

Results: 80 participants were recruited for this study from The Hospital for Sick Children rheumatology clinic, between ages of nine to 17. 55 of the participants identified as female, 26 identified as male, and one identified as non-binary.

O.7: Factors affecting the relationship between physical symptom burden and traumatic stress symptoms in patients with newly diagnosed acute leukemia: a multi-site observational study

Student: Angela Mathews, Supervisor: Gary Rodin

Acute leukemia (AL) is a life-threatening hematologic malignancy that poses a significant threat to both the physical and psychological well-being of patients. Traumatic stress symptoms, which encompass the immediate psychological and physiological response to a traumatic event, such as the diagnosis of a life-threatening cancer diagnosis, can exacerbate the distress experienced by patients. However, the influence of variables that may moderate the association between physical symptom burden and traumatic stress symptoms in AL remains understudied. This work aims to investigate this relationship and identify potential targets for therapeutic interventions. We recruited adult patients with newly diagnosed AL from four cancer care centers in Ontario. Self-report questionnaires assessed traumatic stress symptoms, physical symptom burden, attachment security, spiritual well-being, and patient satisfaction with care immediately following diagnosis. Quantitative data analysis was conducted using descriptive statistics and cross-sectional techniques, including multivariate regression, to determine the frequency of traumatic stress symptoms and the presence, severity, and distress of physical symptoms in patients with newly diagnosed AL, as well as to examine the extent through which the relationship between physical symptom burden and traumatic stress symptoms acts through potential moderating variables. Spiritual well-being was found to moderate the relationship between physical symptom burden and traumatic stress symptoms, and a potential moderating effect was observed for satisfaction with care on the relationship between physical symptom burden and traumatic stress symptoms. The findings generated from this study may be used to identify those at higher risk of traumatic stress symptoms and guide the development and improvement of tailored interventions to address the distinct support needs of this patient population.

P: Regenerative Medicine & Development

P.1: Evaluating the immunogenicity of mouse partial hindlimb allografts for vascularized composite allotransplantation

Student: Jiahui (Angela) Sun, Supervisor: Siba Haykal

Background: Vascularized composite allotransplantation (VCA) is the transplantation of multiple tissues such as skin, muscle, bone, nerve, and vessel, as a functional unit (i.e. hand or face) to patients suffering from major tissue trauma and functional deficits. Though the surgical feasibility has been optimized, issues regarding graft rejection remains. Currently, patients are required to follow potent and life-long immunosuppressing regimens to maximize graft tolerance, thereby putting them at risk for malignancies, opportunistic infections, and cancers.

Objective: In this study, we aim to identify the immune cells infiltrating the allograft and transplant bioengineered allografts to mitigate rejection and ultimately eliminate or decrease the need for immunosuppressants.

Hypothesis: Transplanting bioengineered grafts will decrease graft rejection compared to non-bioengineered grafts.

Methods: A mouse partial hindlimb allotransplant model will be used to assess the immunogenicity of the whole limb, skin, muscle, and bone. Flow cytometry and histological analysis will be used to identify the immune cell population in the allograft, allowing for the deduction of rejection mechanisms occurring in each tissue type. Tissues will then be bioengineered to achieve cell-enriched (e.g. T regulatory cells) grafts prior to transplantation to induce tolerance. Currently we are in the process of defining the immune profile of syngeneic and allogeneic grafts through flow cytometry analysis.

P.2: The association of clonal hematopoiesis and adverse outcomes in solid organ transplant recipients

Student: Tafsia Hussain, Supervisor: Filio Billia

Background: Clonal hematopoiesis (CH), a clonal expansion of hematopoietic stem and progenitor cells with specific somatic mutations, could be the potential basis for a non-invasive prognostic tool for solid organ transplant (SOT) recipients. Today, the long-term survival rate of SOT recipients is limited by the development of multiple complications. To mitigate these complications and increase overall survival, patients are subjected to intense and invasive surveillance. This includes undergoing periodic biopsies to diagnose rejection, viral infections, and malignancies. The procedure itself can cause complications such as infections or bleeding. Most importantly, it significantly increases the care burden of patients.

Purpose: The purpose of this study is to evaluate the prevalence of CH mutations in SOT recipients and its association with survival and adverse outcomes post-transplant including rejection, graft failure, infection, or malignancies.

Hypothesis: CH mutations are associated with increased adverse outcomes and decreased survival in SOT recipients.

Methods: This is a retrospective study, where 1394 blood samples were collected of heart, kidney, liver, or lung transplant recipients at Toronto General Hospital from 2016 through 2022. Genetic sequencing was used to identify patients with CH-associated mutations. In addition, clinical data was collected from the patient's electronic medical record.

Results: Preliminary analysis has been conducted in 101 lung transplant recipients (mean age 54, SD=15, 41% female). In this cohort, CH-associated variants at a variant allele frequency (VAF) of more than 2% was detected in 19 individuals (19%). The most common mutated genes in the cohort were DNMT3A (8/19), ASXL1 (7/19), TET2 (5/19) and PPM1D (5/19). There was no statistically significant difference between lung transplant recipients with or without CH mutations in mortality, cellular acute rejection, chronic lung allograft dysfunction (CLAD), malignancy, and CMV infection ($p>0.05$). The clinical data continues to be collected for the heart, kidney, and liver transplant cohorts.

Conclusion: This study addresses a significant research gap in the association of CH and post-transplant outcomes in a large cohort study and may serve as a potential biomarker for a non-invasive tool to predict the risk of adverse outcomes in transplant recipients.

P.3: Nanotechnology for inner ear drug delivery and middle ear cholesteatoma therapy: overcoming challenges and assessing efficacy

Student: Dina Mahmoud, Supervisor: Trung Le

Background: Inner and middle ear conditions are in a need for an efficient and versatile therapy. The inner ear's deep anatomical location and the blood labyrinth barrier present significant hurdles, compounded by potential deafness upon labyrinth opening for drug instillation. Moreover, cholesteatomas, benign tissue in the middle ear, pose a unique challenge due to their destructive potential within the temporal bone, leading to complications such as hearing loss and facial paralysis. Surgeries remain the only treatment for the previous challenges. In addressing these challenges, our research explored the potential of porphyrin-based nanoparticles known as porphysomes (PS) for inner ear drug delivery and middle ear cholesteatoma eradication. These 100nm spherical nanovesicles, derived from hemoglobin, possess a lipid-rich core ideal for loading and delivery of various drugs. Upon laser irradiation, PS absorb light, emitting fluorescence for analysis, or transform it into heat, functioning as photothermal agents.

Hypothesis: Based on PS's unique characteristics, PS are safe, can be penetrate to the inner ear after local application and are effective for middle ear cholesteatoma treatment.

Purpose: Prove the safety and efficiency of PS as a drug delivery system (DDS) for the inner ear. In addition to investigate PS efficacy as a photothermal agent for cholesteatoma eradication.

Methods: *1. Assessment of safety and uptake of PS ex vivo:* Cochlear explants (n=8) were treated with different concentrations of porphysomes (up to 50 µM). Cytotoxicity was assessed using Alamar blue reagent, hair cell counting, and immunofluorescence. Explants were also incubated with porphysomes for up to 4 hours to observe uptake via confocal laser microscopy (CLSM). *2. Assessment of biocompatibility of PS in vivo:* Baseline hearing and vestibular assessments were conducted. Rats then underwent intratympanic injections (IT) of PS into one ear, with the contralateral ear serving as a control. Evaluations of hearing and vestibular function occurred at 2- and 6-week intervals to analyze short- and long-term effects on auditory and balance capabilities. Rats were then euthanized, and cochleae were extracted for immunohistochemistry analysis. *3. Assessment of PS penetration to the inner ear in vivo:* Rats received (IT) of PS into one ear, with the other ear serving as control. Following this, rats were positioned dorsally for one hour before euthanasia. Perilymph samples and cochlear tissues were collected for fluorescence detection at the PS peak emission wavelength (675 nm) using a fluorometer. Immunohistochemistry was performed on cochlear specimens for further examination. *4. Efficacy of photothermal therapy on human cholesteatoma tissue:* specimens (n=6) were treated with porphysomes for 30 minutes before being exposed to a 660 nm laser at a fluence rate of 2.5 W/cm2. A thermal camera monitored the temperature elevation. Post-irradiation, tissues were subjected to microscopic examination and histological evaluation.

