CSCI-CITAC PRESENTS

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ABSTRACT BOOKLET

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Basic Sciences

ORAL PRESENTATIONS

Dietary supplementation with inulin slows the progression of extracolonic colorectal tumors in mice

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Background: Inulin is fermented by the colonic gut microbiota to produce short chain fatty acids (SCFAs), including butyrate, acetate, and propionate. Butyrate is sensed by the peroxisome proliferator-activated receptor γ (PPAR-γ) and 5-aminosalicylic acid (5-ASA) is an agonist of PPAR-γ. Butyrate and 5-ASA have been reported to alleviate inflammation and inhibit the proliferation of colorectal cancer cells in the gut. We assessed the effect of dietary fiber supplementation on the progression of distance colorectal tumours in a mouse model of subcutaneous colorectal cancer (CRC).
Methods: BALB/c mice received a dietary supplementation with diet supplemented with either, 10% inulin, a fermentable oligosaccharide, 0.6% 5-aminosalicylate (5-ASA), an anti-inflammatory agent, or a control diet lacking fibers. After 2 weeks, mice were subcutaneously injected with CT26 colon carcinoma cells. Tumor development and progression was monitored until 18 days post-injection.
Results: Mice supplemented with inulin and 5-ASA displayed a slower tumor progression and smaller tumors than the mice that received a diet without fibers. Quantification of short-chain fatty acids in the gut lumen showed that butyrate, propionate, and acetate were significantly higher in mice supplemented with inulin versus those receiving no fibers. Serum IL-6 is reduced in response to inulin and 5-ASA supplementation.
Conclusions: At the end point, inulin and 5-ASA, impedes the growth of extraintestinal CRC tumors in mice. Inulin increases short chain fatty acid production in the gut. Both inulin and 5-ASA significantly lowered serum IL-6 levels, indicating inflammatory effect. Dietary interventions may be beneficiary in CRC.

Investigating the role of soluble adenylyl cyclase in the response post-myocardial infarction

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Background: Myocardial infarction (MI) is a leading cause of morbidity and mortality worldwide. Recently, the ubiquitous second messenger cyclic adenosine monophosphate (cAMP) has emerged as a regulator of key response pathways post-MI including apoptosis, inflammation, fibrosis and metabolism. In contrast to conventional transmembrane adenylyl cyclases, soluble adenylyl cyclase (sAC) is responsible for cAMP signaling in cellular compartments. However, the role of sAC in the response post-MI is poorly understood.
Methods: We performed permanent left anterior descending coronary artery ligation to induce experimental MI in sAC knockout ($sAC^{-/-}$) and sAC wild-type mice littermates ($sAC^{+/+}$). We monitored mice for 28 days post-MI for differences in survival. Subsequently, we performed molecular and biochemical characterization of the heart at d3 post-MI, prior to the onset of cardiac rupture events, using 2,3,5-Triphenyltetrazolium chloride and Masson's Trichrome staining, echocardiography and PV-loops. In addition, we collected blood for analysis of circulating systemic cytokines/chemokines.
Results: We observed that post-MI mortality was significantly increased in $sAC^{-/-}$ mice, due to an increase in cardiac rupture events. We found that hearts taken from $sAC^{-/-}$ mice at d3 post-MI have larger infarct size and marked systolic dysfunction. However, there was no differences in fibrosis in $sAC^{-/-}$ versus $sAC^{+/+}$ mice at d3 post-MI. Interestingly, $sAC^{-/-}$ mice at d3 post-MI showed increased circulating levels of pro-inflammatory cytokines including TNFα and CCL5/RANTES.
Conclusion: We have identified a novel role for sAC in preventing cardiac rupture post-MI by improving cardiac function, reducing infarct size and regulating the inflammatory response.

Characterization of novel contributors to superior coloboma and the closure of the superior ocular sulcus

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The superior ocular sulcus (SOS) is a transient developmental fissure that forms within the superior eye. Improper closure of this structure can result in a phenotype in zebrafish that resembles the human condition superior coloboma, an atypical form of ocular coloboma. Coloboma results from improper closure of the choroid fissure and causes 3-11% of congenital blindness in children. The choroid fissure and coloboma of the inferior eye have been widely studied; however, the mechanisms involved in the formation and closure of the SOS and the genetic causality of superior coloboma remain largely understudied. We have previously demonstrated the potential importance of Bmp-dependent ocular dorsal eye patterning in SOS closure. Recently, we have uncovered variants from patients with superior coloboma in the mTOR regulator, *TSC2*; the ventral eye patterner, *VAX2*; and the planar cell polarity (PCP) gene, *SCRIB*. Our current investigations show that loss of *tsc2*, *vax2*, and the PCP gene, *vangl2*, independently, result in SOS closure delays in zebrafish. Through RNA sequencing, we have identified multiple candidate genes whose roles in the formation and closure of the SOS have not yet been studied. Taken together, our results have extended our knowledge of the regulation of SOS closure by implicating the possible roles of the PCP pathway, mTOR signaling, and ventral eye patterning. These results also affirm that the causes of superior coloboma are likely combinatorial and that multiple pathways might be involved in its causality. Therefore, we hope to create an ultimate zebrafish model of superior coloboma through our investigations.

Cortisol levels and number of age-related comorbidities between women living with and without HIV

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Background: Women living with HIV (WLWH) disproportionately experience comorbidities and psychosocial stressors. High cortisol levels from chronic stress can negatively impact health. We assessed whether cortisol levels relate to number of comorbidities among WLWH and HIV-negative controls.
Methods: Groups were age-matched 1:1 within 3 years. Cortisol levels assayed by ELISA from extracted hair (3cm) specimens, then normalized by log-transformation. Demographics and comorbidities were ascertained via survey, including depression and cardiovascular, metabolic, bone, liver, and renal disease. Multivariable linear and Poisson regression models, stratified by HIV status, identified variables independently associated with cortisol levels and comorbidity counts, respectively.
Results: WLWH and HIV-negative groups were similar in age (mean±SD: 51.0±11.2 vs. 51.0±11.0 years) and most clinical/demographic factors; WLWH had lower education and more opioid use. WLWH had more comorbidities than controls (3.23±1.57 vs. 2.47±1.55; p=0.001). In univariable analyses, higher cortisol levels and older age correlated with greater comorbidity counts among WLWH (r=0.23, p=0.02; r=0.47, p<0.0001, respectively); age correlated with cortisol levels among controls (r=0.39, p=0.0002). In adjusted models, age was associated with comorbidity count for WLWH (prevalence ratio=1.02, p=0.01). For controls, comorbidities related to age (1.02, p=0.004) and non-White ethnicity (1.30, p=0.004). higher cortisol levels were independently associated with current cocaine/methamphetamine use among WLWH (p=0.002), and with current opioid use (p=0.012) and low income (p=0.03) among controls.
Conclusions: Older age, but not higher cortisol levels, was associated with more comorbidities among WLWH. Results identified modifiable risk factors for increased cortisol levels and comorbidities, including cocaine/methamphetamine and opioid use and low income.

Comparing two versions of automated insulin delivery systems among adults with type 1 diabetes

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Background: We aim to compare Health Canada approved commercial automated insulin delivery (AID) systems with unregulated open-source do-it-yourself (DIY)- AID systems for glucose management among adults with type 1 diabetes in real-life conditions.
Methods: Planned interim analysis of a prospective non-inferiority, non-randomized, parallel-cohort study involving 45 non-pregnant adults with type 1 diabetes having used an AID for ≥3 months and living in Canada: 15 DIYAID and 30 commercial AID users, meeting a prespecified ratio of 1(DIY):2 (Commercial); 55.6% females, mean age 48.0±14.2 years old with mean diabetes duration of 29.9±15.3 years and HbA1c of 6.7±0.7%. Participants continued using their system as per usual. Four weeks' data from an additional blinded CGM (Dexcom G6) was used to assess effectiveness (Primary outcome: 24-hour time in optimal glucose range (3.9-10.0 mmol/L%).
Results: DIYAIDs were non-inferior to commercial AIDs regarding primary outcome (82.0±8.1% vs. 73.1±9.0%, mean difference 9.0% [95% CI 3.4% to 14.5%], P<0.001 for non-inferiority [non-inferiority margin 5%]), even after adjusting for confounding factors. 24-hour outcomes including mean glucose (7.4±0.8% vs. 8.5±0.9%, P<0.001) and hyperglycemia (>10.0 mmol/L: 14.1±8.4% vs. 25.1±9.6%, P<0.001) were better in DIYAID than commercial AID users while percentage in hypoglycemia was higher (3.8±2.5% vs. 1.8±1.1%, P<0.001) when using DIYAID. The benefits of DIYAID were mostly attributed to the daytime period. No severe adverse event occurs in either group.
Conclusion: In a real-world setting, DIYAID was safe and non-inferior to commercial AIDs for overall glucose management with an increase in hypoglycemia (yet still within recommended range) among adult with type 1 diabetes.

BASIC SCIENCES

Spatially Mapping the Melanoma Immune Landscape of Brain Metastases using Imaging Mass Cytometry

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Up to 75% of metastatic melanoma patients develop brain metastases, and 5% develop leptomeningeal spread, which presents with the worst prognosis of any melanoma brain metastasis (MBM) infiltration pattern. However, the differences in the spatial immune landscape between MBM with and without leptomeningeal spread (LS) remains poorly-understood. To spatially characterize the tumor microenvironment (TME) of MBM, we quantified the expression of 35 protein markers using CyTOF Imaging Mass Cytometry (IMC) in 21 MBMs (9 with LS, 12 without LS). We performed segmentation and cell type assignment to identify lineages that included melanoma, endothelial cells, immune cell subsets and astrocytes to spatially characterize the TME of MBMs with LS. Using a novel cell segmentation and identification technique, we segmented and classified 130,000 cells into 19 cell types. In our MBM dataset, we found the most common cell type after melanoma to be bone marrow derived macrophages (BMDM). In our comparisons between patients that had LS or not, we found that patient samples that did not exhibit LS had significantly more neutrophils, M1-like BMDM, T other cells and less cancer cells. In addition, we found a smaller median distance between cancer cells and neutrophils. Patients that did not exhibit LS have increased immune-cell involvement and neutrophil-cancer cell interactions, which may suggest a role for the immune system in mediating LS. These studies highlight the potential of multiplexed single cell technology to quantify spatial cell-cell interactions within the TME of MBM.

Cell-surface glycan engineering for capturing glyco-immune checkpoint interactions

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Proliferation of tumor cells is dependent on their ability to evade recognition by the immune system. Targeting these mechanisms has thus proven highly effective in the treatment of incurable cancers through modulation of inhibitory immune receptor interactions, such as programmed death-1/ligand (PD-1/PD-L1) binding. However, many cancers are unresponsive to current immunotherapies that target well-characterized interactions. As a result, there is significant interest in exploring alternative immunoevasive mechanisms. One such pathway harnessed by diverse cancer types, is through changing the cell-surface sugar coating of glycans, through increased presentation of sialic acid (Neu5Ac) on cellular glycans. This hypersialylation produces glycoprotein ligands that interact with inhibitory receptors, including sialic-acid- binding immunoglobulin-type lectins (Siglecs) expressed by various immune cells, to suppress immune activation. Despite their importance, Siglec ligands remain poorly defined.
Introduction of photo-cross-linkers into cellular glycans provides an attractive strategy to capture native glycan-binding protein partners to permit identification of the interaction complex. Herein, we present cell-surface exo-enzymatic glycan engineering as a platform for selective introduction of photo-cross-linking probes on to native cellular glycans. Our approach harnesses the inherent specificity of various administered sialyltransferases to install CMP-sialic acid probes through select linkages to target glycan subclasses. This method thus will provide a basis to discover and probe the glycan epitopes and bound conjugates that are critical to Siglec-glycan interactions in a biologically relevant setting to guide the development of novel immunotherapies.

Identification of protein trapping mutations in Topoisomerase IIIα (TOP3A)

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Genomic instability is a fundamental feature of cancer cells, which become dependent on DNA damage response mechanisms to survive. This dependency makes these proteins appealing targets for synthetic lethal therapies. PARP inhibitors, the most successful synthetic lethal therapeutic to date, employ a protein trapping mechanism, stabilizing protein-DNA complexes. This sabotages DNA repair while restricting access for uninhibited proteins, enabling effective toxicity at sub-saturating inhibition. The present study aims to replicate this clinically successful strategy in topoisomerase III alpha (TOP3A). TOP3A resolves double Holliday junctions created in break-induced replication and double-strand break repair. Point mutations are used to model inhibition while maintaining protein interactions. The D148N and R364W mutants, homologous to trapping mutants identified in related topoisomerases, were introduced into TOP3A and expressed in human cell lines. Monitoring DNA damage by detection of gamma-H2AX foci showed TOP3A overexpression significantly increased foci formation. However, the R364W mutant induced more damage than either wildtype or D148N, indicating a unique mechanism. To explore the relationship of this locus to protein structure, we modeled the covalent DNA-bound transition state of TOP3A using a homologous bacterial enzyme structure. This model guided selection of nearby sites for generation of mutant libraries. In summary, we have identified a dominant DNA damage allele of TOP3A. Future work will determine the mechanism of action and contexts in which R364W causes damage. We also aim to identify novel trapping mutations to pinpoint targetable regions of TOP3A. Characterization of mutations could guide inhibitor development, expanding the accessible repertoire of synthetic lethal therapies.

Application of immune checkpoint inhibitors targeting LAG-3 and PD-1 on invariant natural killer T cells

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Background: Invariant Natural Killer T (iNKT) cells are innate lymphocytes critical in combatting viral infection. Our lab showed expression of LAG-3, an inhibitory immune checkpoint, was increased on iNKT cells in HIV infection, correlating with decreased functionality. Another checkpoint, PD-1, was shown to be increased on iNKT cells in HIV infection, correlating to decreased function. We hypothesize that blocking LAG-3 and/or PD-1 via immunotherapeutic blockades will restore iNKT function.
Methods: Utilizing peripheral blood mononuclear cells from HIV-uninfected donors (n=9), efficacy of anti-LAG-3 and anti-PD-1 blockades to enhance iNKT function was assessed via a 10-day *in vitro* iNKT stimulation assay, with enhanced proliferation as the primary outcome, reported as "log2 fold-change".
Results: By ANOVA test, both single PD-1 and dual PD-1+LAG-3 blockade systems showed significantly enhanced proliferation with a mean of 6 and 6.29 log2 fold-change compared to the stimulation without blockade control (3.07 log2 fold-change) ($p=0.0005$). Follow-up analysis of paired two-tailed t-test found the dual anti-PD-1+anti-LAG-3 blockade system significantly enhanced iNKT cell proliferative capacity compared to the single PD-1 blockade ($p=0.013$). Additionally, the single LAG-3 blockade condition trended towards increased proliferation compared to the stimulated without blockade control with a mean of 3.77 log2 fold-change ($p=0.074$).
Conclusions: This study provides proof-of-concept for targeting LAG-3 and PD-1 to enhance proliferative ability of iNKT cells. In the near future, these immunotherapeutic blockades will be applied to HIV-positive samples to assess if HIV-mediated dysregulation of iNKT cells can be reversed and thereby potentially boost viral control in a functional HIV cure approach.

SARS-CoV-2 Accessory Protein ORF8 Decreases Antibody-Dependent Cellular Cytotoxicity

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Viruses use many different strategies to evade host immune responses. In the case of SARS-CoV-2, its Spike mutates rapidly to escape from neutralizing antibodies. In addition to this strategy, ORF8, a small accessory protein encoded by SARS-CoV-2, helps immune evasion by reducing the susceptibility of SARS-CoV-2-infected cells to the cytotoxic CD8+ T cell response. Interestingly, among all accessory proteins, ORF8 is rapidly evolving and a deletion in this protein has been linked to milder disease. Here, we studied the effect of ORF8 on peripheral blood mononuclear cells (PBMCs) using flow cytometry and biolayer interferometry. The impact of ORF8 on ADCC was further characterized using our newly developed ADCC assay. Specifically, we found that ORF8 can bind monocytes as well as NK cells. Strikingly, ORF8 binds CD16a (FcγRIIIA) with nanomolar affinity and decreases the overall level of CD16 at the surface of monocytes and, to a lesser extent, NK cells. This decrease significantly reduces the capacity of PBMCs and particularly monocytes to mediate antibody-dependent cellular cytotoxicity (ADCC). Overall, our data identifies a new immune-evasion activity used by SARS-CoV-2 to escape humoral responses.

High-throughput fabrication and screening of polymeric nanocarriers for specific organ targeting

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Drug delivery systems are crucial to the therapeutic response of a drug, but there are many limitations to those currently available. In recent years, polymeric nanoparticles (PNPs) have been extensively investigated for their potential to improve drug pharmacokinetics, including bioavailability and biodistribution. However, despite the abundance of fundamental research and clinical trials, PNPs have failed to reach the market. This failure is largely attributed to the non- specific distribution of PNPs, wherein >95% of an intravenous administration does not reach the pathological site. This non-specific distribution of PNPs is regulated by their surface composition and opsonization. To prevent opsonization and thus increase half-life, anti-fouling hydrophilic polymers, such as PEG, must be incorporated at the surface of PNPs. However, these polymers have been ineffective in directing the distribution of PNPs to any specific organ, despite increasing their half-life.
Interestingly, polypeptides with recently demonstrated anti-fouling effects have been identified. This has led us to hypothesize that the presence of these polypeptides on the surface of PNPs will improve selective organ targeting while increasing half-life. To test this hypothesis, we have begun constructing a library containing hundreds of PNPs with variable surfaces that mimic these polypeptides. This library will be screened for toxicity in endothelial cells and hepatocytes, and the most promising PNPs will be tested for organ targeting properties *in-vivo* with zebrafish and mouse models. We expect to discover a set of polymeric nanocarriers with specific organ targeting properties which could then be used to encapsulate small molecule drugs, thus improving therapeutic response.

Pcsk9 knockout exacerbates diet-induced non-alcoholic steatohepatitis, fibrosis and liver injury in mice

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Background: The fatty acid translocase, CD36, is a well-established scavenger receptor for fatty acid (FA) uptake in many metabolically active tissues. In the liver, CD36 is known to contribute to the progression of non-alcoholic fatty liver disease by promoting triglyceride accumulation and subsequent lipid-induced endoplasmic reticulum (ER) stress. Given the recent discovery that the hepatocyte-secreted PCSK9 blocks CD36 expression, we sought to investigate the role of PCSK9 in liver fat accumulation and injury in response to saturated FAs with a mouse model of diet-induced hepatic steatosis.
Methods: We investigated the role of PCSK9 on the uptake and accumulation of FAs, as well as FA-induced toxicity, in a variety of cultured hepatocytes. Diet-induced hepatic steatosis and liver injury were also assessed in $Pcsk9^{-/-}$ mice.
Results: Our results indicate that PCSK9 deficiency in cultured hepatocytes increased the uptake and accumulation of saturated and unsaturated FAs. In the presence of saturated FAs, PCSK9 also protected cultured hepatocytes from ER stress and cytotoxicity. Consistently, a metabolic challenge using high-fat diet caused severe hepatic steatosis, ER stress, inflammation and fibrosis in the livers of Pcsk9-/- mice compared to controls. Given that inhibition of CD36 ablated the observed accumulation of lipids in vitro and in vivo, our findings also highlight CD36 as a strong contributor to steatosis and liver injury in the context of PCSK9 deficiency.
Conclusions: Our findings demonstrate that PCSK9 regulates hepatic triglyceride content in a manner dependent on CD36 and may protect against hepatic steatosis and liver injury.

Germline missense variants in *CDC20* result in aberrant mitotic progression and familial cancer

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CDC20 is a coactivator of the anaphase promoting complex/cyclosome (APC/C) and is essential for mitotic progression. The APC/C^{CDC20} complex is inhibited by the spindle assembly checkpoint (SAC), which prevents premature separation of sister chromatids and aneuploidy in daughter cells. Although overexpression of *CDC20* is common in many cancers, oncogenic mutations have never been identified in humans. Using whole-exome sequencing, we identified heterozygous missense *CDC20* variants (L151R and N331K) that segregate with ovarian germ cell tumors in two families. Functional characterization showed these mutants retain APC/C activation activity but have impaired binding to BUBR1, a component of the SAC. Expression of L151R and N331K variants promoted mitotic slippage in HeLa cells and primary skin fibroblasts derived from carriers. Generation of mice carrying the N331K variant using CRISPR-Cas9 showed that, although homozygous N331K mice were nonviable, heterozygotes displayed accelerated oncogenicity of *Myc*-driven cancers. These findings highlight an unappreciated role for *CDC20* variants as tumour-promoting genes.

Oral iron supplementation shapes the gut microbiota recovery and promotes carcinogenesis in APC$^{min/+}$ mouse

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Introduction: Colorectal cancer (CRC) induces anemia in a large proportion of patients and is usually treated with oral iron supplementation. Surgery, the main treatment for CRC, is routinely accompanied by prophylactic antibiotics to avoid infection. However, the combined effect of antibiotics and luminal iron in the gut on the microbiota and intestinal homeostasis remains unknown.
Methods: Wild type (WT) mice were subjected to antibiotic treatment followed by oral iron supplementation at different concentrations. The recovery of the gut microbiota was assessed by 16S rRNA sequencing. Short-chain-fatty-acid (SCFA) concentrations were also assessed in the stool. In addition, APC$^{Min/+}$ mice (CRC model) received fecal microbiota transplantation (FMT) using samples from anemic CRC patients, followed by oral iron supplementation at different concentrations. Tumor burden and gut microbiota composition was assessed by 16S rRNA sequencing.
Results: In WT mice recovering from antibiotics under oral iron supplementation, three bacterial species characterized as CRC markers and/or initiators were more abundant and showed a lack of recovery of fecal concentrations of butyrate, an SCFA that inhibits cancer cell proliferation. APC$^{Min/+}$ mice that received FMT from anemic CRC patient under oral iron supplementation developed more colonic tumors and had a higher proportion of Ki-67-positive cells.
Conclusions: Gut microbiota recovery from antibiotic exposure under oral iron supplementation is frequent in CRC patients, but is also common in the general population. This study identifies possible deleterious effects of the concomitance of these two disruptive agents of the gut microbiota and may lead to modifications in the management of anemia in patients with CRC.

Clinical recessive mutations in PINK1 impact PINK1-TOM complex assembly and Parkin mitochondrial recruitment

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Although most cases of Parkinson's Disease (PD) are idiopathic, mutations in autosomal genes essential within mitochondrial quality control pathways have been linked to the etiology of the disease. PTEN-induced putative kinase 1 (PINK1) is an autosomal recessive gene for familial early onset PD encoding a Ser/Thr kinase that functions in a mitochondrial clearance pathway involving the E3 ubiquitin ligase Parkin, another PD associated gene. Upon mitochondrial damage, Parkin is recruited to the mitochondria and mediates the removal of damaged mitochondria via mitophagy. Importantly, the mitochondrial recruitment of Parkin hinges on the stabilization of PINK1 on the translocase of the outer mitochondrial membrane (TOM) complex. However, despite recent advances in our understanding of PINK1's structure, the biochemical elements within PINK1 that mediate the PINK1-TOM complex interaction and the ensuing stress signaling remains poorly understood. Many mutations in PINK1 have been linked to PD, with the pathophysiology of most remaining uncharacterized. Our work has uncovered elements of the biochemical basis of PINK1-TOM complex formation inspired through the study of some PD-related mutations in the N-terminal domain of PINK1. Using biochemical and cell biological approaches in human immortalized cell lines, we demonstrate that several N-terminal domain mutations impair mitochondrial accumulation of PINK1, the formation of the PINK1-TOM complex, and subsequent Parkin mitochondrial recruitment. These lines of evidence suggest that this PINK1-TOM interaction is crucial for PINK1 mediated mitophagy upon mitochondrial stress. Further research could investigate potential ways to further stabilize PINK1-TOM interactions to promote complex formation and rescue mitophagy in relevant PD patients.

The modulation of intestinal inflammation by *Parabacteroides goldsteinii* in experimental murine colitis

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Inflammatory bowel diseases (IBDs), comprised mainly of Crohn's disease and ulcerative colitis, are characterized by chronic and relapsing intestinal inflammation. Though the etiology of IBDs is not entirely understood, an imbalance of the gut microbiota is likely one contributing factor, making it a potential target for the treatment of IBDs. This study aims to examine how the potential probiotic *Parabacteroides goldsteinii* modulates intestinal inflammation in the context of experimental colitis.
Wild type C57BL/6 mice were divided into three groups. One group was given phosphate-buffered saline (PBS) via oral gavage without any further treatment. The second was treated with 1% dextran sodium sulfate (DSS) for twelve days induce colitis and given PBS every three days. Finally, the third was also treated with 1% DSS for twelve days and given *P. goldsteinii* via oral gavage every three days. Weight loss, stool consistency, and intestinal bleeding were monitored and were used to calculate the Disease Activity Index (DAI) daily. Postmortem, colon length was measured.
Colonization was confirmed at day three after gavage by real-time PCR. Preliminary results show that DSS-treated mice lost significantly more weight by day twelve than mice colonized with *P. goldsteinii*. Additionally, on days eight and twelve, mice colonized with *P. goldsteinii* had significantly lower DAI scores than mice that received only PBS. Finally, mice colonized with *P. goldsteinii* had significantly longer colons than those that received only PBS.
The preliminary data suggests that *P. goldsteinii* may attenuate inflammation in mice treated with DSS, as indicated by lessened symptom severity.

Investigating the cancer-cell extrinsic pro-tumourigenic influence of mutant p53 in Li-Fraumeni Syndrome

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Li-Fraumeni Syndrome (LFS) is a cancer predisposition syndrome associated with germline *TP53* mutations and an elevated lifetime cancer risk. The majority of *TP53* mutations code for missense proteins associated with oncogenic gain-of-function (GOF) properties. One such GOF of mutant p53 (mutp53) is the release of a pro-malignant secretome.

We hypothesize that in LFS, mutp53 promotes a systemic, pro-tumourigenic state through the release of a pro-inflammatory secretome. Our lab has evidence to support this, showing that subcutaneously injected tumours grow to be larger in LFS mice with the GOF R172H mutation ($p53^{R172H/WT}$, $p53R172H^{/R172H}$) compared to p53-wildtype ($p53^{WT/WT}$) littermates. Furthermore, cytokine expression analysis of plasma from $p53^{R172H/WT}$ and $p53^{WT/WT}$ mice revealed that mutp53 mice exhibit higher levels of pro-inflammatory cytokines. We further co-injected immunocompromised mice with fibroblasts induced to express GOF mutp53 with cancer cells and showed that mutp53 induction promoted tumour growth.

Mutp53 activates mTORC1 and anabolic metabolism. Rapamycin, an mTORC1 inhibitor, delays tumour onset and extends lifespan in LFS mice. We hypothesized that mTORC1 activity and resultant increases in anabolic rate are determinants of cancer risk in LFS and that mutp53's oncogenic influence might be diminished through mTORC1 inhibition. We pre-treated $p53^{R172H/WT}$ mice with systemic rapamycin prior to tumour challenge. Rapamycin diminished subcutaneous tumour growth in $p53^{R172H/WT}$ mice, suggesting that mutp53's paracrine influence is mTORC1-dependent.

We have shown that mutp53 promotes tumourigenesis in a cancer cell-extrinsic manner and that this oncogenic influence is dependent on mTORC1. Investigation of underlying mechanisms might reveal additional pharmacology for reducing cancer risk in LFS.

The gut microbiota dictates anastomotic healing in colorectal cancer surgery

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Background: The management of colorectal cancer (CRC) consists of a surgical resection, followed by a reconnection, or "anastomosis", of the remaining bowel ends. Up to 30% of patients may present a poor healing of the anastomosis, and anastomotic leak (AL), a major complication. Our objective is to investigate the possible role of the gut microbiome in anastomotic healing in patients with CRC.
Methods: Preoperative fecal samples were collected from CRC patients undergoing surgery. Fecal microbiota transplantation (FMT) was performed in mice using samples from CRC patients with and without AL. Mice were then subjected to colonic surgery. After 6 days, anastomotic healing and the gut barrier were assessed. The gut microbiota of patients and mice were compared to detect potential differences between the groups.
Results: Mice colonized by FMT with the microbiota of donors with AL displayed poorer healing of the colonic anastomosis macroscopically, and a weaker gut barrier after surgery. The anastomotic wounds of mice receiving the microbiota of AL donors displayed poor extracellular matrix formation after surgery. This was accompanied by higher expression of collagenolytic enzymes, indicative of collagen degradation. Several bacterial species were differentially abundant between the two groups and were associated with the healing process. Mechanistically, vital repair mechanisms were shown to be impaired by the microbiota of patients with leak.
Conclusions: These results suggest a causal role for the gut microbiota in colonic healing after surgery in patients with CRC. Specific bacterial species and their mechanisms of action were identified.

Modulation of gut microbiota to prevent anastomotic tumors and distant metastasis in colorectal cancer surgery

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Background: Colorectal cancer (CRC) recurrence is a leading cause of mortality worldwide. It has been suggested that poor anastomotic healing and leakage (AL) after surgery allows cancer cells to implant at the anastomotic site thereby increasing the risk of local cancer recurrence and metastatic spread. In previous studies, we showed that inulin, a well-known prebiotic improves anastomotic healing by strengthening the gut barrier. Here we further investigated the relationship between promotion of postoperative intestinal healing using prebiotics and anastomotic cancer local implantation and dissemination.
Methods: A retrospective review of AL and non-AL cases after CRC surgery was performed. The effect of dietary supplementation with inulin on the occurrence of local anastomotic tumors was assessed in a mouse model inoculated with tumor cells directly in the gut lumen after surgery. We also investigated in mice whether inulin may prevent metastatic spread and growth of tumor cells in the liver by transplanting CRC cells surgically into the spleen.
Results: Patients experiencing AL displayed significantly lower overall survival and more cancer recurrence and progression compared to non-AL patients. Poor anastomotic healing in mice led to larger anastomotic tumors and peritoneal cancer dissemination. Inulin supplementation significantly inhibited local tumor implantation and metastatic spread and increased the production of butyrate, a metabolite that impedes cancer growth.
Conclusions: AL was associated with worse oncological outcomes in patients and mice. Inulin was shown to reinforce the gut barrier, decrease the implantation of cancer cells at the anastomosis, and to prevent tumor dissemination and progression of liver metastasis.

Augmenting Anti-Cancer Immunotherapy by Targeting the non-Receptor Tyrosine Kinase Fes

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Cancer immunotherapy is an emerging pillar of treatment, but it still faces many barriers due to the immunosuppressive nature of cancer. The non-receptor protein tyrosine kinase Fes, abundantly expressed in antigen-presenting cells (APCs), has been shown to dampen innate immune responses by inhibiting Pattern Recognition Receptor (PRR) signaling required for inflammatory cytokine production. PRRs also bind damage associated molecular patterns released upon induction of immunogenic cell death (ICD). Fes may guard against autoimmunity or endotoxic shock in non-cancer contexts; however, in cancer, its functions in APCs may include attenuating priming of adaptive immune responses, thus acting as an additional barrier to effective cancer immunotherapy. We show that following stimulation of bone marrow derived macrophages (BMDMs) with a PRR agonist, *fes*$^{-/-}$ BMDMs have stronger PRR signaling compared to wildtype BMDMs. Using syngeneic mouse engraftment models of E0771 triple negative-like mammary carcinoma and B16-F10 melanoma, we show increased tumour control and survival in *fes*$^{-/-}$ mice compared to wildtype mice, which is further enhanced by doxorubicin treatment (a potent inducer of ICD). E0771 tumours and spleens isolated from these treated mice showed a genotype-dependent increase in NK and T cell activation in *fes*$^{-/-}$ mice, which was further enhanced by doxorubicin treatment. Tumor bearing *fes*$^{-/-}$ mice also displayed a higher degree of PD1-positive NK and CD8^{+} T cells and demonstrated greater tumor control and survival when treated with anti-PD1 antibody than IgG-treated *fes*$^{-/-}$ mice. Together, these results provide strong rationale for targeting Fes in innate immune cells to enhance cancer immunotherapy.