Results: Our studies suggest the safety of porphysomes up to a 50 µM concentration. Hair cell counts, immunofluorescence, and auditory and vestibular evaluations suggest the biocompatibility of porphysomes within the inner ear, demonstrating no significant adverse effects compared to control groups, ($p>0.05$). Distinct PS fluorescence signal within cochlear explants is observed after 1-hour incubation. In vivo penetration studies in rats indicate a concentration-dependent infiltration into the organ of Corti and perilymph following IT injection. Cholesteatoma tissues subjected to PS exhibited a substantial temperature increase (ΔT of 31±3.76°C, $p<0.05$) upon laser activation, accompanied by visible burn marks when compared to control.

Conclusion: PS are promising DDS for inner ear and non-surgical cholesteatoma treatment. Such multifunctional nanoparticles will allow testing and validating future drug therapies and imaging options that can be useful for a wide range of clinical applications.

P.4: Understanding the mechanisms of human liver regeneration via characterization of circulating small extracellular vesicles

Student: Yilin Sun, Supervisor: Mamatha Bhat

Background: Liver disease affects one out of every ten Canadians potentially leading to end stage scarring resulting in death. Remarkably, the liver is the only major organ in the body that is able to heal itself when insults are removed. A deeper understanding of the liver's unique regenerative capabilities might be pivotal in helping patients avoid hospitalization, liver transplantation, and death.

Purpose & Hypothesis: Our research aims to characterize possible specific markers in circulating small extracellular vesicle (sEVs) associated with normal or impaired liver regeneration. We hypothesize that sEVs in regenerating livers will carry distinct molecules that uniquely modulate regeneration.

Methods: Plasma samples (n=24) were retrospectively obtained from twelve living donor liver transplant (LDLT) recipients undergoing normal regeneration at 1- and 3-months post-transplant. These samples were retrospectively collected from the UHN Multi Organ Transplant Biobank for sEV isolation. Extracted microRNA (miRNAs) from the sEVs were analyzed using Nanostring's nCounter® Gene Expression System. Additionally, significant changes in miRNA expression levels and their potential effects on the expression of their related target genes involved in liver regeneration were investigated by profiling sEV associated miRNAs in a murine model of liver regeneration (n=13). MiRNA associated target genes were compared with the matched transcriptomic data from the same mice (n=6) undergoing liver regeneration.

Results: Analysis of data from both human and murine subjects indicates that miRNAs are dynamically changing over time in sEVs during various stages of liver regeneration. In particular, many MiRNAs target genes are within the Hippo and Cell Cycle pathways. These pathways are integral to the regenerative process. Further validation using in vivo mouse transcriptomic data confirms the intricate regulation of these pathways, particularly highlighting their roles in the termination phase of liver regeneration, where they contribute to the negative regulation of regenerative mechanisms.

Conclusions: Our findings reveal that miRNA abundance in sEVs in both human and mice regenerating livers, is a dynamic process that changes over time. These miRNAs can play a fundamental role in finely regulating regenerative processes. Specifically, these miRNAs are implicated in the modulation of the cell cycle and the Hippo pathway, which are crucial for liver regeneration. This highlights the potential of targeting these miRNAs in therapeutic strategies to enhance liver regeneration.

P.5: Prolonged kidney storage at sub-zero temperatures is safe for porcine auto-transplantation: a world-first in-vivo study

Student: Francisco Calderon Novoa, Supervisor: Markus Selzner

Background: Static cold storage (SCS) at 4°C remains the method of choice for kidney grafts preservation prior to transplantation. However, rapid decline of graft quality limits prolonged SCS to ~18 hours. Sub-zero storage could potentially prolong graft viability by halting cellular metabolism and decay, offering new opportunities for exchange over larger distances or scheduling procedures. Nonetheless, sub-zero storage has been proven hazardous in organ transplantation, since tissue freezing and thawing leads to intracellular ice crystal formation, irreversible membrane damage and cellular death. Different studies have delved into methods of avoiding graft freezing while lowering the temperature, however, there are no studies conducted in large animal models.

Purpose & Hypothesis: The purpose of this study is to determine if changes in storage temperatures for preservers organs can impact the outcomes of transplantation. The working hypothesis is that decreasing the storage temperature to sub-zero values (without tissue freezing) will result in lower metabolism and in term in better preservation of kidney grafts over prolonged periods (24-48 hours). The aim of this study was to determine the feasibility of sub-zero storage without freezing followed by auto-transplantation of porcine kidneys.

Methods: Kidneys were retrieved from 30kg Yorkshire pigs, followed by 5-hour SCS at either 4°C with Histidine-Tryptophane-Ketoglutarate (HTK) (n=5), or at –2 °C (n=5) using a novel platform from Cryostasis© that includes media consisting of Good's buffer, polyols, saccharides, amino acids, and antioxidants. After storage, grafts were transplanted into the right iliac fossa, prior removal of the contralateral kidney. The pigs were then followed-up for 7 days, assessing renal function and ischemia-reperfusion variables such as creatinine, urea, potassium, lactate and AST serum levels, as well as urinary output. Following this set of experiments, two additional sets consisting of 24 (n=6) & 48-hour (n=4) SCS at 4°C with HTK or at –0.5°C with Cryostasis© solution were performed.

Results: All subjects survived the procedure and reached the expected endpoint. All kidneys produced urine shortly upon reperfusion. 5-hour 4 °C and –2 °C groups showed function from day one post op, with peak creatinine levels at 3.05 mg/dL SD ±0.96 mg/dL vs. 3.4 mg/dL SD ± 0.84 mg/dL, respectively. There were no significant differences between groups in serum values, urine output and biochemical assays, and ischemia injury score assessed in PAS-stained biopsies. 24h and 48h experiments showed delayed graft function with recovery after day 4, with creatinine levels peaking at 7.26 SD ± 3.38 mg/dL for the –0.5 °C Cryostasis® group and 8.43 SD ± 1.5 mg/dL for the 4 °C group at 24 hours, and 12 mg/dl SD ± 0.42 vs. 13.7 mg/dl SD ± 1.27 mg/dl for the 48 hour experiments, respectively.

Conclusions: Sub-zero storage is safe and feasible for both short and prolonged static storage of porcine kidneys, leading to preserved function, even after extended times such as 48 hours. This is a proof-of-concept study which shows that preservation at below-zero temperatures is possible. Efforts are underway to determine if additional benefits can be obtained by further lowering the storage temperature.

Q: Regenerative Medicine & Development

Q.1: Unveiling the science of skin disease: exploring VEGF-A and RhoA signaling in keratinocyte cells

Student: Vida Maksimoska, Supervisor: Katalin Szaszi

Background: Keratinocytes, key to skin integrity, regulate wound healing and inflammation. However, dysregulated keratinocyte responses, characterized by excessive cytokine production, can contribute to conditions like atopic dermatitis and abnormal wound healing. To understand how keratinocytes react to inflammation, we used a cultured keratinocyte cell line (HaCat). We exposed them to the potent inflammatory cytokine, Tumor Necrosis Factor (TNF)α and performed an unbiased screen to identify factors released. We found that one of the main proteins produced by stimulated keratinocytes is Vascular Endothelial Growth Factor-A (VEGF-A). While VEGF-A aids tissue regeneration, excess levels disrupt the skin barrier. VEGF-A is known to alter keratinocyte migration, though the underlying molecular mechanisms remain unknown. To obtain new mechanistic understanding of how VEGF-A affects keratinocytes, we studied HaCat migration in a gap closing assay using time-lapse live imaging. Interestingly, we found that VEGF-A released by the keratinocytes is crucial for promoting their migration. This effect required VEGF-Receptor 2 (KDR) and the small GTPase protein RhoA. Rho-family small GTPases are essential regulators of the cytoskeleton controlled by a large family of Guanine Nucleotide Exchange Factor (GEF) proteins. These proteins are excellent candidates for therapeutic targets since they provide stimulus-specific control of downstream effects. While VEGF-A is known to activate RhoA, the specific GEFs mediating this effect are largely unidentified. To close this knowledge gap, we performed a screen using an affinity precipitation assay that isolates activated GEFs from HaCat cells, followed by Mass spectrometry analysis. We identified seven candidate VEGF-stimulated Rho-GEFs, none of which had been studied in detail in keratinocytes. Thus, the specific goal of this proposal is to uncover the role of identified candidate GEFs in keratinocyte migration, differentiation, and immune function.