RNA binding protein dysfunction contributes to prodromal neuronal injury in models of multiple sclerosis

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Background: Multiple sclerosis (MS) is a chronic neurodegenerative disease in which dysfunction of the RNA binding protein (RBP) heterogenous nuclear ribonucleoprotein A1 (A1) has been shown to contribute to neuronal injury. Recent evidence indicates a prodromal period in MS, consisting of early signs/symptoms occurring before the clinical onset of disease, in which increased medical visits, lowered cognitive performance, and evidence of neuronal injury are observed. The contribution of A1 dysfunction to the MS prodrome is unknown.
Methods: Using the experimental autoimmune encephalomyelitis (EAE) model of MS, spinal cord neurons were analyzed by immunohistochemistry for markers of RBP dysfunction, and neurodegeneration prior to (mimicking the MS prodrome) and at the onset of clinical symptoms (5-, 8-, and 11-days post immunization (DPI) and symptom onset).
Results: Compared to naïve, spinal cord neurons from mice 11DPI demonstrated increased A1 nuclear-cytoplasmic mislocalization (*$p < .01$), a feature of its dysfunction, which remained elevated at symptom onset. Similarly, immunoreactivity for SMI-32, a marker of neuronal injury, was elevated in mice 11DPI (*$p < .05$), which persisted to symptom onset. There was a trend of decreasing neuronal cell bodies leading up to, and a significant increase in the expression of necroptosis markers (P-MLKL), a cell death pathway (*$p < .001$) at, the onset of clinical symptoms.
Conclusions: These data identify RBP dysfunction as a potential contributor to prodromal neuronal injury in an animal model of MS, which may be targeted to prevent permanent neurological decline in persons living with MS.

Somatic mutations underlie chemotherapy induced cardiotoxicity

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Background: Childhood cancer survivors (CCSs) have a 7-fold increased risk of cardiovascular death from chemotherapy exposure. Anthracyclines, a class of chemotherapy prescribed to >50% of childhood cancer patients, can lead to irreversible heart damage. This condition is known as anthracycline-induced cardiotoxicity (AIC). Unfortunately, current diagnostic and treatment options for AIC patients are limited.
Methods: We whole genome sequenced patient-matched blood and heart samples from CCSs to understand the genotoxic impact of anthracyclines on different normal tissues.
Results: We found that the blood and heart have distinct mutational profiles due to anthracycline exposure. In addition, we have identified potential anthracycline-associated mutational signatures, highlighting mechanisms by which this chemotherapy agent causes genomic damage.
Conclusions: This is the first genome-wide characterization of somatic mutations underlying chemotherapy-induced cardiotoxicity. It advances our understanding of the mechanisms of AIC and heart-specific biomarkers of cardiotoxicity. Ultimately, my goal is to alleviate the cardiac sequela of cancer therapy and ensure that CCSs are lifetime survivors.

Structure, immune evasion, and receptor binding of the highly mutated SARS-CoV-2 Omicron BA.1/BA.2 spike glycoproteins

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Background: The BA.1 sub-lineage was the first of the Omicron (B.1.1.529) SARS-CoV-2 variants to establish global dominance in early 2022 and was replaced by the BA.2 sub-lineage shortly thereafter. Both variants possess highly mutated spike glycoproteins with distinct mutational signatures. We hypothesized that these mutations could threaten humoral immunity and impact receptor engagement via structural changes. Therefore, we performed a systematic study to unravel the structural, functional, and antigenic consequences of these mutations to better inform treatment and vaccination efforts.
Methods: We assessed neutralization of the ancestral (D614G), BA.1, and BA.2 pseudo-typed viruses by sera from 205 individuals with diverse vaccination and sequence-confirmed infection histories. Spike proteins corresponding to the BA.1 and BA.2 Omicron sub-lineages were investigated by cryo-electron microscopy (cryo-EM), receptor binding, and antibody binding analyses.
Results: Both BA.1 and BA.2 pseudotypes displayed evasion of serum neutralization from individuals with vaccine, convalescent, or hybrid immunity. We observed evasion of BA.2 pseudotypes by BA.1 convalescent sera, suggesting an antigenic drift. Accordingly, antibodies isolated from BA.1 convalescent patients exhibited decreased BA.2 spike protein binding relative to BA.1. Structural comparison between BA.1 and BA.2 spike proteins highlights a dramatic reorganization of the BA.2 amino terminal domain. Analysis of receptor-spike interfaces revealed a network of interactions established by mutated residues which balance antibody evasion while maintaining or enhancing affinity for both human and murine receptors.
Conclusion: Our analysis identifies structural and functional mechanisms underlying the antigenic drift in the rapidly evolving Omicron variant landscape, providing important insights as vaccine formulations are updated to target these variants.

HIV-1 downmodulates SLAM ligands to evade NK cell responses

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NK cells are the first to initiate antiviral immune response and are crucial mediators to clear HIV-1 infected cells. NKs effector functions are activated by the engagement of the Fc-gamma receptor (FcγRIIIa/CD16) and/or activating/co-activating receptors (aR). HIV-1 accessory proteins Nef and Vpu contribute to NK cell evasion by downmodulating ligands of aR, such as NKG2D, DNAM-1 and NTB-A. NTB-A belongs to the Signaling Lymphocyte Activating Molecule (SLAM) family, which increases and maintains NK cell effector functions. Vpu-mediated cell-surface downregulation of NTB-A prevents NK cell degranulation via homophilic interaction. This contributes to prevent the elimination of infected cells by NK Antibody-Dependent Cellular Cytotoxicity (ADCC). Considering the role of SLAMs in stimulating NK cell functions, we evaluated the modulation of CD48, the ligand of the SLAM receptor 2B4, by HIV-1. Using a panel of infectious molecular clones defective or not for Nef and/or Vpu expression, we found that both proteins act in concert to downregulate CD48. To better understand the implication of CD48 and NTB-A downmodulation, we used a redirection assay to study the role of their receptor in stimulating NK cell effector functions. We observed redundancy in the ability of NTB-A and 2B4 to promote NK cell degranulation. Lastly, using an ADCC assay, we validated the importance of NTB-A and CD48 downmodulation by HIV-1 in evading ADCC responses. These results uncover a new evasion mechanism by HIV-1 to escape NK cell effector functions and suggest a model where downmodulation of multiple SLAM ligands is required to fully evade NK cell responses.

Characterization and prevention of radiation-induced malignancies in Li-Fraumeni Syndrome

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Li-Fraumeni Syndrome (LFS) is a genetic disorder associated with a significant risk of early-onset cancer. This condition is driven by germline mutations in the TP53 gene which has a broad spectrum of functions including the transcriptional regulation of the radiation response. Given that aberrant TP53 function contributes to radiation vulnerability, there is caution with the use of radiotherapy to treat primary LFS tumors in order to prevent the formation of secondary, radiation-induced malignancies. To this end, therapeutic options for LFS are often limited, posing substantial constraints for the treatment of patients who may otherwise benefit from primary tumor irradiation. Metformin, a commonly prescribed anti-diabetic drug, is associated with lower cancer incidence in populations worldwide. Recent studies have shed light on the potential for metformin to protect against radiation injury in normal tissue, as well tumor development in LFS models; hence, **we hypothesize** that metformin can reprogram the radiation response and delay the onset of radiation-induced malignancies in LFS. To characterize the effects of metformin on normal tissue biology and tumor development following ionizing radiation in LFS, mice harboring a hotspot *Tp53* mutation are treated with metformin prior to receiving localized irradiation. Skin tissue is collected longitudinally for transcriptomic profiling, and tumor development is monitored in a second cohort. Skin and tumors are sequenced to characterize the effects of metformin on the biology of radiation-induced LFS tumors. This work will advance our understanding of the chemopreventive effects of metformin, with the potential to broaden the treatment options available to LFS patients.

Gardnerella subgroup contributions to the cervicovaginal microbiome and immune milieu

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Background: Bacterial vaginosis (BV) remains a common yet poorly understood condition with limited interventions and high recurrence rates. Cervicovaginal microbiomes associated with BV have been linked to increased risk to HIV and pregnancy complications – highlighting the need to solve the BV-enigma. *Gardnerella* species have been implicated in the pathogenesis of BV, although their effects on mucosal immunity remain unclear. Here microbiome analysis using *cpn*60 barcoding was used to resolve four *Gardnerella* subgroups and additional bacterial taxa in cervicovaginal secretion samples to examine microbiome associations with mucosal immune responses and BV in a longitudinal cohort.
Methods: Cervical cytobrush and cervicovaginal secretion samples were collected across multiple visits from a longitudinal cohort of Kenyan women at low risk to HIV (N=41). BV was diagnosed using the Nugent criteria. Cervicovaginal secretions were used for microbial profiling and cytokine quantification. Cervical cytobrush samples were used to measure $CD4^+$ T-cells and activated subsets. Linear mixed models were used for analysis.
Results: Non-*Lactobacillus* dominant microbial communities were associated with increases in pro-inflammatory cytokines ($p<0.05$). Divergent associations emerged with the CXCR3 chemokine IP-10, whereby *Gardnerella* subgroup A dominant and polymicrobial communities were associated with reduced levels of this chemokine ($p<0.0001$). These communities were also more associated with Nugent-BV compared to the other *Gardnerella* subgroup dominant microbial communities. Significant associations between microbial communities and total T-cell counts and related activated subsets were not identified.
Conclusions: These findings further our understanding of *Gardnerella* contributions to the cervicovaginal microbiome and mucosal immunity – critical to solving the BV enigma.

Chitinase-3 Like 1 creates an immunosuppressive tumor microenvironment that supports breast cancer progression

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Breast cancer remains the most prevalent cancer and a major cause of mortality affecting women worldwide. Chemotherapy along with radiation and surgery have long been the standard of care for breast cancer patients. However, devastating side effects, resistance to treatments and relapse are major concerns for patients and physicians. Recent rise of immunotherapies has revolutionized cancer care and brought new hope of using the immune system to halt breast cancer progression.
Recently, our lab showed that that the transcription factor Signal Transducer and Activator of Transcription 3 (Stat3) is dispensable for tumor initiation, but essential for suppressing the immune system and promoting tumor progression. Transcriptional profiling of Stat3 WT and knockout mice indicates that the secreted cytokine Chitinase-3-like- 1 (Chi3l1) is the mediator of Stat3-induced immunosuppression. Elevated levels of Chi3l1 are correlated with poor prognosis and worse outcomes for breast cancer patients.
Our data demonstrates that Chi3l1 is a direct Stat3 target that is secreted by hyperplastic epithelial cells to reprogram the tumor microenvironment. Chi3l1 knockout mice exhibit a severe defect in tumorigenesis and slower tumor progression as a result of an active anti-tumor immune response that clears early hyperplastic lesions. Moreover, RNAscope analysis revealed an increase in interferon γ producing CD4+ T helper cells. Increased T cell infiltration and interferon signaling contributed to an increase expression of the checkpoint PD-L1, raising the possibility for combinational treatments. Thus, our results indicate that Chi3l1 is a major immunosuppressive cytokine that promotes breast cancer progression by inhibiting T cell recruitment and activation.

Methylation quantitative trait loci (mQTL) in the human placenta

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Background: Placental dysfunction is implicated in various adverse pregnancy outcomes such as maternal pre-eclampsia and fetal growth restriction. The polygenic nature of these complex diseases may have prevented genome-wide association studies from identifying reproducible genetic variants. An approach to enrich for loci functional inferences is to integrate genetic data with DNA methylation (DNAme) data through a quantitative trait loci (QTL) approach.
Method: SNP genotype and DNAme data were obtained from two independent cohorts: the United States-based NICHD cohort, and the Vancouver EPIC cohort. Data were collected using the SNP array and Illumina 450K or 850K array. Standard preprocessing and QC steps were applied, with the notable exception that we retained SNPs that deviated from Hardy-Weinberg equilibrium (HWE). Cis-mQTLs were identified via the MatrixeQTL package.
Results: In the NICHD cohort (n = 289 placentas), 75,286 mQTLs were identified (FDR<0.05, delta beta > 0.075), 38.5% of which were located on chromosome 6 (contains *HLA* genes). SNPs that deviated from HWE accounted for 2.78% the mQTL hits, and were retained as their genotype frequencies were consistent with population substructure rather than genotyping error. mQTLs were enriched in enhancer regions and depleted in promoters. Gene set enrichment analysis showed immune-related pathways. In the Vancouver cohort (n = 47), we replicated 21.0% of these hits.
Conclusion: Methylation quantitative trait loci (mQTL) are widespread in human placentas from healthy pregnancies. Immune-related genetic variants may play an important role in placental gene expression variation. Future work will test the association of these loci with pregnancy outcomes.

Improved expansion of induced pluripotent stem cell-derived hematopoietic progenitors cells using stem cell agonist cocktails

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Induced pluripotent stem cells (iPSC) are a renewable and scalable cell source to generate patient-specific induced hematopoietic progenitor cells (iHPC) for diverse applications. A bottleneck hindering translation is the inefficient production of iHPC to meet clinical requirements. Stem cell agonist cocktails (SCAC) have been developed and shown to improve expansion of cord-blood hematopoietic stem cells (HSC). SCAC promote strong expansion through the additive and synergistic effects of four small molecules. We hypothesized that supplementation of iHPC cultures with SCAC would increase the yields of iHPC. We also wished to determine whether GAS6, a growth factor shown to promote expansion of HSC cultures, could increase expansion. First, we generated $CD34^+CD43^+$ iHPC at high purity (≥80%) using the monolayer differentiation strategy. Next, we assessed iHPC expansion over two weeks in the presence or absence of four SCAC (X2A, SM6, SMA or X2A). All SCAC had a significant impact on iHPC growth, with iHPC expanded by 6- (SMA and UM171), 15- (SM6) and 25- (X2A and X2A+GAS6) fold while retaining high levels of $CD34^+$ $CD43^+$ expression (n = 2). The frequencies of $CD34^+CD45^+CD45RA^-CD90^+$ cells, normally enriched in HSC, were also higher with SCAC supplementation. The multilineage potency of the expanded iHPC was assessed using the colony-forming unit assay. All SCAC-expanded iHPC gave rise to erythroid and granulocyte-macrophage progenitors. Although expansion protocols are being refined, these results suggest that SCAC improves iHPC expansion while retaining potency. Optimization of iHPC expansion and subsequent immune cell derivatives yields is key to advancing scalable iPSC therapy products.

Perinatal iron deficiency alters nephrogenesis and patterns of cellular senescence and apoptosis in developing kidneys

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Background/Purpose: Iron deficiency (ID) commonly occurs during pregnancy, resulting in intrauterine growth restriction and altered organogenesis. Previously, we have shown that adult offspring exposed to perinatal ID exhibit renal dysfunction and hypertension. Here, we sought to assess how perinatal ID may alter kidney development through cellular senescence and apoptosis.
Methods: Eight-week-old Sprague Dawley dams were fed an iron-restricted (3-10 mg/kg) or iron-replete (37 mg/kg) diet prior to and throughout gestation. Offspring were euthanized and kidneys were collected on postnatal day (PD)1 and PD28. Apoptosis was assessed by TUNEL and Caspase 3 activity assays. Cellular senescence was assessed via senescence-associated β-galactosidase (SA-βGal) activity. Nephron endowment was assessed with an acid maceration protocol.
Results: ID resulted in neonatal anemia, decreased birth weight, and decreased kidney weight in box sexes ($P<0.05$). Nephron endowment was reduced by 20% in male perinatal ID offspring ($P<0.001$), but was unchanged in female offspring; thus, nephron density per mg kidney tissue was increased by perinatal ID in females far greater than in males ($P<0.001$). On PD1, TUNEL staining and caspase 3 activity was increased in male ($P=0.01$), but not female, perinatal ID offspring. Irrespective of sex, perinatal ID exhibited reduced SA-βGal activity on PD1 ($P<0.01$), in contrast to PD28 where perinatal ID increased activity ($P<0.01$). On PD28, no differences were observed in apoptosis markers in male or female offspring.
Conclusions: Perinatal ID causes alterations in cellular health and survival in the developing kidney, particularly in males, which may play a role in altering nephron endowment and long-term function.

Apo-metallothionein exists primarily as a folded structure under physiological conditions

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For the first time, we report experimental data that reveal detailed structural properties of apo-metallothionein-3. Our research offers the use of widely applicable techniques such as mass spectrometry, protein modification reactions from both an equilibrium and kinetics perspective, as well as ion mobility mass spectrometry and molecular modeling calculations in determining the structure of a fluxional protein that is not able to be characterized by traditional methods such as NMR and X-ray crystallography. Human metallothioneins are cysteine-rich proteins that regulate essential metal concentrations and protect against toxic metals. Metallothionein isoform 3 is constitutively expressed in neurological tissue and protects the cell from oxidative stress, which is a key component of neurodegenerative disease progression. Due to the lack of formal structure, structurally significant data on the metal-free form of metallothionein-3 have remained inconclusive, despite the growing knowledge of substantial accumulation of these apoproteins in human tissue. We have determined that apo-metallothionein-3 cooperatively forms tightly compact structures under physiological conditions, important for rapid reaction with metals and protection of the constitutively expressed peptide prior to metalation.

CLINICAL RESEARCH

Unresolved Immune Dysfunction is Lethal in Both COVID-19 and non-COVID-19 Sepsis

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Background: Severe COVID-19 and non-COVID-19 pulmonary sepsis share pathophysiological and clinical features. To what extent they also share gene expression trajectories throughout hospitalization is unknown.
Methods: To compare gene expression trajectories of COVID-19 and non-COVID-19 sepsis patients in the intensive care unit (ICU), we performed temporal differential expression analysis on whole blood transcriptomic profiles from 47 COVID-19 and non-COVID-19 septic patients.
Results: Mortality, not COVID-19 status, was associated with the largest percentage of gene expression variability in the first week of ICU. Non-survivors had more than double the number of "persistent" genes (genes that stayed up/downregulated at both timepoints) when compared to survivors. These included persistently downregulated genes in the T-cell receptor signaling complex and persistently upregulated genes in interleukin-1 (IL-1), IL-4/13, IL-6, and TNFα signaling pathways, suggesting unresolved immune dysfunction in non-survivors irrespective of COVID-19 status. In survivors, these pathways were enriched at ICU admission but were no longer enriched a week later, suggesting resolution of these processes. Interestingly, COVID-19 and non-COVID-19 septic patients differed only at ICU admission, with higher interferon responses in COVID-19 patients, but became indistinguishable a week later at the gene expression level.
Conclusions: Transcriptional evidence of persistent immune dysfunction was associated with 28-day mortality in both COVID-19 and non-COVID-19 septic patients. COVID-19 patients and non-COVID-19 sepsis patients became highly similar on a gene expression level after one week in the ICU, suggesting a shared common end pathological process. These findings can inform development of potential therapeutics for both sepsis and COVID-19.

Association between vitamin B12 levels and subclinical neurological deficits: evidence to reconsider current healthy targets

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Vitamin B12 is an enzymatic cofactor critical for methylation and for isomerization reactions in amino acid catabolism and fatty acid metabolism. Patients with B12 deficiency classically have been described to evidence hematological as well as neurological deficits. The typical threshold for clinical B12 deficiency, 148 pmol/L, was calculated as three standard deviations below the mean B12 level of the population in the U.S.A, independently of clinical observations. We hypothesized that lower B12 levels within the specified "normal" range may still be associated with subtle neurological deficits. We enrolled 247 healthy volunteers (females: n = 155 [55%], age: 74 ± 6.5 years) with a mean B12 blood concentration of 408 pmol/L (as measured by automated chemiluminescence assay in the serum). Participants with a B12 level below the mean demonstrated an association between higher averaged multifocal visual evoked potentials latency (electrophysiological marker of myelin integrity) and lower B12 blood levels (P = 0.012). We also found an association for slower computer-based spatial processing speed with lower B12 values that is age dependent (P = 0.013). Interestingly, higher B12 levels correlated positively (*P*= 0.005) with higher blood tau levels (blood-based markers of axonal integrity). This correlation was only evident for holoHC (*P*= 0.019), the biologically inactive fraction of B12, but not the biologically available B12 on transcobalamin, holoTC. In conclusion, healthy elder subjects present subtle neurological changes at both ends of the B12 spectrum. This may implicate that our current understanding of optimal serum B12 may have to be revisited.

Tracking epilepsy-related gray matter atrophy across the lifespan: an ENIGMA study

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Rationale. Magnetic resonance imaging (MRI) analysis can measure brain atrophy in temporal lobe epilepsy (TLE) and idiopathic generalized epilepsy (IGE)[1]. We investigate further the association between aging and brain atrophy in the common epilepsies[2].
Methods. *Participants*. As part of ENIGMA-Epilepsy, we analyzed T1w MRI data in 885 healthy individuals, 769 and 113 IGE patients.
Young and old differences. Median age (35) divided participants between young and old. Cortical thickness and subcortical volume in TLE and IGE patients were compared to controls across both cohorts.
Sliding age-window analysis. Using a window range of ± 2 years from the age of interest the mean values for CT and SV regions were calculated for each age. The mean CT and SV values for each unique age were calculated, then multiplied by normally distributed weights to yield a weighted average for each brain region. This was repeated for every age of interest for TLE and IGE patients.
Results. The sliding age-window analysis revealed an accelerated decline with aging for TLE patients across cortical and subcortical regions and a steady decline for IGE patients. Older TLE patients showed greater reductions in frontal and parietal cortical volumes and contralateral hippocampal atrophy than controls (Fig 2A). In contrast, differences between younger TLE patients and controls were limited only to the ipsilateral hippocampus, thalamus, and bilateral parietal regions.
Conclusion. Age is associated with greater gray matter decline across a broad subcortico-cortical territory in epilepsy. The nonlinear progression in epilepsy throughout adulthood highlights the need for further distinction between younger and older patients.
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Eye-tracking based emotion identification in youth with cerebral palsy

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Emotions are a significant contributor to well-being. Motor impairments and the co-occurrence of neurological disabilities in individuals with cerebral palsy (CP) often lead to complex communication needs. To this end, many individuals with CP are unable to speak aloud their thoughts and feelings. Current eye-tracking research has published evidence connecting features of eye-movement to emotion; however, they have focused on typically developing (TD) populations. Individuals living with CP could benefit from eye-tracking technology, which has the potential to collect, interpret, and relay biological signals without direct physical interaction with the device. This study will involve the collection of eye-tracking data while individuals with CP view images to connect features of eye-gaze with each image's validated affective rating. The end goal of this research is to create an emotion recognition system that conveys emotions felt by individuals with CP to unfamiliar communication partners.

In this first phase of the project, both TD and CP cohorts will view pictures from the International Affective Picture System. Each image will be displayed for six seconds while eye-movement data is collected. Participants will then be asked to rate their emotions using the Self-Assessment Manikin, a three-part visual scale aiming to express the PAD dimensions of emotion. pleasure, arousal, and dominance. This process will be repeated for twenty images until the protocol is complete. Features such as pupil dilation, saccade velocity, and fixation duration will be statistically analyzed to differentiate amongst emotions. Several supervised machine learning techniques will be explored to automate this process.

Clinical interpretation of partial duplication involving neurodevelopmental genes

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Background : Chromosomal microarray is routinely used in the evaluation of neurodevelopmental disorders to investigate copy number variants (CNVs). However, CNVs encompassing neurodevelopemental (ND) genes partially are still a challenge to interpret in the clinical setting. We hypothesized that partial duplications of these genes are benign in contrary to deletions.
Methods : To explore this hypothesis, we studied the transmission of CNVs in the 2008-2016 cohort of microarrays from CHU Sainte-Justine. We compared the proportion of inherited and *de novo* CNVs by Fisher's test.
Results : There is no difference in the proportion of *de novo* between the partial duplications of ND genes (0%) and non ND genes (2%). The proportion of complete duplications of ND genes *de novo* (44%) is higher ($p=3.9x10^{-11}$) than the complete duplications of non ND genes (14%). The complete duplications of ND genes are more *de novo* (44%) than the partial duplication of ND genes (0%, $p= 1.6x10^{-5}$). In contrary, the proportion of *de novo* for complete (81%) and partial (74%) deletions of ND genes was similar and their comparison with the complete and partial deletions of non ND genes was statistically significant ($p= 1.3x10^{-23}$ and $p=1.7x10^{-13}$).
Conclusions : Complete CNVs of ND genes are more often *de novo* which suggest that they are also triplosensitive. The partial duplications of ND genes are rarely *de novo* and their transmission is similar to the non ND genes in contrary to the deletions. In conclusion, the partial duplications of ND genes can be interpreted as benign.

Estimating individual treatment effect on disability progression in multiple sclerosis using deep learning

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Disability progression in multiple sclerosis remains resistant to treatment. The absence of a suitable biomarker to allow for phase 2 clinical trials presents a high barrier for drug development. We propose to enable short proof-of-concept trials by increasing statistical power using a deep-learning predictive enrichment strategy. Specifically, a multi-headed multilayer perceptron is used to estimate the conditional average treatment effect (CATE) using baseline clinical and imaging features, and patients predicted to be most responsive are preferentially randomized into a trial. Leveraging data from six randomized clinical trials (n=3,830), we first pre-trained the model on the subset of relapsing-remitting MS patients (n=2,520), then fine-tuned it on a subset of primary progressive MS (PPMS) patients (n=695). In a separate held-out test set of PPMS patients randomized to anti-CD20 antibodies or placebo (n=297), the average treatment effect was larger for the 50% (HR, 0.492; 95% CI, 0.266-0.912; p=0.0218) and 30% (HR, 0.361; 95% CI, 0.165-0.79; p=0.008) predicted to be most responsive, compared to 0.743 (95% CI, 0.482-1.15; p=0.179) for the entire group. The same model could also identify responders to laquinimod in another held-out test set of PPMS patients (n=318). Finally, we show that using this model for predictive enrichment results in important increases in power.

A miR-340-5p signaling network promotes neuronal recovery following axonal injury

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The regeneration of damaged neurons in the mammalian central nervous system (CNS) is severely limited, owing to a decrease in the intrinsic regenerative capacity of neurons throughout development, and the presence of growth inhibitory substances in CNS lesions. This paucity of regeneration leaves individuals sustaining injury to the CNS (such as spinal cord injury) with lifelong impairments. In contrast, neurons in the dorsal root ganglion (DRG) retain regenerative capacity following injury, prompting inquiries into how CNS neurons can be reprogrammed to regenerate. Whereas some studies have focused on modulating individual gene targets to enhance axon regeneration, an alternative approach is to modulate multiple genes in neurons simultaneously. MicroRNAs (miRNAs) are small, non-coding RNA molecules that target and downregulate multiple mRNAs in concert, serving as powerful regulators of multi-gene programs in cells. We conducted a systematic literature search to identify existing single cell RNA sequencing (scRNA seq) datasets from regenerating DRGs. We applied a novel *in silico* approach to these datasets to predict miRNAs that regulate large numbers of regeneration-associated genes. Neurite regeneration is promoted by modulating the levels of four candidate miRNAs. Inhibiting miR-340-5p promoted the most dramatic regeneration *in vitro*, and this phenotype was preserved in the presence of growth inhibitory substances. MiR-340-5p regulates numerous pathways associated with cell survival, neurotrophin sensitivity, and neurite outgrowth. Inhibiting miR-340-5p upregulated TrkB, and sensitized neurons to BDNF, which improved neuronal survival following axotomy *in vitro. In silico* approaches can identify molecular targets for regeneration that are missed when only using experimental approaches.

Evaluating neurological impairment in critically ill adults on acute kidney replacement therapy: A feasibility study

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Background: Long-term kidney replacement therapy (KRT) and critical illness have been independently linked to acute and prolonged cognitive impairment, and structural brain pathology. Although the cause is unknown, poor regional cerebral oxygenation (rSO2) may be a contributing factor. We assessed the feasibility of exploring the association between intradialytic rSO2 and neurological outcomes.
Methods: We assessed feasibility of enrollment, data capture, and long-term follow-up. We enrolled patients who initiated continuous KRT (CKRT) or intermittent hemodialysis (IHD) in the ICU. rSO2 was monitored continuously during the first 72h of CKRT or throughout each IHD session. We measured acute neurological impairment by daily delirium screening using the Confusion Assessment Method (CAM-ICU-7), and long-term neurocognitive outcomes at 3- and 12-months using the Kinarm Standard Tests™, Repeatable Battery for the Assessment of Neuropsychological Status, and brain magnetic resonance imaging.
Results: Of 484 ICU patients, 26 met screening criteria. Two declined, and 13 met at least one exclusion criteria (neurological illness, outside of 12h enrollment window from KRT initiation). Eleven patients were enrolled. Eight died in ICU, one died two months after discharge, and one declined follow-up. Data capture rates were high: rSO2/vitals in ICU (91.3%), delirium screening in ICU (100%), demographics (100%). Longitudinal testing was completed in 50% of patients who survived to follow-up.
Conclusions: It is feasible to collect rSO2 and delirium data in critically ill patients undergoing KRT, but high mortality and difficulty with enrollment limits the number available for follow-up. Future studies should take this into consideration for sample size planning.

Cerebral autoregulation dysregulation as a mechanism underlying delirium: a precision medicine approach to ICU delirium

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Background: Cerebral autoregulation (CA) is a mechanism that acts to maintain consistent cerebral perfusion across a range of blood pressures. Impaired CA is associated with delirium. Although blood pressure maintenance within the target CA range reduces postoperative delirium rates in cardiac surgery, the impact of individualized blood pressure targeting to reduce ICU delirium has not been elucidated. Our goal was to determine whether derivation of personalized blood pressure targets (MAPopt) was feasible in ICU patients.
Methods: Critically ill adults were enrolled if they had shock and/or respiratory failure requiring invasive mechanical ventilation >24hrs. Patients' blood pressure and regional cerebral oxygenation were monitored for up to 72hrs. Patients were screened daily for delirium up to 30 days using the CAM-ICU. Impaired CA and MAPopt were determined using previously described algorithms to calculate area outside MAPopt.
Results: 113 patients were assessed. 80 (71%) patients experienced delirium. Mean (± SD) values over 72 hours were: duration of disturbed CA (209 ± 243 minutes), MAPopt (77.6 ± 9.2 mmHg), and proportion of area outside MAPopt (34.8 ± 11.0%). MAPopt targets did not differ based on delirium status, however, they were above the recommended target of 65mmHg in the majority (72%) of patients.
Conclusions: We demonstrate feasibility to calculate personalized MAPopt targets. In the majority of patients, optimal targets were higher than current management guidelines. The one- size-fits-all approach may put patients at risk of cerebral hypo- or hyperperfusion. This work paves the way for interventional studies assessing whether applying patient-specific MAP targets improves ICU delirium.

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Choosing a prosthetic valve in chronic dialysis: Survey of Canadian nephrologists, cardiologists, and cardiac surgeons

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Background: Little evidence guides prosthetic valve selection in patients receiving dialysis. Society guidelines provide minimal or no recommendations.
Methods: Nephrologists, cardiologists, cardiac surgeons and methodologists developed the questionnaire. Domains include: respondents' demographics, patient and physician involvement in decision making, and important risks and benefits of prosthetic valves. We report responses using descriptive statistics.
Results: We administered this survey to physicians affiliated with the division of nephrology, cardiology, or cardiac surgery across 6 Canadian institutions. Overall, 115 of 315 eligible physicians completed the survey (response rate: 36.5%). We asked all physicians how influential individual factors were when recommending a prosthetic valve. Physicians across specialties favoured mechanical valves when future reoperation would carry high risk, in presence of another indication for anticoagulation use, low bleeding risk, a history of warfarin use and good INR control, or good medication adherence. Similarly, physicians favoured bioprosthetic valves for older patients (>65 years old), high bleeding risk, increased frailty, possible future pregnancy, eligible for kidney transplant or future transcatheter aortic valve replacement, high risk of endocarditis, and history of calciphylaxis. Physician groups favoured factors similarly, with nephrologists more neutral in preference for one valve over the other.
Conclusion: Overall, we present 3 key findings. Most cardiologists discuss prosthetic valves and their options, whereas nephrologists leave this discussion to cardiac surgeons. Second, cardiologists and nephrologists are not involved or minimally involved in choosing a prosthetic valve in patients on dialysis. Third, cardiologists and cardiac surgeons discuss similar factors during prosthetic valve selection, except for future TAVR eligibility which more surgeons discuss.