Hypothesis: VEGF-A activates RhoA through specific GEFs in keratinocytes, which augment cell migration and cytokine release, and modulate cell differentiation to promote normal keratinocyte function. Dysregulation of specific GEFs may contribute to keratinocyte dysfunction.

Methods: To determine the role of specific RhoA GEFs in keratinocyte function we will utilize new inhibitors and genetic knockdown techniques to study GEF activation and impact on signaling pathways, migration, cytokine production, and differentiation. We will also use various biochemical and cell biology approach to explore how VEGF-A activates these GEFs, and to study their role in inducing specific signaling pathways, and in migration, cytokine production and differentiation. To verify the role of GEF-H1 and Larg in complex skin models,we will use the MatTek EpiDermFT tissue model and expose it to various cytokines that are upregulated in inflammatory skin diseases. We will use pharmacological inhibitors of RhoA, GEF-H1 or Larg to assess changes in the development of the skin disease outcomes. This will help validate the role of GEF-H1 and Larg in skin models, and as druggable candidates. To confirm GEF-H1 and Larg as potential targets for skin disease treatment, we will utilize mouse models of AD and chronic wounds to assess the activation states for RhoA, GEF-H1 and Larg proteins. In these models we seek to determine the activation state of GEF-H1 and Larg; and to use their inhibitors to establish potential beneficial effects on the skin barrier.

Results: Our investigation into the roles of GEF-H1 and Larg in keratinocyte function revealed their activation by VEGF-A and their significant involvement in signaling pathways crucial for migration, cytokine production, and differentiation. Utilizing inhibitors and genetic knockdown techniques, we demonstrated their impact on keratinocyte behavior, including changes in RhoA activation and expression. These findings collectively underscore the promise of GEF-H1 and Larg as druggable targets for managing skin diseases. Our studies will obtain new knowledge of the molecular events that govern keratinocyte functions and skin re-epithelization

process. We anticipate providing valuable insights into key processes of skin healing and identify new druggable targets for skin disease. This information is key for advancements in treating skin disorders such as AD and psoriasis.

Q.2: Characterizing a novel transgenic mouse model expressing a post-translational modification enzyme implicated in Alport syndrome

Student: Samantha Ricardo, Supervisor: Moumita Barua

Background: Alport syndrome (AS) is a rare monogenic disorder which results from pathogenic variants in *COL4A3*, *COL4A4*, and *COL4A5*. The Barua lab has identified differential expression of a post-translational modification (PTM) enzyme in a *Col4a3* knockout (KO) model.

Purpose: We generated a transgenic mouse overexpressing the PTM enzyme. We hypothesize the upregulation of this PTM enzyme is involved with GBM stabilization in Alport syndrome.

Methods: Mice were generated by pronuclear microinjection. Copy number analysis was performed using digital droplet PCR (ddPCR). Gene and protein expression of the transgene from kidneys collected at 4 weeks was determined by Western blot and ddPCR. Kidney tissue was also analyzed by histology.

Results: Two founder transgenic lines, PTM-TG1 and PTM-TG2, were created. PTM-TG1 showed 12-25 copies, while PTM-TG2 had 2 copies. There was no evidence of PTM enzyme gene or protein overexpression in PTM-TG1. This was consistent across tissues including isolated glomeruli, kidney, spleen, and lung. PTM-TG2 mice exhibited increased gene and protein PTM enzyme expression in kidney tissues, though currently insufficiently powered for statistical significance. Female compared to male mice in PTM-TG1, PTM-TG2, and wildtype groups displayed higher levels of PTM enzyme gene and protein expression in the kidney.

Conclusion: PTM-TG2 displays evidence of transgene overexpression while PTM-TG1 does not, possibly indicating a compensatory mechanism that is silencing overexpression in PTM-TG1. Our results also suggest sex-specific differences in PTM enzyme expression in the kidneys. In the future, PTM-TG2 will be crossed with *Col4a3* knockout mice to assess effect on renal phenotype.

Q.3: Investigating the role of mitochondrial metabolism in human kidney development and maldevelopment

Student: Caoimhe Costigan, Supervisor: Norman D. Rosenblum

Background: Kidney maldevelopment is a major cause of kidney disease in children. Mechanisms leading to maldevelopment are not well understood. The mature kidney is reliant on mitochondrial energy production; during development differentiation of epithelial elements is dependent on a transition from glycolytic to oxidative energy production. Many children with inherited metabolic, particularly mitochondrial, conditions have kidney disease, some with malformation. Yet, little is known about the role of mitochondrial dysfunction in kidney development.

Purpose: To understand the role of mitochondrial metabolism in normal kidney development and investigate whether mitochondrial dysfunction contributes to abnormal kidney development.

Hypothesis: Mitochondrial metabolism is central to normal kidney development in humans.

Methods: We are generating kidney organoids (KO) from induced pluripotent stem cells (iPSCs) in which to study metabolism using gene expression analysis and assays for functional cellular respiration. We will expose KOs to pharmacological inhibitors of metabolism to determine the impact of bioenergetic pathway disruption on kidney development. Finally, we are generating KO from urine-iPSCs (UiPSCs) of patients with kidney malformation and to analyse for mitochondrial dysfunction.

Results: Transcriptomic analysis suggests that these KOs demonstrate an expected transition from glycolytic to oxidative metabolism as differentiation proceeds. We are optimising assays to measure the functional contribution of mitochondrial metabolism to development. We are assessing the impact of pharmacological inhibitor treatments and are generating patient-specific UiPSCs-derive KO models of maldevelopment in which to test our hypothesis.

Conclusions: Further results are outstanding but preliminary data suggests kidney organoids are a viable model in which to study bioenergetic mechanisms in the context of kidney development.

Q.4: Harnessing the endogenous stem cell response after spinal cord injury

Student: Laureen Hachem, Supervisors: Michael Fehlings, Charles Tator

Background: The adult spinal cord contains a population of ependymal-derived neural stem/progenitor cells (epNSPCs) which are normally quiescent but are acutely activated after spinal cord injury (SCI). Once activated, epNSPCs serve as critical players in promoting endogenous regeneration and baseline functional recovery. However, activation of epNSPCs remains limited to the acute injury period and thus, strategies that harness their regenerative potential after subacute/chronic SCI hold promise in enhancing endogenous repair and regeneration. A major barrier to unlocking the therapeutic benefits of epNSPCs has been a limited understanding of the mechanisms that regulate their activation post-SCI. Recently, we discovered that excitotoxic levels of glutamate, a hallmark in the pathophysiology of acute SCI, promote epNSPC proliferation and survival in vitro.

Purpose: Herein, we characterize the downstream signaling pathways involved in the activation of epNSPCs in response to glutamate excitotoxicity and target this mechanism in vivo to enhance the endogenous regenerative capacity of the injured spinal cord.

Methods: epNSPCs isolated from the central canal region of the adult spinal cord were treated with glutamate in the presence or absence of pharmacological inhibitors of glutamate receptors in vitro. Pathway analysis was conducted using immunohistochemistry, RNA sequencing and Western Blot. In vivo, cervical SCI was induced in adult rats. One-week post-SCI, animals were randomized to receive CX546 (a positive AMPA receptor modulator), or vehicle-control. Animals underwent weekly behavioral testing and spinal cords were extracted for histological analysis.