Intrathecal versus alternate routes of delivery for HER2-targeted therapies for HER2+ breast cancer leptomeningeal metastases

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Background: Leptomeningeal metastasis (LM) is a debilitating condition associated with metastatic cancers, including breast cancer (BC). When oncogenic drivers are identified, targeted therapies (TT) represent an appealing therapeutic strategy. However, the efficacy of TT for LM is unknown as LM patients are routinely omitted from clinical trials.
Methods: We conducted a systematic review and meta-analysis of individual patient data to evaluate the effectiveness of HER2-TT in HER2+ BC LM in accordance with PRISMA guidelines. TTs evaluated included trastuzumab (intrathecal (IT) or intravenous (IV)), trastuzumab-emtansine, trastuzumab-deruxtecan, and lapatinib. Primary outcome was overall survival (OS).
Results: Of 7791 abstracts screened, 45 publications and a total of 117 patients were included in the final analysis. Patients receiving IT trastuzumab (N=87) exhibited a median progression-free survival (mPFS) and mOS of 7.0 and 7.2 months, respectively, while patients receiving IV trastuzumab (N=14) exhibited a mPFS and mOS of 8.08 and 10.0 months, respectively (PFS: P=0.5915, HR: 0.783, 95% CI: 0.455-1.446; OS: P=0.1812, HR: 0.815, 95% CI: 0.501-1.327).
Conclusions: Together, the results of this study demonstrate that HER2-TT is active in patients with HER2+ BC LM. Patients with HER2+ BC LM should not be treated with intrathecal trastuzumab outside of a clinical trial protocol, an assertion that is supported by the lack of demonstrable survival benefit alongside the morbidity and potential for additional adverse events associated with intrathecal therapy. Univariate and multivariate analyses will be presented.

CLINICAL EPIDEMIOLOGY

A versatile, fast and unbiased method for estimation of gene-by-environment interaction effects on biobank-scale datasets

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Current methods to evaluate gene-by-environment interactions (GxE) on biobank-scale datasets are limited due to constraints such as high data dimensionality (p>>n), weaker signals than marginal genetic and environmental effects, computational burden, and common sources of heritability biases. Our method, MonsterLM, enables multiple linear regression on blocks of up to 30,000 SNPs and their interactions to estimate GxE on genome-wide datasets without requiring assumptions about genetic architecture. We show that MonsterLM provides unbiased estimates of variance explained by GxE effects across a range of MAF and LD quantiles (MAF>0.01; LD r2 < 0.9) and is robust to common biases including varying SNP effect sizes and collider bias. We applied MonsterLM to the UK Biobank to test for GxE of waist-to-hip ratio (WHR) with eleven complex traits, including ten blood biomarkers and height (N=297,529-325,989), and two dichotomous disease traits (N=324,858-325,989). We identified significant genome wide GxE with WHR for eight biomarkers and both dichotomous diseases, with variance explained by interactions ranging from 0.009 to 0.071. Generally, >50% of GxE was attributed to variants without significant marginal association with the phenotype of interest. Conversely, 5% or less of variants contributed to >50% of GxE. We observed modest improvements in polygenic score prediction by additionally incorporating GxE for some biomarkers. Our results imply an important contribution of GxE to complex trait variance, driven largely by a restricted set of variants distinct from loci with strong marginal effects.

Impact of DNA methylation of breast adipose tissue on the efficacy of aromatase inhibitors treatments

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Aromatase inhibitors (AI) are the standard adjuvant treatment for breast cancer patients who have hormone-dependent tumors. However, a significant proportion of these patients do not fully respond to AI treatment. AI decrease estrogen levels by blocking their peripheral synthesis from androgens, which occurs primarily in adipose tissue in post-menopausal breast cancer patients. Factors related to adipose tissue function could therefore influence the response to AI treatment. DNA methylation is a reversible biological signal that regulates gene expression and cell functions, and thus, DNA methylation status of breast adipose tissue may affect AI efficacy. Following a prospective cohort study design, we recruited 150 women with invasive breast cancer receiving an AI as adjuvant treatment. Breast adipose tissues were sampled during surgery, fasting blood samples and anthropometric measurements performed before and after 6 months of AI treatment and prognostic factors retrieved from medical records. We will measure DNA methylation in breast adipose tissue using a genome-wide methylation assay (Illumina) and validate the findings using bisulfite sequencing PCR and real-time PCR. AI efficacy will be estimated using plasma estrogen levels measured with mass spectrometry. Associations will be estimated using multivariate generalized linear models. This translational research project will enable the identification of modifiable factors that affect AI efficacy and provide clinicians with a tool to assess each patient risk of recurrence prior to AI treatment and to tailor treatments accordingly. In addition, generated data will serve to identify new therapeutic targets for breast cancer treatment.

Factors Influencing Recurrence in Medial Breast Cancer after Skin Sparing Mastectomy and Immediate Breast Reconstruction.

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Background: Skin sparing mastectomy (SSM) with immediate breast reconstruction (IBR) has been established as a safe option for curative-intent surgical resection. Prior studies have shown that medial location of the primary tumour is associated with increased risk of local recurrence. The purpose of this study is to determine the factors associated with recurrence and survival in individuals with breast cancers located in the inner quadrants (medial) who underwent SSM with IBR.
Methods: A retrospective chart review was done on individuals with medial breast cancer who received SSM with IBR in British Columbia between 1980 and 2012 using the Breast Cancer Outcomes Unit Database, one of the largest North American databases dedicated to breast cancer treatment.
Results: Of 136 individuals with medial breast cancer undergoing SSM with IBR, with a mean follow-up duration of 19.25 years, 27.9% experienced local recurrence and 42.6% overall recurrence. Factors associated with recurrence were: T-stage (44.8 vs. 22.4% with T2 disease, p=0.02), transverse rectus abdominis muscle (TRAM) flap reconstruction (48.3 vs 29.5%, p=0.00395), prior breast surgery (87.9 vs 63%, p= 0.002), and prior radiation therapy (74.1 vs 38.5%, p<0.0001). LR was associated with higher mortality (OR 2.78 95%CI:).
Conclusions: For patients with medial tumors undergoing SSM with IBR, potential risk factors for recurrence are T-stage, TRAM flap reconstruction, prior breast surgery, and prior radiation therapy. Local recurrence is associated with poor survival.

The *Support-Pro* Online Platform: Healthcare Professionals Increased Knowledge to Support People with Type 1 Diabetes

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Background: Despite the high prevalence of type 1 diabetes (T1D), previous literature has shown that many healthcare professionals (HCPs) may not be aware of recent developments in T1D management and new treatment options. The *Support-Pro* online self-training platform was created to fill this gap. Using practical training modules, HCPs can acquire knowledge on up-to-date topics that are important to support people with T1D (PWT1D).
Methods: In this non-randomized, open label study, HCPs were asked to self-assess their knowledge level pertaining to diabetes management and care using questionnaires at baseline and after using the platform for 3 months (NCT04859205).
Results: 142 HCPs were given access to the *Support-Pro* platform and 66 participants (mean years of practice 14±10; 44% dietitians; 30% nurses; 23% pharmacist; 3% physician/ resident) completed the 3-month questionnaire. After 3 months on the platform, the percent of HCPs who felt their knowledge to be adequate to advise PWT1D significantly increased from baseline in the following areas: blood glucose monitoring (86% to 94%, $p=0.020$), technology (17% to 48%, $p=0.005$), pharmacotherapy (45% to 65%, $p=0.045$), nutrition (53% to 65%, $p<0.001$), acute (61% to 74%, $p=0.003$) and chronic (70% to 82%, $p=0.028$) complications prevention, and acute complications management (33% to 59%, $p=0.026$).
Conclusions: The *Support-Pro* online training platform is an effective tool to increase HCPs knowledge on diabetes management and care. It could lead to better care for PWT1D.

Timing of complications following carotid endarterectomy for symptomatic and asymptomatic carotid artery stenosis

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Background: Current decision-making for safe discharge following carotid endarterectomy is based on clinical judgment after routine overnight observation. The aim of this study is to examine the time to early postoperative complications and identify risk factors of early complications to assess the safety of implementing same-day discharge following carotid endarterectomy.
Methods: A retrospective cohort study of patients undergoing carotid endarterectomy from 2015 to 2020 at two academic hospitals was performed (n = 440). Detailed information regarding patient characteristics, primary complications including 30-day and 1-year death, stroke or transient ischemic attack (TIA), and secondary complications including 30-day myocardial infarction (MI), other cardiac complications, and reintervention were extracted.
Results: Of 440 patients, 383 (87.0%) patients presented with symptomatic carotid artery stenosis. There was no mortality within 30-days postoperatively and death within 1-year occurred in 4 (0.9%) patients. The overall incidence of 30-day and 1-year stroke/TIA was 9 (2.0%) and 11 (2.5%). Within 30 days postoperatively, MI occurred in 3 (0.6%), other cardiac complications in 2 (0.5%), and reintervention in 17 (3.9%) patients. Stroke/TIA within 24 hours involved 5 patients, of which 1 (20.0%), 3 (60.0%), and 1 (20.0%) occurred within the first 6 hours, 7-12 hour, and 13-24 hour intervals respectively. MI occurred once within 6 hours and another within 13-24 hours. All other cardiac complications (n = 2) and reintervention (n = 10) within the 24-hour window occurred in the first 6 hours.
Conclusion: Majority of complications occur within the first 12 hours postoperatively and it may be unsafe to discharge patients before that window.

Content validity for a patient-reported outcome measure assessing cough severity in patients with chronic cough

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Background: Cough severity represents an important subjective endpoint to assess the impact of therapies for patients with refractory or unexplained chronic cough (RCC/UCC). As existing instruments assessing the severity of cough are neither widely available nor tested for its measurement properties, we aim to develop a new patient-reported outcome measure addressing cough severity.
Methods: We generated an initial draft of items through a systematic survey of the literature and patient focus groups. Focus groups involved 16 adult patients with RCC/UCC and were analyzed using directed content analysis. Discussions among an international panel of 15 clinical experts in chronic cough led to consensus on a final set of items.
Results: We identified 61 eligible studies reporting on patients' experience with chronic cough. Data from both the systematic survey and patient focus groups provided 48 unique items arranged under broad domains of urge-to-cough sensations and cough symptom. Feedback from expert panel members confirmed the appropriateness of items and domains, and provided an additional subdomain related to cough triggers. The final conceptual framework comprised 51 items in the following domains: urge-to-cough sensations (subdomains: frequency and intensity) and cough symptom (subdomains: triggers, control, frequency, fit/bout duration, intensity, quality, and associated features/sequelae).
Conclusions: Our studies inform item generation and content validity of a novel patient-reported outcome measure for use in both health research and clinical practice. Future studies will address items and domains that are most important to patients for item reduction.

Association of Parity with Weight Loss in the Ottawa Hospital Weight Management Program

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Purpose: We aim to evaluate the association of parity and weight loss success in diet-adherent women in the Ottawa Weight Management Program.
Hypothesis: In our database of > 5057 patients, we hypothesize that women with one or more pregnancies (parity) will have a lower rate of weight loss compared to nulliparous counterparts.
Methods: This study contained 1626 women with previous full-term pregnancies who completed the 6-week Optifast 900®diet component of our behavioural program. A linear regression model assessed association of parity and weight loss. A Welch Two Sample t-test assessed the impact of parity on age of onset of obesity. Association of parity and ATP criteria/metabolic syndrome was determined with a Chi-squared test. A Spearman's rank correlation test assessed the relation between age at first pregnancy and age of onset of obesity.
Results: The age of onset of obesity and age of first pregnancy are uncorrelated (P=0.2). Older patients lost less weight (B=-0.02, P<0.001). Women with multiple full-term pregnancies had a higher age of onset of obesity (P <0.001 Mean=18.12, SE=0.28) and more weight lost (B=0.11, P=0.04). The number of children (multiparity) is trivial to the rate of weight loss (P= 0.96). Parous women are more likely to be diagnosed with metabolic syndrome (P=0.0008) or show an increased number of ATP criteria (P=0.006).
Conclusion: Parity is associated with an increased rate of weight loss in women. Our study is funded by CIHR.

Is infratentorial stroke associated with isolated brainstem death among patients suspected of neurologic death?

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Background: Isolated brainstem death (IBD) occurs in patients who have a clinical examination consistent with death by neurologic criteria (DNC) despite ancillary test evidence of preserved supratentorial perfusion. IBD it is not considered compatible with DNC in most jurisdictions, but there are currently no documented risk factors for this condition. Our objective was to investigate the association between infratentorial stroke and IBD among patients suspected of neurological death.
Methods: We enrolled consecutive adult patients admitted to the intensive care unit after a severe brain injury who were deeply comatose despite the absence of sedation in a prospective 15-site cross-sectional study on DNC determination (INDex trial). Patients underwent sequential clinical examination for DNC and a CT-perfusion scan. Infratentorial stroke was identified based on brain injury etiology and non-contrast CT scans, whereas IBD was diagnosed when the clinical examination was consistent with DNC and there was preserved supratentorial perfusion despite absent infratentorial perfusion. We built a generalized linear model with a logit link function and intercept random effects for participating site, controlling for potential confounders using inverse probability weighting based on a propensity score.
Results: Among 274 included patients, the median [IQR] age was 60.0 [47.0-69.0] years; 130 (47%) were female. Thirty (11%) patients had an infratentorial stroke. The prevalence of IBD was 1.8% (95% CI: 0.6-4.2%). Infratentorial stroke was associated with IBD (aOR: 16.81, 95% CI: 3.95-71.51).
Conclusion: Infratentorial stroke is associated with IBD among patients suspected of neurologic death, suggesting this may be a risk factor for IBD.

Elevated vascular transformation blood biomarkers in Long-COVID Indicate angiogenesis as a key pathophysiological mechanism

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Key Words: Long-COVID, Vascular Transformation, Angiogenesis, Biomarkers, Machine Learning

Background: Long-COVID is characterized by prolonged, diffuse symptoms months after acute COVID-19. Accurate diagnosis, targeted therapies, and suspected mechanisms for Long-COVID are lacking. We investigated vascular transformation biomarkers in Long-COVID patients.
Methods: A case-control study utilizing Long-COVID patients post-infection (median 98.5 days), with multiplex immunoassay measurement of sixteen blood biomarkers of vascular transformation, including ANG-1, P-SEL, MMP-1, VE-Cad, Syn-1, Endoglin, PECAM-1, VEGF-A, ICAM-1, VLA-4, E-SEL, thrombomodulin, VEGF-R2, VEGF-R3, VCAM-1 and VEGF-D.
Results: Fourteen vasculature transformation blood biomarkers were significantly elevated in Long-COVID outpatients, versus acutely ill COVID-19 inpatients and healthy controls subjects ($P<0.05$). A unique two biomarker profile consisting of ANG-1/P-SEL was developed with machine learning, providing a classification accuracy for Long-COVID status of 96%. Individually, ANG-1 and P-SEL had excellent sensitivity and specificity for Long-COVID status (AUC=1.00, $P<0.0001$; validated in a secondary cohort). Specific to Long-COVID, ANG-1 levels were associated with female sex and a lack of disease interventions at follow-up ($P<0.05$).
Conclusions: Long-COVID patients suffer prolonged, diffuse symptoms and poorer health. Vascular transformation blood biomarkers were significantly elevated in Long-COVID, with angiogenesis markers (ANG-1/P-SEL) providing classification accuracy of 96%. Vascular transformation blood biomarkers hold potential for diagnostics, and modulators of angiogenesis may have therapeutic efficacy.

Readiness assessments for youth with chronic health conditions transitioning from pediatric to adult care

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Background: The transition from pediatric to adult health care for adolescents and young adults (AYA) with chronic conditions is a vulnerable and complex time, associated with gaps in care, poor treatment adherence, and increases in emergency room visits. Various tools are available to assess AYA readiness (knowledge of illness and self-management skills) to transition, such as the Transition Readiness Assessment Questionnaire (TRAQ), that are intended to help identify who is most at risk. However, the readiness scales do not capture the differential challenges in accessing adequate care related to sociocultural, social determinants, and identity factors.
Proposed objective: To examine the association between TRAQ scores and health outcomes for AYA with chronic conditions and identify sociocultural, social determinant and/or identity factors associated with transition readiness.
Methods: A patient-oriented, mixed methods study will be conducted, with quantitative priority. This study will be guided by the social-ecological model of AYA readiness for transition (SMART) model to identify factors impacting transition readiness. This model refers to the reciprocal interactions between AYA and their surrounding systems, including family, caregivers, healthcare providers, and medical system. Data will be obtained from a cohort of AYA enrolled in the Transition Navigator Trial (TNT), an ongoing pragmatic randomized controlled trial evaluating the effectiveness of a patient navigator to decrease emergency room utilization in AYA undergoing transitions in care in Alberta.
Significance: Results from this study will generate new evidence regarding risk assessments of youth with chronic health conditions transitioning to adult care, with a focus on social-ecological factors.