Results: Excitotoxic levels of glutamate lead to calcium influx in spinal cord epNSPCs via AMPARs and this change in calcium in concert with Notch signaling drives the proliferation of epNSPCs via pCREB, and induces astrocytic differentiation through Hes1 upregulation. Positive modulation of AMPARs subacutely after SCI enhances epNSPC proliferation, astrogliogenesis, neurotrophic factor production and promotes neuronal survival and functional recovery.

Conclusion: We uncover an important mechanism by which glutamatergic signaling via AMPARs regulate the growth and phenotype of spinal cord epNSPCs. Pharmacological modulation of AMPAR signaling offers a novel, translational strategy to regulate the fate of epNSPCs and harness their regenerative potential after SCI.

Q.5: Extending ex vivo lung perfusion with the addition of a kidney

Student: Fei Yu Gao, Supervisor: Marcelo Cypel

Background: Lung transplantation remains the only treatment option for end-stage lung diseases. However, donor lungs are frequently associated with injury and unsuitable for transplant, resulting long waitlists. *Ex vivo* lung perfusion (EVLP) allows injured lungs to be treated prior to transplantation. However, continuous EVLP is limited to 12 hours prevents the use of treatments requiring longer durations. Some of the challenges to prolonging continuous EVLP include the accumulation of lactate, sodium, and potassium in the perfusate.

Purpose: Our objective is to overcome these challenges and extend EVLP up to 72h by adding a kidney to EVLP. The kidney plays a role in metabolite balance and has exhibited a protective effect in other multi-organ ex vivo perfusion systems.

Hypothesis: The addition of a kidney can extend EVLP duration to 72h with improved lung function and reduced lung injury compared to 24h isolated EVLP.

Methods: Ten donor pig lungs will undergo EVLP either with or without a perfused kidney. Donor organs are harvested from pigs with minimal ischemia time. The Toronto EVLP protocol will be followed with the addition of washed erythrocytes from donor pigs. Lung functional assessments, lung injury, and metabolomics will be analyzed between lung only and lung-kidney groups.

Results: Results are still in progress. Pilot experiments show the kidney can produce urine in a parallel circuit setup with standard EVLP perfusate.

Conclusions: Prolonging EVLP duration can facilitate the logistics of lung transplantation. Extending EVLP duration provides the opportunity to employ longer *ex vivo* lung treatment interventions to improve donor lung injury.

Q.6: Devleopment of a small animal model of atrophic nonunion for the evaluation of xenogeneic endothelial progenitor cell therapy

Student: Matthew Raleigh, Supervisor: Aaron Nauth

Background: Up to 10% of all long bone fractures do not heal and result in a nonunion. The standard treatment for these injuries involves taking bone from elsewhere and transplanting it to the nonunion site. However, this technique is associated with significant rates of morbidity and persistent nonunion. As an alterative, our group has been developing an endothelial progenitor cell (EPC) therapy for nonunions. Several pre-clinical studies have demonstrated the efficacy and reliability of this treatment. Unfortunately, translational research using human EPCs is lacking, limiting clinical testing and potential translation of this promising therapy. To perform this work, and properly evaluate the therapeutic potency of human EPCs for bone healing, an animal model of nonunion capable of accepting human (i.e., xenogeneic) EPCs is required.

Purpose: To develop and test an immunodeficient small animal model of atrophic nonunion capable of accepting xenogeneic EPC for the treatment of nonunions.

Hypothesis: The recipient's immune status will be inversely proportional to the degree of bone healing generated by xenogeneic EPCs in a small animal model of atrophic nonunion.

Methods: EPCs were isolated from the bone marrow of male and female Yorkshire pigs and cultured. Porcine EPCs were then delivered via a collagen scaffold to a surgically generated non-healing bone defect in animals with one of three congenital or induced immune deficiencies:

1. RNU: Rowett Nude ($Foxn1^{-/-}$) T cell deficient rats (n = 6)

2. IS: Immune Supressed Fischer 344 rats treated with 1.33 mg/kg/d tacrolimus (n = 5)

3. SRG: Sprague Dawley ($Rag2^{-\backslash-}$, $Il2rg^{-\backslash-}$) B-, T-, and NK cell deficient rats (n = 5)

In the IS group, immune suppression began the day of treatment and continued for 28 days. All animals were kept for 10 weeks with standardized x-rays obtained at 4-, 6-, and 10-weeks following treatment. Bone healing was determined by evaluation of the final 10-week x-rays.

Results: Ten weeks following porcine EPC treatment, 4/5 (80%) SRG animals demonstrated complete union, while 2/5 (40%) IS animals demonstrated partial union. No animals (0/6, 0%) in the RNU group demonstrated union, as did the remaining animals in the SRG and IS groups.

Conclusions: The SRG rat appears to be an ideal small animal model for the study of xenogeneic EPCs in the treatment of nonunions. This is likely due to its severe immunodeficiency and greater xenograft tolerance in comparison to the less immunocompromised RNU and IS animals.

R: Neuroscience & Brain Health

R.1: Examining the impact of a national policy change legalizing recreational cannabis use on injuries in canada (cannabis C-45)

Student: Sarah Paleczny, Supervisor: Michael D. Cusimano

Background: On October 17th, 2018, Canada implemented recreational cannabis legalization (RCL). Cannabis use has since increased across certain demographic groups. Acute cannabis use can result in impaired judgement and psychomotor capacity, and psychosis, increasing the risk of intentional and unintentional injuries.

Purpose: To examine whether RCL affected rates of hospitalizations and emergency department (ED) visits for all intentional and unintentional injuries across Canada, and whether this differed between demographic groups.

Hypothesis: RCL did not significantly change overall rates of hospitalizations and ED visits for intentional and unintentional injuries across Canada in all populations.

Methods: An interrupted time series analysis using individual-level patient data from population data repositories was conducted using monthly rates of: (i) hospitalizations for injuries from all Canadian provinces and territories except Quebec (since April 2006), and (ii) all ED visits for injuries from Ontario and Alberta (since April 2010) and Yukon (since April 2015), until February 2022.

Results: RCL was not associated with significant level (immediate) and trend (over time) changes in rates of hospitalizations and ED visits for intentional and unintentional injuries overall across Canada (level change $\beta1$=0.80, 95% CI: [-2.02, 3.63], p=0.5772; trend change $\beta2$=0.09, 95% CI: [-0.20, 0.37], p=0.5539) including in females, males, and Canadians from urban and rural areas. There was a significant trend (over time) increase only in monthly rates of ED visits for all injuries in males from Yukon post-RCL (level change $\beta1$=7.32, 95% CI: [-193.03, 207.68], p=0.9431; trend change $\beta2$=19.90*, 95% CI: [2.59, 37.20], p=0.0275).

Conclusions: RCL did not significantly increase injury rates on a national level. However, injury rates increased among certain sub-populations pre-disposed to higher cannabis usage (male residents of Yukon territory). These results may help guide the implementation of targeted safety programs surrounding cannabis use to ensure its safe implementation across all populations and geographies in Canada.

R.2: Assessing "LDX" induced restoration of inhibitory signaling after traumatic spinal cord injury

Student: Omar Imad Hassan, Supervisor: Michael Fehlings

Introduction: Traumatic spinal cord injury (SCI) is a life-threatening condition which threatens the physical, social, and vocational well-being of patients. The initial injury in SCI involves the mechanical disruption of neural tissue and is followed by secondary injury which impedes recovery in the spinal cord around the lesion site. Furthermore, functional losses extend beyond the lesioned area, disrupting synapses in anatomically preserved tissue, worsening SCI symptoms and recovery. Thus, restoring functional signaling in perilesional tissue is a promising target for therapeutics to improve patient quality of life. However, the regeneration of signaling in the perilesional area is limited by the difficulty of integrating new synapses in the excitatory/inhibitory (E/I) imbalanced environment of the injured spinal cord. The downregulation of K+/Cl- cotransporter type 2 (KCC2) and upregulation of Na- K-2Cl cotransporter 1 (NKCC1) in inhibitory interneurons makes them depolarizing rather than repolarizing in effect and results in impaired inhibitory neurotransmission. To this aim, restoring balanced E/I signaling through NKCC1 inhibition has the potential to both re- establish functional neurotransmission as well as provide the inhibitory signaling necessary to integrate new synapses for functional recovery.

Hypothesis: "LDX", a pharmacological inhibitor of NKCC1, will cross the BSCB and enhance functional recovery during task-specific rehabilitation following traumatic SCI, concomitantly restoring E/I balance and enhancing new synapse integration.

Method: Female Wistar rats (4 weeks) will receive a clip compression-contusion injury at the C6/C7 level of the spinal cord as the model for patients with SCI. 60 rats will be randomized into 5 groups (n=12) as a blind study: 1) sham group, 2) vehicle control group, 3) "LDX" administration and clip-induced injury, 4) vehicle administration and rehab, 5) "LDX" administration and rehab. Administration will be 4mg/kg of "LDX" or vehicle bidaily through intraperitoneal injections for 12 weeks, beginning 1-week post-SCI. Sham rats will undergo laminectomy without clip-induced injury whereas vehicle will have the full clip- induced injury. Self-directed rehabilitation will be done through the robotic Whishaw task. At multiple intervals, neurobehavioral tests and blood draws will be conducted until 12 weeks post-SCI, when the animals will be sacrificed for electrophysiological and histological assessments.

Aim 1: Assessing locomotor improvement. Neurobehavioral tests of grip strength, trunk balance (inclined plane), locomotion (Basso, Beattie and Bresnahan scale), gait (Catwalk XT), forelimb reaching (Wishaw task) will be performed to see forelimb and hindlimb recovery. Neuropathic pain (von Frey and tail flick tests) will also be assessed.

Aim 2: Determining the restoration of inhibitory signaling and "LDX" BSCB penetrance. Electrophysiological assessments including motor evoked potentials and somatosensory evoked potentials of the cervical circuits as well as whole-slice patch clamping will assess inhibitory signal recovery. Histological staining to assess neuronal survival and synapse prevalence around the lesion. Degree of penetrance will be measured by running spine tissue through high-performance liquid chromatography with UV detection; localization of the drug in other organs will also be conducted with the same method.

Predicted Results: LDX and rehabilitation will achieve a better outcome than solely rehabilitation and vehicle, with greater locomotion, balance, forelimb, and hindlimb recovery. This will be due to sufficient penetration of the BSCB and inhibition of aberrant NKCC1 Cl- transport. At this stage of the project, conclusions cannot be drawn.

Novelty & Significance: The model for SCI used in this study is highly clinically relevant with 60% of all traumatic SCI cases occurring at the cervical level. This is the first study to utilize concomitant rehabilitation and NKCC1 inhibition therapy in SCI. Other studies of different NKCC1 inhibitor drugs have been limited in effect after BSCB repair due to poor permeability. "LDX"'s neutral pH, low affinity for hydrogen bonding, and no carboxyl group presents it as a hopeful candidate over its predecessors for better BSCB penetrance. Hence, in comparison to other proposed SCI treatments, this concomitant therapy closely addresses patient requirements for long-term neuromodulation and rehabilitation therapy after SCI — beyond the acute phase of injury.

R.3: Co-culture with healthy cells in patient-derived diseased retinal organoids improves photoreceptor outer segment formation

Student: Kristen Ashworth; Supervisor: Brian Ballios

Background & Purpose: No pre-clinical model exists to investigate human-to-human donor-host cell interaction and engraftment in cell therapies for inherited retinal disease (IRD). I propose a chimeric retinal organoid ("chimeroid") model, differentiated from the co-culture of healthy human stem cells and IRD patient-derived induced pluripotent stem cells (iPSCs).

Hypothesis: Chimeric association between disease and healthy human photoreceptors will promote the recovery of a normal outer retinal phenotype through non-cell autonomous mechanisms.

Methods: Red fluorescent protein (RFP)-tagged H9 human embryonic stem cells were co-cultured with green fluorescent protein (GFP)-tagged patient-derived iPSCs (from patients with *CRB1* or *USH2A*-related inherited retinal disease (IRD) mutations), in various H9-to-IRD cell mixing ratios (1:3,1:1,3:1). Throughout retinal organoid differentiation, immunohistochemistry was used to assess the rescue of disease photoreceptors by healthy cells within the chimeroid outer retinal border.

Results: H9- and IRD-only organoids were first established to compare differences between pure healthy and pure disease phenotypes. At Wk24 of retinal organoid growth, *CRB1*-organoids had reduced photoreceptor outer segment (OS) formation compared to healthy organoids. In Wks28, 30 and 34, *USH2A*-organoids had a significantly shorter OS length compared to healthy organoids ($p<0.05$), with significantly decreased localization of mature cone photoreceptor-specific markers (Arr3, L/M-Opsin). For all chimeric experiments, cell mixing proportions were maintained in mature (Wk24) H9:*CRB1* and H9:*USH2A* chimeroids. By Wk24 in H9:*CRB1* chimeroids, OS formation and expression of retinal cell-specific markers (Rho, Arr3) were increased in regions of the outer retinal border where H9-RFP photoreceptors were integrated.

Conclusions: The co-culture of healthy and disease cells promotes improved disease photoreceptor OS formation in mature human retinal chimeroids. As H9:*USH2A* and H9:*CRB1* chimeroids reach late maturation (Wk34), fluorescence activated-cell sorting and qRT-PCR will be used to assess transcriptomic changes in disease and healthy photoreceptors, elucidating mechanisms through which healthy cells rescue disease OS formation. This will provide insight into human donor-host cell interactions during retinal cell engraftment to inform new IRD cures.

R.4: Direct lineage reprogramming of A1 astrocytes for the generation of oligodendrocyte lineage cells

Student: Hiba Taha, Supervisor: Maryam Faiz

Background: Multiple sclerosis (MS) is a demyelinating nervous system disease caused by immune cell targeting of myelin-producing oligodendrocyte lineage cells (OLCs). Despite the success of immune-based therapies, there are currently no treatments that restore myelin. Direct lineage reprogramming (DLR) is a new therapeutic strategy that removes unwanted donor cell types involved in disease progression by converting them into functionally important target cell populations. This ability to selectively remove cells that drive disease pathology is a major advantage of DLR. Astrocytes are a heterogeneous population of cells that play key roles in MS pathology. Specifically, C3-expressing ('A1') astrocytes are present in human and mouse models and have been shown to promote neuronal degeneration, highlighting their potential as a donor cell type for therapeutic manipulation. However, whether this ('A1') subtype is amenable to reprogramming is unknown.

Purpose: We previously demonstrated that different transcription factors (TFs; TF1, TF2 and TF3) involved in OLC development convert 'pan' astrocytes to iOLCs. Here, we tested a model for the conversion of a well-defined C3+ 'A1' astrocyte phenotype to iOLCs.

Hypothesis: I hypothesize that the delivery of TF1, TF2, or TF3 to A1 astrocytes will give rise to iOLCs *in vitro*.