Next-Generation Sequencing of Non-Small Cell Lung Cancer at a Quebec Health Care Cancer Centre

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Lung cancer is the leading cause of cancer death in both men and women. It is estimated that 30,000 Canadians will be diagnosed with lung cancer in 2022 and 20,700 will die from their disease. Quebec has the highest lung cancer mortality out of all provinces in Canada, believed to be caused by higher smoking rates. Molecular testing for lung cancer is standard of care due to the discovery of actionable driver mutations that can be targeted with tyrosine kinase inhibitors. To date, no detailed molecular testing characterization of Quebec lung cancer patients using next generation sequencing (NGS) has been performed. The aim of this study was to describe the genomic landscape of lung cancer patients (n = 997) who underwent NGS molecular testing at a tertiary care center in Quebec and to correlate it with clinical and pathology variables. Overall, our cohort had a substantially higher prevalence of KRAS mutations (39.2%) versus another Canadian NGS study (32.3%) and the MSK-IMPACT Caucasian cohort (24.6%). On the other hand, our cohort had less EGFR variants (16.1%) compared to the other Canadian study (24.2%) and the MSK-IMPACT study (29.1%). It remains important to assess institutional rates of actionable driver mutations to help guide governing bodies, fuel clinical trials and create benchmarks for expected rates as quality metrics.

Development of a National Synoptic Operative Report for Thyroid Surgery

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Background: A national review of narrative operative reports for thyroid surgery performed on benign and/or malignant disease showed no reporting consistency amongst providers. This study aimed to develop a nationally standardized synoptic operative report for thyroid surgery.
Methods: Canadian surgeons, radiation oncologists, endocrinologists, and pathologists who manage thyroid disease ranked reportable thyroidectomy elements on a 1–5-point Likert scale of importance. Items that scored ≥ 4.50 were automatically included while a national advisory committee (11 surgeons, 7 non-surgeons) voted on inclusion of items scoring 3.50-4.50 using the Modified Delphi Method. Inclusion was defined by 88% agreement after 2 rounds of voting and a multidisciplinary meeting finalized the synoptic operative report.
Results: 32 surgeons and 14 non-surgeons who manage thyroid disease in Canada ranked 136 reportable elements of which 17 items (≥ 4.50) met inclusion. Top ranked general items included type of procedure (4.93 +/- 0.25) and status of the recurrent laryngeal nerves (4.71 +/- 0.79). Cancer specific items such as invasion of structures (4.76 +/- 0.68), gross extrathyroidal extension (4.74 +/- 0.71), and residual macroscopic disease (4.74 +/- 0.71) were particularly favored. Voting by the national advisory committee added 7/70 elements that scored 3.50-4.50 including the status of Level VI dissections. A final multidisciplinary discussion removed 3 items resulting in a 21-item synoptic operative report of which 5 items are cancer specific.
Conclusions: We developed a nationally standardized synoptic operative report for thyroid surgery that may enhance user consistency, satisfaction, and benefit quality of postoperative patient care.

Risk stratification for hypertriglyceridemia using common genetic variation, BMI, and sex in 22q11.2 deletion syndrome

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Elevated triglyceride levels are a heritable and modifiable risk factor for cardiovascular disease. Mild-moderate hypertriglyceridemia is approximately twice as prevalent among individuals with a 22q11.2 deletion (40%) compared to the general population (22%). Using whole genome sequencing data available for an adult cohort of 157 individuals with a 22q11.2 deletion, we examined the genetic and phenotypic contributors of triglyceride and other lipid levels in this patient population who have an elevated baseline risk for mild-moderate hypertriglyceridemia. We found that common variant-derived lipid polygenic risk scores were significantly associated with their corresponding lipid level (all $p<0.01$) in individuals with a 22q11.2 deletion after adjusting for age, sex, BMI, and other relevant covariates. Furthermore, we found that among individuals with a top 50th percentile triglyceride polygenic risk score and who are obese (BMI≥30), 67.9% had mild-moderate hypertriglyceridemia compared to 39.7% of individuals without both these risk factors (odds ratio=3.18, $p=0.01$). The highest prevalence for mild-moderate hypertriglyceridemia was found in males with both these polygenic risk and obesity risk factors (76.9%), whereas the prevalence in females without both these risk factors (21.7%) dropped to approximately the general population level. Collectively, these results support the potential clinical utility of the triglyceride polygenic risk score for risk stratification of mild-moderate hypertriglyceridemia as part of a cardiovascular disease prevention strategy for individuals with an elevated baseline risk conferred by the 22q11.2 deletion.

TRANSLATIONAL RESEARCH

Extracting 3D prostate geometry using optically-tracked 2D transrectal ultrasound

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Transrectal ultrasound-guided prostate biopsy remains the standard of care for diagnosing prostate cancer in many low-resource healthcare settings where other imaging modalities, such as MRI, are not available. The technically challenging nature of capturing and interpreting transrectal ultrasound imaging limits the diagnostic utility of this navigation method. The objective of this research was to extend the diagnostic utility of transrectal ultrasound guidance during prostate biopsy using accessible technologies including optical tracking, deep learning and open-source software. A system capable of reconstructing the 3D geometry of a patient's prostate based on spatially-tracked 2D ultrasound images was designed, implemented and tested. The system used deep learning to segment the prostate in spatially-tracked transrectal ultrasound images, with the combined segmentations co-localized and used to generate a convex hull of the prostate. A user study was performed to assess the quantitative reconstruction performance of the algorithm, with participants completing a survey to capture their qualitative user experience. The system showed comparable results to a mechanically-tracked method of reconstructing the prostate, despite being implemented using substantially more accessible materials and software. The ability to visualize the 3D anatomy of the prostate in the absence of a 3D imaging modality, such as MRI, may assist clinicians in avoiding critical anatomy and better inform biopsy core placement. Low-cost navigational tools that extend the functionality of readily-available technologies have an important role to play in democratizing access to reliable prostate cancer diagnosis.

Identification and differential usage of a host metalloproteinase entry pathway by SARS-CoV-2 Delta and Omicron

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The severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) binds to angiotensin converting enzyme 2 (ACE2) via its spike glycoprotein (S), delivering its genome upon S-mediated membrane fusion. SARS-CoV-2 uses two distinct entry pathways: 1) a surface, serine protease-dependent or 2) an endosomal, cysteine protease-dependent pathway. In this study, we found that SARS-CoV-2 S evolved to expand its protease usage, rendering S sensitive to activation by TMPRSS13 and matrix metalloproteinases (MMPs). Importantly, we found that MMP-2 and MMP-9 played a role in activating SARS-CoV-2 S fusion activity for syncytia formation, cell-cell fusion and viral entry in cells expressing high MMP levels. MMP-dependent viral entry required cleavage at the S1/S2 junction in viral producer cells and differential processing of variants of concern S dictated its usage. We found that Delta S was efficiently processed and preferred the metalloproteinase-dependent entry when available. In contrast, the Omicron S had reduced processing, exhibited lower cell-cell fusion activity, and was unable to use the metalloproteinase-dependent viral entry. Altogether, we identified a MMP-2/9-dependent mode of activation of SARS-CoV-2 S. As MMP-2/9 are released during inflammation and severe COVID-19, they could exacerbate S-mediated cytopathic effects such as syncytia formation and expand tropism by allowing entry in serine protease deficient cells. Therefore, usage of the MMP-dependent viral entry by current and future circulating SARS-CoV-2 variants could have profound implications in disease severity, outcome, and potential sequelae following recovery. Targeting MMPs, serine proteases, and cathepsins may be useful to reduce SARS-CoV-2 infection and COVID-19 severity.

A bicortical neuroprosthesis to improve locomotor recovery following incomplete spinal cord injury: a preclinical study in the cat

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Introduction: We developed a neuroprosthesis whereby electrical microstimulation is delivered to the motor cortex during ongoing locomotion to improve motor recovery after an incomplete spinal cord injury. In the present study, we tested the impact of a bicortical neuroprosthesis in a preclinical cat model of large contusion SCI that paralyzes both legs, as an important step toward clinical translation.
Methods and Material: Electromyographic electrodes were implanted within six hindlimb muscles bilaterally and a 16-microelectrode array were lowered into layer V of each hindlimb motor cortex. In a second surgery, a spinal contusion was performed at T10 with a controlled impact force of 700 kdyne. Once cats recovered weight-supported locomotion, they were trained on a treadmill training for three weeks, with or without cortical stimulation. Intracortical stimulation was delivered with precise time resolution during the leg flexion phase of gait. Kinematic parameters during treadmill walking such as step height and foot drag were analyzed. We also rated success rates to clear obstacles during treadmill walking.
Results: Before SCI, stimulation of the leg motor cortex elicited various contralateral hindlimb movements. After SCI, phase-coherent cortical stimulation increased contralateral hindlimb flexion and reduced dragging during locomotion. Bicortical stimulation allowed generating a bilateral walking pattern. Preliminary data on four experimental cats and three control cats suggests that cortical neuroprosthesis therapy improves the performance on voluntary motor tasks that persist four weeks after stimulation is discontinued.
Conlusion: these preliminary results suggest that a bicortical neuroprosthesis may improve the motor recovery of patients with incomplete paraplegia. Further preclinical validation in the cat and other animal models is ongoing.

Continuous Subzero-Balance Ultrafiltration Extracts Several Inflammatory Cytokines during Pediatric Cardiac Surgery with Cardiopulmonary Bypass

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Background: Cardiopulmonary bypass (CPB) is associated with systemic inflammation (alternate complement pathway activation of C3a and C5a) in pediatric patients undergoing cardiac surgery. Intra-operative ultrafiltration has been hypothesized to remove inflammatory factors (< 66 kDa) from the patient's circulation during CPB. This study aimed to identify a comprehensive collection of inflammatory mediators extracted by continuous subzero-balance ultrafiltration (SBUF).
Methods: Pediatric patients undergoing cardiac surgery with CPB and SBUF were enrolled in a prospective observational cohort study. At the end of CPB, arterial blood (End-CPB Plasma) and ultrafiltration effluent samples (End-CPB Effluent) were analyzed with Luminex® to yield the concentrations of 39 inflammatory mediators from the complement, cytokine, chemokine, leukocyte adhesion and pulmonary vasoconstriction pathways. Sieving coefficients ([End-CPB Effluent Mediator] / [End-CPB Plasma Mediator] x 100%) were calculated to quantify the degree of mediator extraction. Results presented as median (IQR).
Results: Twenty patients were enrolled with an age of 4.0 (0.2–12.0) months, weight of 5.2 (3.4–8.1) kg and a spectrum of congenital heart diseases. Twenty-two mediators were extracted by SBUF with a range of Sieving coefficients (0.1%-1019%). Sieving coefficients for C3a and C5a were 1019% and 46% respectively. Mediator extraction by SBUF was significantly associated with molecular mass < 66kDa (Chi2 with Yates' correction = 16.0, $p < 0.0001$).
Conclusion: SBUF extracts twenty-two circulating inflammatory mediators from the complement, cytokine, chemokine, and leukocyte adhesion pathways throughout pediatric cardiac surgery with CPB. Further translational investigations are required and ongoing, to assess the clinical impact of this potentially therapeutic immunomodulatory mechanism.

Clinical activity of mitogen-activated protein kinase-targeted therapies in patients with non-V600 BRAF-mutant tumors

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Purpose: Non-V600 mutations comprise approximately 35% of all BRAF mutations in cancer. These oncogenic drivers can be classified into three classes according to molecular characteristics. Consensus treatment strategies for class 2 and 3 BRAF mutations have not yet been established.
Methods: We performed a systematic review and meta-analysis with published reports of individual patients with cancer harboring class 2 or 3 BRAF mutations, to assess treatment outcomes with mitogen-activated protein kinase (MAPK) pathway targeted therapy (MAPK TT) according to BRAF class, cancer type, and MAPK TT type. Coprimary outcomes were response rate and progression-free survival.
Results: A total of 18,167 studies were screened, identifying 80 studies with 238 patients who met inclusion criteria. This included 167 patients with class 2 and 71 patients with class 3 BRAF mutations. In both univariate and multivariable analyses, response rate and progression-free survival were higher among patients with class 2 compared with class 3 mutations, findings that remain when analyses are restricted to patients with melanoma or lung primary cancers. MEK ± BRAF inhibitors demonstrated greater clinical activity in class 2 compared with class 3 BRAF-mutant tumors than BRAF or EGFR inhibitors.
Conclusion: MAPK TTs have clinical activity in some class 2 and 3 BRAF-mutant cancers. BRAF class may dictate responsiveness to current and emerging treatment strategies, particularly in melanoma and lung cancers. Together, this analysis provides clinical validation of predictions made on the basis of a mutation classification system established in the preclinical literature. Further evaluation with prospective trials is needed.

Directed Acyclic Graphs; a Tool for Clinical Research in Surgery

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Surgical literature has become increasingly dependent on observational studies to answer important clinical questions due to the evolution of large electronic health record databases. However, associations presented in observational studies are prone to a number of biases that are not fully controlled for due to lack of available of data. A simple but sophisticated technique to address this issue that has caught momentum in health research is the use of causal directed acyclic graph (cDAG). cDAGs incorporate letters and arrows to describe causal relations between variables, that should or should not be adjusted for when quantifying a causal relationship between two variables. They are quick and easy to create and require minimal understanding of the statistical theory whilst representing data in a visual manner. It is a useful method for clinicians researchers to communicate their understanding of relationships between study variables to the research team.
We provide an easy-to-understand tutorial about cDAGs to clinicians who conduct or review observational studies. First, we introduce important principles as biases such as confounding, colliders, mediators, and selection bias through components of a cDAG (Fig. 1). Then, we work through a case study involving a hypothetical research question investigating the risk of hernia recurrence with mesh hernia repairs and discuss what biases may be present in such a study and how they would be represented in a cDAG (Fig. 2).

Fig. 1

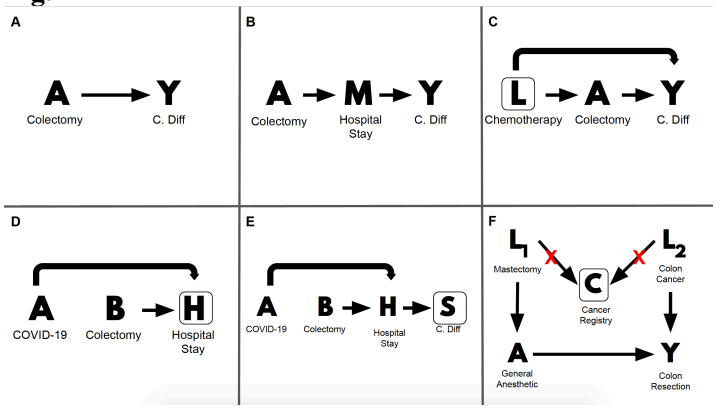
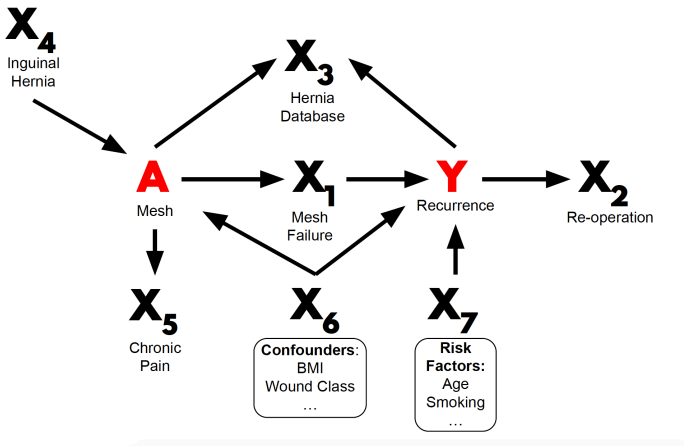


Fig. 2



Modulation by PCSK9 of the immune recognition of colorectal cancer liver metastasis

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Background: Colorectal cancer liver metastasis (mCRC) are refractory to immunotherapies though effective against other metastatic solid cancers. The proprotein convertase subtilisin/kexin type 9 (PCSK9), discovered by Dr. M. Chrétien's team in 2003, causes the internalization of the LDL receptor on hepatocytes, but could also internalizes the major histocompatibility complex class 1 (MHC-I) from the surface of cancer cells preventing tumor recognition by T lymphocytes. Our goal is to assess whether PCSK9 contributes to immune evasion of liver metastasis.
Methods: 1) Correlations between the oncological outcomes of 250 patients following mCRC resection and the expression of PCSK9 at a) intra-tumoral levels (RNAseq and immunohistochemistry), b) plasmatic levels (ELISA). **2)** Secretion of PCSK9 by cancer cell lines (ELISA) and impact of PCSK9 on MHC-I downregulation (in vitro, FACS). **3)** Proof of concept in humans studying the immunological characteristics of metastasis after preoperative chemotherapy combined or not with an anti-PCSK9.
Results: In RNAseq analysis of 52 resected mCRC, we observed that PCSK9 expression was lower in metastasis classified as immune-reactive compared to non-immune reactive metastasis. Unlike melanoma, cancer lines of the gastrointestinal tract secrete PCSK9. In vitro, the MHC-I of these lines does not seem downregulated by PCSK9. High plasmatic PCSK9 may be associated with poorer survival in patients with resected mCRC. We have developed an immunohistochemical labeling technique for PCSK9 on mCRCs.
Conclusions: Our study generates key dataset showing that PCSK9 blockade may enhance mCRCs' response to immune checkpoint inhibitors. Our results suggest an association between PCSK9 abundance and mCRC immuno-resistance.