Methods: Previous studies characterize A1 astrocytes at very low plating densities (< 1000 cells/cm^2). Due to DLR requiring a high throughput starting cell culture, we first investigated whether the A1 signature is retained at high plating densities in preparation for DLR. Cortical astrocytes were isolated from postnatal day (P)0-P7 C67bl6 mice by immunopanning in serum-free conditions at low (<1000 cells/cm^2) and high (>3000 cells/cm^2) cell plating densities. Immunopanned (IP) astrocytes were induced to an A1 state by the addition of the cytokines IL-1α, TNFα and C1q. Cell morphology was analyzed prior to and following induction. We selected a panel of genes previously associated with the A1 signature to confirm A1^{hi} gene expression profiles using droplet digital PCR (ddPCR). A1 function was assessed using a live/dead survival assay with CalcienAM (live) and EthD1 (dead). Next, we tested A1 conversion to OLCs using three different transcription factors (TFs; TF1, TF2 and TF3) involved in OLC development. TFs linked to a GFP (LV-GFAP::TF-ZsGreen) or a GFP control (LV-GFAP::ZsGreen) were delivered to A1 astrocytes using lentiviruses driven by the astrocyte promoter, GFAP. Following viral transduction, cells were assessed for OL lineage and astrocyte markers (OPC: SOX10, NKX2.2, PDFGRα; nfOL: O4; mOL: MBP) 12 days post-transduction. Reprogramming efficiency was calculated as the iOL cell type+ DAPI+/total DAPI+ cells.

Results: We found that A1 astrocytes converted to iOLCs in the presence of OLC-promoting culture conditions that could be enhanced with the ectopic expression of TFs. These results suggest DLR of pathological 'A1' astrocytes to new iOLCs can be used as a novel 'two-birds-with-one-stone' therapeutic strategy for demyelinating diseases.

Conclusions: Altogether, this suggests that DLR of A1 astrocytes is feasible and our results lay the foundation for future applications of astrocyte to oligodendrocyte DLR in CNS disease.

S: Other

S.1: Quality of surveillance in patients with completely resected gastroenteropancreatic neuroendocrine tumours

Student: Gordon Taylor Moffat, Supervisor: Monika Krzyzanowska

Background: The incidence and prevalence of gastroenteropancreatic neuroendocrine tumors (GEP-NETs) are increasing worldwide. Surgery remains the only curative modality. Because of limited data on patterns of recurrence, real-world surveillance practices and duration vary widely. In 2018, the Commonwealth Neuroendocrine Tumour Research Collaboration (CommNETs) published consensus surveillance guidelines for patients with completely resected GEP-NETs.

Purpose & Hypothesis: Our purpose was to assess surveillance practices of patients with completely resected GEP-NETs at the UHN and adherence to the CommNETs guidelines practices to close gaps in the quality of care provided and patient outcomes. We hypothesize that our practices do not align with the natural history of the disease and the most recent guideline recommendations.

Methods: We conducted a retrospective cohort study of patients with GEP-NETs seen for a new patient appointment at the UHN from 2019-2022. Patients were included if they had a completely resected GEP-NET and followed on surveillance at our center. Demographic and tumor characteristics, surveillance practices, and clinical outcomes were abstracted. Summary statistics and a descriptive comparison of surveillance practices were completed. Adherence was defined as the completion of recommended guideline investigations.

Results: Out of 1305 assessed patients, 259 patients met eligibility and included. The main reasons for exclusion were metastatic disease or not followed at the UHN. The primary tumour sites were pancreatic (n=146, 56%), small bowel (n=47, 18%), appendiceal (n=36, 14%), rectal (n=21, 8%), and colon (n=9, 4%). One hundred and eight patients had stage 1 disease (42%), 63 patients had stage 2 disease (24%), and 88 patients had stage 3 disease (34). One hundred and sixteen patients had a WHO tumour grade 1 (45%), 36 patients had grade 2 (50%), and thirteen patients had grade 3 (5%). The median follow-up was 58 months. Most patients had two appointments per year with yearly ordering of imaging in 72% of patients, chromogranin A in 72% of patients, and urine testing in 9% of patients. Adherence to CommNETs guideline was 23% (48/206). There was better adherence in females (55%), appendiceal tumours (40%), and grade 1 (60%) and stage 1 (50%) disease. Provider factors for adherence were statistically significant in a Fixed Effects Model and Fischer-Exact tests, and account for approximately 25% of variation (Psuedo R^2 Model). A generalizing estimating equation multivariate logistic regression produced a positive odds ratio of adherence for moderately differentiated tumours and those patients followed by medical oncology and a less favorable odds ratio of adherence for male sex and stage 2 or 3 tumours. A trend towards over ordering investigation were observed with an estimated cost of $565,795.70 between 2019 to 2022.

Conclusions: Adherence to the CommNETs consensus guidelines was low at our centre, suggesting the guidelines had a minor impact of surveillance practices and providing an area for improvement in the process of care and resource utilization.

S.2: Bridging the implementation gap: best practices for implementing smoking cessation referrals in ontario regional cancer centres

Student: Isabella Devuono, Supervisors: Nadia Minian, Peter Selby

Background: Despite well-documented evidence indicating that smoking cessation (SC) interventions could increase the treatment and survival of patients diagnosed with cancer by 40%, the implementation of such interventions in oncology centres in Ontario, Canada is suboptimal (Canadian Partnership Against Cancer, 2021).

Purpose: Our purpose is to identify the best practices for implementing referrals to SC in Ontario regional cancer centres. By conducting a rapid review, we aim to identify the implementation strategies that affect the success or failure of SC interventions.

Methods: In collaboration with an academic librarian at the Centre for Addiction and Mental Health (CAMH), we developed our search strategy and identified a systematic review, identical in scope to what we intended to explore. However, this published review identified, up to 2020, implementation and clinical outcomes that supported the uptake of SC interventions. Following the declaration of COVID-19 as a pandemic, many services underwent a transition to virtual or remote care. Recognizing the evolving landscape, we deemed it necessary to revisit this review and ascertain whether novel strategies have emerged that correlate with enhanced screening, advice dissemination, and referral processes during this period. Our focus is solely on implementation strategies, including articles beyond their search from 2020–2023. We searched MEDLINE (Ovid), EMBASE (Ovid), and Cochrane Library. The article inclusion for eligibility was based on the population (P), intervention (I), comparator (C), and outcome (O) framework. The PICO framework is (P) patients with cancer who smoke or healthcare professionals, both situated within a cancer centre; (I) implementation strategies for implementing SC interventions; (C) other implementation strategies or no comparator; (O) implementation outcomes (with or without clinical outcomes).

Results: 21 studies met our inclusion and the implementation outcomes were clustered into nine domains, using the Expert Recommendations for Implementing Change tool. To date, the title and abstract screening, full-text review, data extraction, and quality assessment are complete. A narrative synthesis will be utilized to summarize the implementation strategies used for SC within the context of cancer centres and to identify strategies that led to successful implementation outcomes. Our findings will be presented in tabular format with descriptions for each strategy. We are committed to knowledge sharing through manuscripts, conferences, and presentations and anticipate the recommendations to be adopted within Canada and internationally. Ultimately, the goal is to improve treatment effectiveness and overall survival for patients with who smoke.

S.3: Presence of cardiovascular risk factors amongst individuals with schizophrenia receiving general-population smoking cessation treatment: an observational study

Student: Salsabil Siddiqui, Supervisors: Peter Selby, David CAstle

Background: Schizophrenia (SCZ) is a debilitating disorder associated with heightened prevalence of cardiometabolic comorbidities, contributing to a markedly reduced life expectancy. For SCZ patients with concurrent nicotine dependence, which comprise over 50 percent of this population, substantially worsened cardiovascular risk is anticipated, however exact associations have not yet been characterized.

Purpose & Hypothesis: The primary objective was to determine the odds of diabetes mellitus amongst cigarette smokers with schizophrenia, bipolar disorder, depression, or anxiety, relative to control smokers without psychiatric disorders. Secondary objectives were to highlight associations with hypertension, high cholesterol, obesity, heart disease, and stroke. Smokers with SCZ were hypothesized to exhibit significantly greater odds of diabetes compared to controls.

Methods: Data from 104,317 participants of the Smoking Treatment for Ontario Patients (STOP) program were analyzed. All analyses were conducted using IBM SPSS and Stata software. Six logistic regression models were employed. Analyses were adjusted for covariates including participant age, gender, education, income, cigarette consumption, alcohol use, and other substance use. Multiple imputation was used to address missing patient data. Results were presented as odds ratios (ORs) with 95% confidence intervals (CI).