Computational processing of routine EEG to predict seizure recurrence

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Background: The presence of interictal epileptiform discharges (IEDs) on routine EEG predicts seizure recurrence. However, 29–55% of routine EEGs do not show IEDs in patients with epilepsy (PWE). We aim to evaluate the performance of computational markers to predict seizure recurrence after routine EEG.
Methods: We selected all patients undergoing 30-minute EEG at the CHUM in 2018. Exclusion criteria were absence of follow-up after EEG, unclear epilepsy diagnosis at last follow-up, and electrical seizure(s) on EEG. Medical charts were reviewed for seizure(s) occurrence after EEG. EEGs were segmented into non-overlapping 10s windows. Several features were extracted for each electrode and each time window. The features capture distinct linear or non-linear properties of the EEG signal. We trained a boosted trees machine learning model on multichannel EEG segments to predict the occurrence of seizure(s) during follow-up. We averaged predictions over windows to obtain one prediction per EEG. We tuned hyperparameters with 5-fold cross-validation on a validation ensemble (80% of data) and evaluated the best model on a separate testing set (20% of data).
Results: 656 EEGs from 534 subjects were included; 345 (65%) were from PWE. 527 EEGs (80%) did not capture IEDs. Median follow-up was 92 weeks (IQR 40–126). The machine learning algorithm could predict seizure recurrence with above chance performance (area under the receiver-operating characteristic curve [AUC ROC] 0.64, 95%CI 0.59–0.69), even in absence of IEDs (0.62, 0.56–0.67).
Conclusion: A computational biomarker could potentially increase the prognostic yield of routine EEG in the clinical setting.

Matching violence risk assessment with tailored management in psychiatry: Usefulness of the DUNDRUM toolkit

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Most ward-based mental health workers are exposed to patient violence during their career, which may cause physical injury and lasting psychological issues. While most patients won't become violent during hospitalization, the roughly 23% who do will require tailored clinical interventions. Without a therapeutic approach, security responses to violence (i.e., restraints or seclusion) may lead to fear or distrust, compromising collaboration in care. These problems are magnified in secured forensic hospitals, which care for psychiatric patients involved with the justice system.

Violence risk assessment tools are crucial to evaluate clinical needs and then tailor a treatment plan to the patient – this is called "matching". Despite the plethora of assessment tools available, there still exists no validated method for matching. The DUNDRUM toolkit offers great promise for improving this process, with its design rooted in care planning and its optional patient self- assessments. This study will evaluate the tool's implementation for the first time in Canadian psychiatry, with an emphasis on clinical outcomes and facilitating shared patient-clinician management.

A mixed-method study will be conducted, first by interviewing professionals and patients to assess satisfaction with implementation methods and the acceptability of self-assessments. Second, through analyzing the DUNDRUM's effects on outcomes such as violence and seclusion rates in a forensic hospital. The predictive value of clinician and patient ratings will notably be compared.

This study answers international calls to move the risk assessment field into evaluating clinical outcomes and will inform the ongoing reorganization of forensic services in Quebec, which is based on the DUNDRUM.

Effect of Tet2-Deficiency on Neutrophil Immune Function

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Background: An estimated 660,000 Canadians have Clonal Hematopoiesis (CH), a blood disorder characterized by leukocyte dysfunction leading to increased risk of leukemia and cardiovascular disease. Emerging evidence suggests that CH also increases susceptibility to bacterial infections, but the mechanisms are not understood. Previous work by our lab showed that Ten-Eleven-Translocation methylcytosine dioxygenase 2 (*TET2*) mutations, which are common in CH, dysregulate neutrophil function. Thus we aimed to understand the link between Tet2-deficient neutrophils and elevated infection risk in CH.
Methods: We used the *Tet2*$^{flox/flox}$ *Vav-iCre* knockout mouse model to assess the impact of Tet2 deficiency. Neutrophils were isolated from bone marrow through immunomagnetic selection, and purity was validated with flow cytometry. Live cell imaging was performed to evaluate neutrophil phagocytosis and motility in response to challenge with *Staphylococcus aureus*. Bulk RNA sequencing was performed on purified neutrophils at baseline and after treatment with lipoteichoic acid from *S. aureus* to assess their transcriptomic response to bacterial pathogens.
Results: Tet2-deficient neutrophils show reduced phagocytic activity ($p = 0.034$, t-test) and motility ($p = 0.004$, t-test) in response to *S. aureus* challenge. RNAseq data showed that pathways related to chemotaxis, motility, and neutrophil development were downregulated Tet2-deficient neutrophils. Interestingly, anti-viral immune response pathways were upregulated in Tet2-deficient neutrophils.
Conclusions: These findings suggest that Tet2-deficiency impairs neutrophil immune effector function in response to bacterial pathogens such as *S. aureus* which likely contributes to the elevated risk of bacterial infection in CH. Overall, this study advances the understanding of how CH dysregulates immunity.

Design of Ultrasound-Augmented Tools for Instrumented Spinal Surgery Navigation

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Spinal navigation improves instrumented procedures but has outstanding limitations including a radiation, inaccessibility, and rigid-body registration. We propose replacing optical tracking with self-localizing tools that have ultrasound embedded in their sidewalls. Recently, Polymer-based Capacitive Micromachined Ultrasonic Transducers make manufacturing these tools feasible, but optimal design configurations are unknown. This work aimed to determine the geometric and algorithmic parameters for an effective device.
A computer simulation, based on a human lumbar-CT, evaluated the localization performance of 500 000 design configurations. We assessed five geometric parameters (guide diameter, guide length, number of ultrasound strips, number of elements per strip and array configuration) and three algorithmic parameters (state-estimation method, parallelization, and the cost-function). Algorithms included unscented Kalman filtering and optimization such as interior- point and quasi-Newton methods. Localization performance was characterized by spatial accuracy, convergence rate, tolerance to measurement uncertainty, capture range and localization range. Simulations demonstrate the new registration method is feasible and submillimetrically accurate. However, only certain configurations facilitate accurate pose estimation. The best localization algorithm used a multi-start interior-point optimization. A cylindrical cannula (diameter: 10mm, length: 150mm) with a curved nose and ten circumferentially arranged ultrasound strips (length: 30mm, 32 elements each) allows for fanned-out ultrasound directionality and performed best. The final design was validated with initial estimation errors and displacements up to the range of the ultrasound distance measurements.
The simulated accuracy suggests that physical prototyping will be successful and supports continued development. Future work will prototype the proposed design, improve the ultrasound physics simulation and integrate complimentary navigation.

Note: A preliminary version of this work was presented at the 2022 CAOS (Computer-Assisted Orthopaedic Surgery) Annual Meeting

Highly multiplexed imaging reveals the spatial immune landscape of highly and minimally invasive brain metastases

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Cancer metastasis to the brain is a common complication of advanced disease with limited therapeutic options. Inefficient treatment is influenced, in part, by the unique brain microenvironment. Brain metastases (BrM) grow in two distinct patterns, either as highly invasive (HI) or minimally invasive (MI) lesions. HI-BrM are associated with poor prognoses compared to MI-BrM; however, differences in the immune microenvironments between these two lesion types remain largely unknown. Here, we leverage single cell technologies with spatial resolution to assess the tumor immune microenvironment of HI and MI-BrM. We performed imaging mass cytometry on 119 BrM samples from 46 patients. Samples represent BrM from various primary sites, including cancers of the lung, breast, and skin. Analyzed tissues include patient-matched samples from the brain-tumor interface ('margin') or the centre of the metastatic lesion ('core'). We performed single cell analysis of over 350,000 cells to identify 20 different cell lineages, activation states, and spatially-defined cellular neighbourhoods. HI- BrM were found to be characterized by abundant astrocytes at the margins, while MI-BrM displayed greater immune infiltration when compared to HI-BrM, both at margins and within cores. Spatial analyses revealed 9 distinct cellular neighbourhoods (CNs). Tumor cores showed similar CN distribution across primary types, while margin samples revealed enrichment of macrophage-rich and vascular-niche CNs in melanoma BrM compared to lung or breast BrM. This study provides the first spatially-resolved single-cell dataset of the BrM microenvironment in MI and HI-BrM. The immune-rich microenvironment of MI versus HI-BrM suggests potential immune-regulation of BrM invasion, which warrants further investigation.

Examining residency match outcomes of MD+ trainees: results from a pan-Canadian survey

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Background: MD+ training programs serve to prepare their trainees to pursue a clinician-scientist career pathway, and following their completion, the subsequent training phase is typically postgraduate medical education (PGME). Entry into PGME programs is typically governed by a competitive match process. Examining outcomes of MD+ trainees in the residency match is essential to determining the effectiveness of MD+ training programs in fulfilling their mandate and in identifying opportunities for curriculum development.
Methods: We conducted a survey of graduates from Canadian MD+ programs (combined MD-MSc or combined MD-PhD) spanning the 2016-2021 graduating classes. Our survey comprised 17 optional questions targeted towards participation and outcomes in residency match processes. Data were expressed as categorical variables (N with percentages) and compared using Chi-squared testing. Exploratory ordinal regression was also performed. Analyses were performed using R (version 1.1.456).
Results: We received 49 unique responses. Most trainees (89.8%) entered the Canadian Resident Matching Service (CaRMS) match, with the remainder participating in international match processes or foregoing PGME altogether. 89.6% of match participants were placed in their top choice specialty and 70.8% in their top choice location. A majority (60.4%) of respondents indicated a desire to pursue a career as a clinician-scientist upon completion of their training.
Conclusions: Residency match outcomes of MD+ graduates are largely positive, however, there remains a large disparity between the number of graduates and the number intending to pursue research-focused careers. Our work has important implications for advocacy, curriculum design, and mentorship within MD+ programs.

Inulin impacts tumorigenesis promotion by colibactin-producing *Escherichia coli* in *Apc*$^{Min/+}$ mice

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Introduction: The prebiotic inulin has previously shown both protective and tumor-promoting effects in colorectal cancer. These discrepancies may be due to polyketide synthase-positive (pks+) *Escherichia coli* promoting carcinogenesis through the production of colibactin, a genotoxin that induces double-strand DNA breaks (DSBs). In this study we investigated the impact of inulin on the genotoxicity of colibactin-producing bacteria.
Material & Methods: *E. coli* strains Nissle (EcN), and NC101 (EcNC101) were grown in medium supplemented with inulin. Colibactin expression was assessed by luciferase reporter gene expression and Caco2 cells were used to assess colibactin-induced genotoxicity by γ-H2AX immunofluorescence analysis. *Apc*$^{Min/+}$ mice received 2% dextran sodium sulfate (DSS) followed by oral gavage with EcNC101 and were fed a diet supplemented with 10% cellulose (control diet) or 10% inulin for four weeks.
Results: Inulin enhanced EcNC101-induced expression of colibactin and DSB levels in Caco2 cells. Inulin supplementation in *Apc*$^{Min/+}$ mice led to enhanced EcNC101 colonization and tumor progression.
Conclusion: The presence of colibactin producing *E. coli* in the gut influences the outcome of inulin supplementation in CRC progression. Further studies are needed to investigate the interaction between dietary supplements and cancer-promoting bacteria.

Genetic Characterization of a Familial Mutation of *MAX* Associated with Pheochromocytoma, Paraganglioma and Neuroendocrine Tumours

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Background: A 30 yo man was diagnosed with a pheochromocytoma based on hypertension, imaging and biochemistry. He underwent a 14 susceptibility gene panel for PPGLs (Invitae, USA) that revealed a germline pathogenic nonsense heterozygous *MAX* variant (c.223C>T p.Arg75*). The patient's father (61 yo) was found to carry the same germline mutation which led to the diagnosis of an unsuspected functional abdominal paraganglioma and two neuroendocrine tumors (NETs) in his pancreas and his duodenum that were operated. Our objective was to determine the causal role of the familial germline *MAX* pathogenic variant in the development of PPGLs and NETs.
Methods: Leucocyte DNA was extracted from blood cells and tumoral DNA was extracted from FFPE tissues. All 5 exons of the *MAX* gene (NM_002382.4) were studied by Sanger Sequencing. The amplicons were directly sequenced using the Applied Biosystems 3730xl DNA Analyzer (McGill University, Genome Quebec, QC, Canada).
Results: The germline *MAX* mutation (c.223C>T p.Arg75*) was confirmed in both leucocyte DNA and all tumoral DNA (son : PHEO, father: PGL and NETs). The wild-type *MAX* allele was lost in the tumoral DNA of the father's PGL. A second somatic pathogenic variant of *MAX* c.263T>C (p.Leu88Pro) was identified in the duodenal NET and was predicted to be likely deleterious (CADD score 31).
Conclusion: We report the rare association of a familial germline *MAX* mutation presenting with PPGLs and NETs. A second hit in the *MAX* gene in the duodenal NET supports the hypothesis that *MAX* mutations might be associated with multiendocrine tumors.

Chemokine expression predicts T cell-inflammation and improved survival with checkpoint inhibition across solid cancers

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Background: Novel predictors of T cell-inflammation may identify a broader subset of tumors with immune checkpoint inhibitor (ICI) responsiveness. Our group has identified four chemokines (c-Score; *CCL4*, *CCL5*, *CXCL9*, *CXLC10*) able to predict a T cell-inflamed phenotype in primary and metastatic pancreatic tumors. Here, we test whether this signature can predict T cell-inflammation and response to ICI across additional tumor types.
Methods: Using multi-omic data from 6,455 patients spanning 25 tumor types from The Cancer Genome Atlas, we searched for associations between the c-Score and metrics of antitumor immunity. We also investigated the prognostic ability of this signature using real-world data from a pan-cancer cohort of 82 patients in the Personalized OncoGenomics Program treated with ICIs.
Results: The majority of tumor types displayed sub-populations with high expression of the 4-chemokines (c-Score^{hi}) and transcriptional hallmarks of the cancer-immunity cycle. Immunomodulatory genes (*PD-L1*, *PD-1*, *CTLA-4*,) and cancer-immunity cycle-associated genesets (MHC I expression, cytolytic activity) and were significantly overexpressed ($p<0.05$) in the c-Score^{hi} cohorts. High TMB was associated with only a subset of c-Score^{hi} tumors. Among patients treated with ICIs, those with c-Score^{hi} tumors had a longer median time to progression (103 versus 72 days, $p=0.012$) and overall survival (382 versus 196 days, $p=0.038$). The c-Score outperformed TMB for overall survival prediction.
Conclusions: Sub-populations of T cell-inflamed patients exist across tumor types and may therefore respond favourably to ICI. The c-Score has the potential to select a wider spectrum of patients that may benefit from ICIs.

miR-375/YAP coordinate complex molecular networks underlying well-differentiated neuroendocrine neoplasms

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Background: Well-differentiated neuroendocrine neoplasms, termed "carcinoids", are variably aggressive and mechanistically ill-defined tumors. microRNAs (miRNAs) negatively regulate gene expression and thereby alter complex molecular networks often involved in tumorigenic processes. In carcinoids, miR-375 and its predicted target gene, yes-associated protein (YAP), are correlated with tumorigenic development, but the impact of miR-375/YAP on complex molecular networks has not been characterized.
Methods: Lentiviral CRISPR/Cas9-mediated miR-375 depletion and lentiviral YAP overexpression were applied to lung (H727) and pancreatic (BON1) carcinoid cells. RNA-sequencing and subsequent pathway analysis was performed, and targets of miR-375 were predicted using the Bio-miRTa algorithm. Network analysis will be performed and cross-referenced with drug-targetable pathways from the Cancer Therapeutics Response Portal.
Results: miR-375 depletion was associated with upregulated pathways related to secretion and exocytosis, while downregulated pathways were related to Notch signalling and cell cycle regulation. Out of 2,677 predicted target genes of miR-375, YAP was the second highest ranked target. YAP overexpression was associated with upregulated pathways enriched in exocytosis and cytoskeletal organization, while downregulated pathways were enriched in hormone secretion. Overlapping transcriptomic changes from miR-375 depletion and YAP overexpression revealed common pathways controlling neuroendocrine functions including neural differentiation, exocytosis, and secretion. Further analysis is expected to identify hub genes mediating cross-talk between key molecular pathways and therapeutically relevant pathways.
Conclusions: Our preliminary results provide evidence that miR-375/YAP coordinate complex molecular pathways relating to neuroendocrine differentiation and tumorigenesis in lung and pancreatic carcinoid cells. Further analysis is expected to improve understanding of carcinoid development and illuminate therapeutic targets.

Prediction of kidney transplant function with machine learning from computational ultrasound features

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Prognosis of kidney function in post-transplant patients is important when considering invasive investigations, intervention, or re-transplantation. Ultrasound imaging is a non-invasive tool that may contain subtle textures associated with kidney function. To address this, we developed a prediction model utilizing machine learning and computational image features to predict decline in estimated glomerular filtration rate (eGFR), a key measure of kidney function. Post-transplant ultrasound scans and eGFR values from N = 819 transplant patients were obtained. A multi-stage pipeline was built to first automatically segment the cortex, medulla, and central echo complex from ultrasound. Imaging features (104 total) related to shape, intensity statistics, texture and ultrasound speckle were computed. A random forest (RF) classifier was trained to predict 5-year eGFR decline from the feature set. For comparison, validation was repeated with using only clinical variables and with the Kidney Failure Risk Equation (KFRE). Predictive features were identified by feature-wise decrease in impurity and a mean validation AUC (± standard deviation) of 0.81 ± 0.03 was achieved. Comparison AUCs were 0.62 ± 0.04 for the clinical variable model and 0.67 ± 0.03 for the KFRE model. 2-dimensional elongation, cluster shade, and Nakagami speckle shape were the most predictive features. This study provides support that computational image features combined with machine learning may potentially serve as a non-invasive eGFR decline prediction tool to aid post-transplant care.

Ketone therapy reduces systemic and organ inflammation and dysfunction in sepsis

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Introduction: Sepsis is a dysregulated inflammatory response to an infection resulting in multi-organ injury. Currently, there are no effective treatments to reduce inflammation and prevent the inflammation-mediated damage during sepsis. Thus, new approaches are needed manage inflammation and improve patient outcomes. Herein, we tested the efficacy of a ketone therapy that increases circulating ketones via ketone esters. Ketones are small molecules produced by the liver in carbohydrate-deprived states, such as fasting. While ketones are classically known to be a metabolic source of energy, they also have non-metabolic effects, such as inhibiting inflammation. Thus, we hypothesized that ketones have anti-inflammatory effects which will protect against septic organ dysfunction and inflammation.
Methods: 8-week-old mice orally received vehicle or ketone ester (KE) for 3 days. On day 3, mice received saline or lipopolysaccharide (LPS) to induce systemic inflammation and sepsis, and assessments were performed 24 hours post-injection.
Results: LPS-treated mice had higher blood ketones compared to controls, suggesting that ketones may be an innate defense mechanism, and this response was further augmented in KE-treated septic mice. While LPS-treated mice had an induction of systemic pro-inflammatory cytokines (e.g., IL-1β, IL-6, interferon-γ) these cytokines were significantly lower in KE-treated septic mice. Similarly, LPS induced notable inflammation in the heart, kidney, and liver, most of which were reduced in KE-treated septic mice. LPS-induced cardiac dysfunction and renal fibrosis was also lower in KE-treated septic mice.
Conclusion: Together, these data show that ketone therapy may be a novel approach to reducing inflammation and organ dysfunction in sepsis.

Attention based multi-instance learning for improved glioblastoma detection using mass spectrometry

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Background: Glioblastoma multiforme (GBM) is the most common and most lethal primary brain tumour in adults with a 5-year survival rate of 5%. The current survival rate and standard of care has remained largely unchanged due to degree of difficulty in surgically removing these tumours, which plays a crucial role in survival, as better surgical resection leads to longer survival times. Novel technologies need to be identified that allows for greater resection accuracy.
Methods: In our current study, we curate a database of ex-vivo GBM and normal brain tissue that is used to train and validate a multi-instance learning model for GBM detection using rapid evaporative ionization mass spectrometry which allows for real time tissue typing. Resected specimens were collected by a surgeon, inspected by a pathologist, and sampled with electrocautery device.
Results: The data consisted of 276 burns obtained from normal tissue, and 321 burns from tumor tissue. A multi-instance learning model was adapted and used to learn molecular signatures of GBM. A 4-fold cross-validation approach, stratified by patients, was used for training and evaluation of the model. The models were robust and were able to predict GBM at an improved AUC of 0.93, and an accuracy of 93%, as compared with our baseline models.
Conclusion: This is the first study that applies deep learning to REIMS data for tissue characterization in brain tumours, and can be an effective way to make real-time decisions and personalize treatment plans for effective management of GBM.

ITGA5 is a novel immunotherapeutic target against treatment refractory medulloblastoma

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Medulloblastoma (MB) is the most common type of malignant pediatric brain cancer. Current standard of care (SOC) involves maximal safe resection and neuraxis radiotherapy and chemotherapy in individuals older than 3 years. To date, these cytotoxic SOC combined with craniospinal irradiation led to devastating neurocognitive and developmental deficits impacting quality of life for pediatric patients. The biological heterogeneity of MB is highlighted by the existence of four distinct molecular subgroups (WNT, SHH, Group 3, and Group 4). Group 3 and Group 4 have the poorest patient outcomes because of their aggressive, metastatic nature, and so often remain treatment refractory to SOC. Group 3 has a poor prognosis due to its high incidence of leptomeningeal spread and an overall survival rate of less than 50%. The cytotoxic nature and lack of response in specific subtypes to SOC underscores the urgent need for developing and translating novel treatment options including immunotherapies. In our earlier work, we have developed a therapy-adapted patient derived xenograft model of the Group 3 MB as the tumor cells undergoes therapy *in vitro* and *in vivo*. N-glycocapture surfaceome profiling of the MB cells through this therapy-adapted model identified Integrin α5 (ITGA5) as one of the most differentially expressed targets found at recurrence when compared to engraftment and untreated timepoints. Through shRNA knockdown and small molecule inhibition, we identify ITGA5 expression marks a MB cell subpopulation with increased self-renewal ability both *in vitro* and *in vivo.* Access to recurrent MB (rMB) post-therapy allowed us to investigate the changes in the surfaceome of MB cells using proteomics profiling to identify promising rMB-specific targets for rational development of novel immunotherapies.

SARS-CoV-2 Omicron subvariants Spike recognition and neutralization elicited after the third dose of mRNA vaccine

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For several months, different sub-variants of Omicron, including the BA.2, BA.4, BA.5, BA.4.6 and BA.2.75 variants, have emerged and are currently circulating all around the world. These variants carry a large number of mutations in their Spike glycoprotein sequences, the main target of the SARS-CoV-2 vaccines, raising concerns about vaccine efficacy. In this study, we evaluate the ability of plasma from a cohort of health care workers (HCW) that received three doses of mRNA vaccine, to recognize and neutralize the Spike of these different subvariants of Omicron. The HCW have been divided into three groups: naïve individuals who were never infected with SARS-CoV-2, previously infected (PI) individuals who were infected during the first wave of COVID-19 in early 2020 and before vaccination, and breakthrough infection individuals (BTI) who were infected after vaccination likely by the Delta variant or an Omicron sub-variant. We observe that the different sub-variants of Omicron are significantly less well recognized and neutralized than the D614G Spike and this is highly marked for the Spike of the BA.4 and BA.5 variants. We also note that SARS-CoV-2 naïve vaccinated individuals present weak humoral responses against these Omicron Spikes, even after three doses of mRNA vaccine, and they decreased rapidly over time. In contrast, PI elicit strong responses that remain stable over time. Interestingly, breakthrough infection in naïve vaccinated individuals leads to the same level of responses than observed in PI individuals. These results indicate that hybrid immunity generates better humoral responses against this subvariant that vaccination alone.

Dominant interfering variants in *IKZF2* cause a syndrome characterized by developmental abnormalities and immune dysregulation

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Helios (*IKZF2*), a member of the Ikaros family of transcription factors, is a zinc finger protein involved in embryogenesis and the development and regulation of the immune system. Helios has been mainly recognized for its role in the development and function of T-lymphocytes, in particular regulatory T cells. Nonetheless, the expression and function of Helios extends beyond the immune system. During embryogenesis, Helios is expressed at varying levels in a wide range of tissues in the developing embryo. The dynamic expression of Helios in various tissues during development and its role in regulatory T cells makes it a strong candidate for causing widespread immune-related and developmental abnormalities in humans. Here, we investigated an individual with syndromic features including facial dysmorphism, developmental/congenital abnormalities, sensorineural hearing loss and immune dysregulation resulting from a *de novo* novel heterozygous variant in critical and highly conserved DNA-binding zinc fingers of Helios. Sequencing revealed a tandem duplication of exon 5 coding for zinc fingers 2 and 3 which are highly conserved and critical for DNA binding activity of Helios (p.Gly136_Val192dup). As these critical zinc fingers determine the sequence specificity of Helios, we hypothesized $Helios^{Gly136\_Val192dup}$ to have altered binding to canonical target sequences. Our functional studies showed that $Helios^{Gly136\_Val192dup}$ is expressed, but its activity as a repressor of *IL2* expression (known target of Helios) is compromised in a dominant negative manner. Our study is the first to describe dominant negative defects in *IKZF2*, causing a novel multi-system syndrome with developmental and immune-related abnormalities in humans.

Predicting the Dose Distribution of Multi-Lesion Lung Radiation Treatment using Generative Adversarial Networks

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Introduction: Stereotactic radiotherapy (SBRT) is a high dose radiation technique used to treat simultaneous lung metastases. For each treatment, a radiation distribution must be created by a medical physicist to determine whether a plan is efficacious and safe, which is a time-consuming operation and prevents physicians from comparing alternative prescriptions. To solve this, we created a machine learning model to automatically create dose distributions in real time.
Methods: A retrospective review of patients who received SBRT treatment for two or more lung lesions between January 2014 and May 2020 was conducted at the London Regional Cancer Program. The data was divided into training (80%) and testing (20%) sets. A generative adversarial network (GAN) was trained to generate a dose distribution from the patient's CT scan, their contours of tumours and healthy organs, and an initial dose estimation. A novel loss function was implemented.
Results: Plans from 67 patients were collected (47 two lesion plans). The mean squared error (MSE) of the best performing network across all plans in the test set was 5.21Gy^2. Synthetic plans for two lesion cases were more accurate than plans with greater than two lesions (MSE 4.42Gy^2 vs. 6.47Gy^2). The novel loss function improved performance compared to standard adversarial loss.
Discussion: This work is the first application of generative machine learning techniques to predict SBRT dose distribution in multi-lesion plans. Using the model, radiation oncologists are able to compare different prescriptions to select the one with the best tradeoff between tumour control and radiation toxicity.

OTHER

Drivers of family doctor shortages across Canada as a function of medical student graduates per province

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Background: Family physicians provide comprehensive care for members of the community. There is a shortage of Canadian family care practitioners, driven in part by an apparent lack of desirability of the profession due to overbearing expectations, lack of resources, stagnant payment, and high clinic operating costs. An additional and yet-unexplored aspect of the family physician shortage is the shortage of medical student graduates, as the number of spots in medical schools in Canada has remained mostly stagnant for the past 15 years, with only few exceptions, and no new medical schools have been opened.
Methods: StatsCAN and the Canadian Medical Association Journal (CMAJ) were used to identify provincial populations and the number of physicians. The Association of Faculties of Medicine of Canada (AFMC) Canadian Medical Education Statistics report was used to identify the number of seats for medical schools across Canada.
Results: Family physician shortages are the highest in the Territories (>55%), QC (21.5%), and BC (17.7%). Among the provinces, ON, PE, MB, and QC have the fewest family doctors per population. Among the provinces that offer medical education, BC and ON have the fewest medical school seats per population, while NS, NL, and QC have the most.
Conclusions: BC has the smallest medical class size as a function of population, and a high percentage of residents without family doctors. Surprisingly, QC has a relatively large medical class size as a function of population, but with a high percentage of residents without family doctors.

FaBiSearch: change point detection for structure of multivariate high-dimensional time series

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Functional magnetic resonance imaging (fMRI) time series data presents a unique opportunity to understand the behavior of temporal brain connectivity, and models that uncover the complex dynamic workings of this organ are of keen interest in neuroscience. We are motivated to develop accurate change point detection and network estimation techniques for high-dimensional whole-brain fMRI data. To this end, we introduce factorized binary search (FaBiSearch), a novel change point detection method in the network structure of multivariate high-dimensional time series in order to understand the large-scale characterizations and dynamics of the brain. FaBiSearch employs non-negative matrix factorization, an unsupervised dimension reduction technique, and a new binary search algorithm to identify multiple change points. In addition, we propose a new method for network estimation for data between change points. We seek to understand the dynamic mechanism of the brain through these techniques, particularly for two fMRI data sets. The first is a resting-state fMRI experiment, where subjects are scanned over three visits. The second is a task-based fMRI experiment, where subjects read Chapter 9 of Harry Potter and the Sorcerer's Stone. For the resting-state data set, we examine the test-retest behavior of dynamic functional connectivity, while for the task-based data set, we explore network dynamics during the reading and whether change points across subjects coincide with key plot twists in the story. Further, we identify hub nodes in the network and examine their dynamic behavior. We make all the methods discussed available in the R package fabisearch on CRAN.

Cortical Network Disruption in Schizophrenia

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A variety of changes in neural architecture and function accompanies schizophrenia, the nature of which is still an active subject of research. Current models based on fMRI and diffusion weighted imaging (DWI) describe a remodeling of the cortical architecture, suggesting cortical dysconnectivity as a possible explanation for schizophrenia symptoms. Many of these models derive from graph theory analysis, a mathematical framework used to describe networks. One model proposes that central cortical nodes are highly susceptible to damage due to their extensive connectivity. As the disease progresses, the brain may reroute itself to bypass these hubs. This model predicts a shift in connectivity strength toward peripheral regions of the cortex. Here, we test this hypothesis through a network analysis of the structural connectivity network. N=120 patients and N=60 healthy controls were recruited from an established cohort enrolled in the Prevention and Early Intervention Program for Psychoses (PEPP) in London, Ontario. DWI tractograms were created using an MRTrix pipeline, and translated to a non-directional association matrix weighted using a qT1-derived myelination coefficient. Using these matrices, the peripherilization of connectivity is assessed using graph theory metrics. Numerous edges were found to be disrupted, however, we did not find a correlation between this disruption and the centrality of the edges, unlike previous reports, although more central hubs are slightly more likely to have a disrupted edge. Thus, while we've found more evidence schizophrenia is a disorder if dysconnectivity, the relation of that dysconnectivity to the brains topological structure remains unclear.

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