Results: Relative to controls, smokers with schizophrenia exhibited significantly greater odds of presenting diabetes (OR=1.67; 95% CI=0.41, 0.61; p<0.001), high cholesterol (OR=1.54; 95% CI=0.35, 0.52; p<0.001), and obesity (OR=1.66; 95% CI=0.43, 0.58; p<0.001). Odds of hypertension (OR=1.08; 95% CI= -0.01, 0.16; p=0.08) and stroke (OR=1.13; 95% CI=-0.05, 0.29; p=0.17) were marginally greater, but lower for heart disease (OR=0.89; 95% CI=-0.25, 0.02; p=0.09); these findings were not significant.

S.4: Improving prenatal autoimmune congenital heart block detection with novel maternal autoantibody biomarkers

Student: Stephanie W. Benjamin, Supervisor: Robert Hamilton

Background: Autoimmune congenital heart block (aCHB), is a rare fetal heart disorder, characterized by complete heart block in fetuses of mothers with autoimmune disease. Typically diagnosed between 18-24 weeks of gestation through fetal heart rate or ultrasound screening, aCHB presents with fetal heart block in the absence of structural heart malformation. Its incidence ranges from 1 in 15,000 to 20,000 general pregnancies, with 1 to 2% in anti-Ro antibody positive maternal autoimmune conditions such as Lupus or Sjögren's Syndrome, and 18% in pregnancies of mothers with prior aCHB history. Fetal mortality rate in aCHB is ~20%, and morbidity often requires permanent pacing for affected infants. While, anti-Ro autoantibodies are usually present in aCHB cases, most anti-Ro positive autoimmune pregnancies will not have aCHB. A more reliable marker and predictor of aCHB is required for improved detection early in pregnancy.

Hypothesis: Given the passive placental transference of maternal autoantibodies in aCHB and heart rhythm alterations exclusively occurring in the fetal heart, we hypothesized that maternal autoantibodies cross-react with fetal cardiac proteins and sought to characterize these targets.

Methods: We analyzed maternal sera from both a discovery and a validation cohort consisting of mothers with aCHB offspring and mothers with healthy offspring, all anti-Ro positive, and sampled at various gestational ages. Autoantibody biomarkers were screened using two-dimensional (2D) gels of cardiac proteins sourced from normal 20-week gestation fetal heart tissues and iPSC-derived cardiomyocytes. The identities of twelve potential protein targets identified from these gels by mass spectrometry underwent confirmation through western blotting of commercial proteins exposed to sera from affected and unaffected pregnancies. Four targets consistently identified affected pregnancies prior to aCHB onset. The earliest autoantibody target of aCHB underwent further analysis. Confirmation of this specific target was achieved through a 'reverse' Western Blot process, where gels loaded with a recombinant protein antigen were exposed to maternal sera to validate the presence of corresponding autoantibodies.

Results: Across gestations of 7 weeks to full term, 2D gel analyses revealed an expanding array of maternal autoantibody targets to fetal cardiac proteins. Early pregnancy sera collected before 18 weeks of gestation consistently identified four cardiac protein targets: Na+/K+ transporting ATPase subunit alpha-1 (AT1A1), cardiac-type myosin-binding protein C (MYPC3), endoplasmic reticulum chaperone BiP (BIP), and annexin A1 (ANXA1). Antibodies to AT1A1 were exclusively identified in 2D gels as early as 7 weeks, accurately predicting aCHB pregnancies. Autoantibodies to AT1A1 in maternal sera were confirmed through 'reverse' Western Blots of recombinant AT1A1. Anti-Ro autoantibodies alone, were present in both affected and some unaffected pregnancies.

Conclusions: Our study highlights the potential for early aCHB detection through novel maternal autoantibody biomarkers, particularly targeting the fetal AT1A1 cardiac protein. The exclusive presence of anti-AT1A1 autoantibody binding at 7 weeks of gestation suggests a possible role in initiating tissue damage within the conduction system, contributing to aCHB. An enzyme-linked immunosorbent assay (ELISA) for reliable aCHB prediction using maternal sera is being developed for potential translation to clinical laboratory testing. This advancement may facilitate more effective *in utero* management of aCHB by informing therapeutic interventions.

S.5: Novel statistical approaches to enhance outcome prediction after traumatic spinal cord injury

Student: Ayesha Quddusi, Supervisor: Michael G. Fehlings

Background: Traumatic spinal cord injury (TSCI) continues to be a devastating injury contributing to mortality and morbidity around the world. Novel statistical approaches utilizing supervised and unsupervised machine learning have been used to model outcome predictions in TSCI that have the potential to optimize management and treatment of patients. The TSCI outcomes for which prediction modelling are measured and documented non-uniformly in the literature. Most models are internally validated, with a paucity of external and comparative validation.

Purpose: The purpose of this study is to perform comparative validation of three outcome prediction modelling techniques including group-based trajectory modelling, latent class analysis, and cluster analysis.

Hypothesis: Prediction modelling techniques may or may not demonstrate superiority over each other for TSCI outcomes.

Methods: We combined prospectively collected data sets spanning the last 30 years from multiple sites across North America. The data was cleaned, and outcome measures were harmonized. In the first step of this study, we described the data set by conducting a trends analysis across last 3 decades that included demographics, etiology, and the most used outcome measure, American Spinal Cord Injury Association Impairment Score (ASIA Grade) at 6 months after the initial injury. The 3 decades were divided as follows, Decade 1 (1990-1999), Decade 2 (2000-2010), and Decade 3 (2011-2020). We also analyzed if the ASIA Grade outcomes differed significantly between each decade using a multivariate analysis.

Results: The total number of patients in the dataset was 2645. Overall, the average age of patients increased from 33.8 to 45.5 to 48 years across the 3 decades. Motor vehicle accidents (MVAs) decreased while falls increased as the mechanism of injury over the 3 decades. Cervical spine showed an increase in the most frequently affected spinal region while thoracic spine showed a decrease. Based on baseline ASIA Grade incomplete injuries increased while complete injuries decreased over the 3 decades. Before conducting multivariate analysis, the proportion of patients who showed improvement in ASIA Grade at 6 months following injury increased across the 3 decades. After controlling for age, early surgery, and spinal region, the difference in proportion of patients who showed ASIA Grade improvement at 6 months was not significant.

Conclusions: Over the last 3 decades, the average age of TSCI patients has increased, etiology has moved from MVAs to falls, incomplete injuries are the more frequently seen sub-type of TSCI than complete injuries, and the region of spinal cord affected is increasingly the cervical spine. These findings have implications for prevention strategy development, and management. On multivariate analysis, there was no difference seen in ASIA score-based outcomes, which may reflect the inherent challenges of ASIA as an outcome measure. In the next steps, the data set will be further cleaned and harmonized to look at outcome measures including functional outcome measures, and upper extremity motor score before under-taking the modelling and comparative validation.

S.6: Investigating the levels of cortical gamma-aminobutyric acid (GABA) in social anxiety disorder (SAD)

Student: Sonja Elsaid, Supervisor: Bernard Le Foll

Background: Individuals with Social Anxiety Disorder (SAD) experience anxiety and avoidance in social settings, which leads to significant dysfunction in all aspects of life. The implicated pathophysiology may be associated with dysregulations in the 'fear neurocircuitry', which includes the regions of the limbic system, thalamus, dorsomedial prefrontal cortex (dmPFC), anterior cingulate cortex (ACC), and dorsolateral prefrontal cortex (dlPFC). In recent studies, downregulation of the major inhibitory neurotransmitter, γ-aminobutyric acid (GABA), was observed in the thalamus and dmPFC/ACC in the SAD group compared to the healthy controls (HCs), while no differences in glutamate and glutamine; glutamix (Glx) were noted either in ACC or dmPFC/ACC. However, these imbalances have not been replicated in dmPFC/ACC or investigated in dlPFC.

Purpose & Hypothesis: Our primary objective was to investigate levels of GABA and Glx in dmPFC/ACC in individuals with SAD compared to their sex-age-matched HCs. Our secondary objective was to conduct the same comparison in dlPFC. We hypothesize that compared to HCs, decreased GABA will be observed in participants with SAD in dmPFC/ACC. Secondly, we predict that in dlPFC, decreased GABA will also be noted in the SAD group.

Methods: Twenty-six participants (mean age: 25.3 ± 5.0), and 26 HCs (mean age: 25.2 ± 4.4) completed the Liebowitz Social Anxiety Scale (LSAS), which was the primary outcome measure, and were scanned with a GE MR750 3T scanner with the 32-channel head coil (Nova Medical) to determine the levels of GABA plus macromolecules (GABA+) and Glx in dmPFC/ACC and dlPFC. GABA+ and Glx were acquired with MEGA-PRESS (MEscher-GArwood Point RESolved Spectroscopy) proton magnetic resonance spectroscopy (^{1}H MRS) sequence, with the following parameters: TE/TR=68/1500ms, 384 averages, and acquisition time 10.4 min. GABA+ and Glx were reported in institutional units (i.u.).

Results: Participants with SAD scored significantly higher on total LSAS (SAD: 98.1±13.6 vs. HCs: 7.8 ± 6.3; $t = 30.8$; $p < 0.001$) In dmPFC/ACC, no between-group differences were noted either in the levels of GABA+ ($t = 0.79$; $p = 0.436$), or Glx ($t = 0.66$; $p = 0.513$), however, a significant negative correlation was observed between levels of GABA+ and LSAS total scores (Pearson's correlation coefficient (r) = - 0.70; $p < 0.01$) and between Glx and LSAS ($r = - 0.57$; $p < 0.01$). In dlPFC, the levels of GABA+ were higher in the SAD group ($t = 2.21$; $p = 0.032$), while no between-group differences were seen in Glx ($t = 0.36$; $p = 0.719$). Contrary to dmPFC/ACC, no significant correlations with the primary outcome measure were recorded in the dlPFC.

Conclusion: Elevated GABA+ in dlPFC in SAD may indicate decreased GABA+ degradation. Given that dlPFC is involved in selecting which sensory information should be brought to the conscious awareness, dlPFC impairments may lead to 'mistakenly' interpreting social cues as threatening. Decreases in both GABA+ and Glx with the increasing severity of SAD may indicate neuronal and glial cell damage in dmPFC/ACC, as SAD progresses. Disturbances in the dmPFC/ACC have been associated with the improper inhibition of the limbic system and consequently the 'misinterpretation' of the sensory signals. The implied aberrances in neurocircuitry may have been manifested as symptoms of SAD. The demonstrated disturbances in dmPFC/ACC and dlPFC could serve as molecular targets for novel pharmacological treatments for SAD.

S.7: Examining the influence of social determinants on medical visit attendance, emergency visits, and clinical outcomes among children with eye cancer: a retrospective analysis

Student: Omer Jamal, Supervisor: Helen Dimaras

Background & Aims: There is limited evidence on the impact of social determinants of health (SDH) on rare pediatric eye cancer (R-PEC) outcomes in Canada. We examined the association of R-PEC patient SDH with (a) medical visit attendance, (b) age and stage at diagnosis, (c) clinical outcomes, and (d) emergency visits.

Methods: This retrospective cohort study between 1-June-2018 and 6-October-2023 included R-PEC patients managed at The Hospital for Sick Children and resided in Ontario. Data collected included: sociodemographic variables, diagnosis details, medical visit attendance and clinical outcomes. Postal code was used to deduce neighborhood income quintile, Ontario marginalization index (OMI), geographic location, distance from hospital, and urbanicity. Pearson Chi-squared analysis and multivariable regression with adjusted odds ratios (aOR) and 95% confidence intervals (CI) were performed (significance was set at $p<0.05$).

Results: There were 324 study subjects with R-PECs affecting the retina (64.2%), optic nerve (28.7%), orbit (5.2%), eyelid (0.9%), and other structures of the eye (0.6%). Rescheduled or no-show medical visits were associated with: highest quintile (most marginalized) of the OMI dimensions material resources ($p=0.049$, aOR=1.576, 95% CI=1.003-2.477) and household dwellings ($p=0.015$, aOR=1.112, 95% CI=1.021-1.211); living >75km from the hospital ($p=0.028$, aOR=1.109, 95% CI=1.011-1.216); and non-white race ($p<0.001$, aOR=1.758, 95% CI=1.051-2.942). Higher stage at diagnosis was associated with: the highest quintile of the OMI dimensions material resources ($p=0.046$), household dwellings ($p=0.015$), age labor force ($p=0.004$), and racialized and newcomer populations ($p<0.001$); low neighborhood income quintile ($p=0.038$); and non-white race ($p<0.001$). Older diagnosis age was associated with: highest quintile of the OMI dimensions material resources ($p<0.001$), household dwellings ($p<0.001$), age labor force ($p=0.013$), and racialized and newcomer populations ($p=0.002$); living >75km from the hospital ($p<0.001$); low neighborhood income quintile ($p=0.017$); rural residence ($p<0.001$), and non-white race ($p<0.001$). Greater visual impairment was associated with the highest quintile of the OMI dimensions material resources ($p=0.003$), household dwellings ($p=0.013$), and racialized and newcomer populations ($p=0.042$); low neighborhood income quintile ($p<0.001$); rural residence ($p<0.001$); and non-white race ($p<0.001$). Having >1 emergency room visit was associated with: low neighborhood income quintile ($p=0.022$); highest quintile of the OMI dimension racialized and newcomer populations ($p=0.002$), and non-white race ($p=0.041$).

Conclusions: Addressing unfavorable SDH could serve to improve clinic attendance, age and stage at diagnosis, final visual outcome and reduce emergency room visits among patients with R-PECs.

S.8: SHH ACTIVATION IN DEDIFFERENTIATION DURING TUMOR PROGRESSION IN MPNST

Student: Rebeca Yakubov, Supervisor: Gelareh Zadeh

Background: Malignant peripheral nerve sheath tumors (MPNST) are highly aggressive sarcomas with little progress on outcomes and treatment strategies. Previously, unsupervised analyses of methylome and transcriptome profiles of 108 peripheral nerve sheath tumors uncovered two subgroups of MPNSTs that predict progression-free survival, MPNST-G1 (characterized by SHH-pathway activation) and MPNST-G2 (characterized by WNT/ß-catenin/CCND1-pathway activation). Further, single nuclear RNA sequencing revealed that MPNST-G1 and MPNST-G2 cells resemble neural crest-like and Schwann cell precursor-like cells, respectively.

Purpose & Hypothesis: To examine whether the activation of the SHH pathway in MPNST-G1 cell lines induces overexpression of factors known to play canonical roles in early neural crest cell specification (TWIST1, SNAI2, PAX3, PAX6, SOX9, OTX2) and to investigate the role of Src activation on SHH pathway-induced dedifferentiation. We hypothesize that MPNST-G1 cells will exhibit SHH pathway activation, synergizing with Src to induce the overexpression of early neural crest cell (ENCC) transcription factors.

Methods: SHH pathway activation, expression of ENCC transcription factors, and effects of inhibitors were analyzed in MPNST-G1 cells using RT-PCR, western blotting, Alamar Blue assay, Trypan Blue assay, and Soft Agar Transformation assay.

Results: Compared to MPNST-G2 cells, MPNST-G1 cells displayed SHH-pathway activation, SMO-dependent activation of Src, and elevated expression of the ENCC transcription factors. Sonidegib (SMO inhibitor) and Dasatinib (Src inhibitor) were able to revert dedifferentiation.

Conclusions: The SHH-pathway cooperates with Src to promote expression of important transcription factors in dedifferentiation. This finding provides insights into the transformation process of MPNST and novel therapeutic options.