James Hogg Lecturer: Dr. Dana Devine
“The Impact of Blood Donor Variation on Transfusion Product Quality”

Guest Speaker: Dr. Naveena Singh
“The Shifting Landscape of Endometrial Carcinoma”

Guest Speaker: Dr. Natalie Prystajecky
“At the Intersection of Research and Clinical Laboratory Service - COVID-19 Experience”

Keynote Speaker: Dr. Ralph Hruban
“The World is Not Flat! A “Clearer” Three-dimensional View of Pancreatic Cancer”
Message from the Chair

I would like to begin by acknowledging that we are gathered on the traditional and unceded territory of the Coast Salish People.

Pathology Day is a significant event in the departmental calendar as it provides us with an opportunity to showcase and celebrate the wide spectrum of scholarly activities undertaken by our trainees and faculty. This gathering allows us to recognize the outstanding contributions in research and in service given by all members of the department. Pathology Day also serves as another important function; it gives us the chance to connect, socialize, and to share and learn more about each other, as well as gaining an appreciation for the breadth of scholarly activities that take place in our department.

Recent times have been difficult as the world battled with an unprecedented pandemic. Yet all of you were able rise above the challenge, remaining active and productive in your academic endeavors. This event marks the first time in three years that we are getting together again in person, united as one team despite our dispersed geographic locations.

This year we are extremely privileged to have Dr. Naveena Singh and Dr. Natalie Prystajecky as our guest speakers, Dr. Dana Devine as the speaker of James Hogg Lecture, and Dr. Ralph Hruban from John Hopkins University as our Keynote Speaker.

I would like to extend my sincere thanks to the members of the organizing committee including Dr. Will Lockwood, Dr. Sophia Wong, Dr. Corree Laule, Dr. Tony Ng, Dr. Suzanne Vercauteren, Heleena Mistry, Genevieve MacMillan, Rachel Cederberg, Shazia Masud as well as all the other individuals whose efforts make Pathology Day a success.

Hoping you all have a wonderful Pathology Day!

Dr. Zu-hua Gao, MD, PhD, FRCP, FCAHS
Professor and Department Head

I would like to begin by acknowledging that we are gathered on the traditional and unceded territory of the Coast Salish People.
Acknowledgements

Pathology Day is a team effort and we would like to extend our thanks to everyone who contributed to the 2022 edition. Heleena Mistry and Genevieve MacMillan have been instrumental in handling the administrative and practical details of Pathology Day. Debbie Bertanjoli designed the website and managed the website tools in addition to preparing the abstract book.

We also wish to express our gratitude to the many department members who contributed their time and expertise to reviewing abstracts, moderating the oral sessions, and judging the oral and poster presentations:

Staff: Genevieve MacMillan, Debbie Bertanjoli, Shelby Douglas, Juliana Li, Jenny Tai, Jen Xenakis, Ivy Zhang, Heather Cheadle, Shelley BerKow, Mayumi Shimada

Grad Students: Maria Elishaev; Farnaz Sahragard; Rachel Cederberg; Tetiana Povshedna, Aaron Mah, Guadalein Tanunlioung, Marie-Soleil Smith

Students: Rosie Papp, Lauren Rutherford, Riya Gandhi

Judges:

ABSTRACT REVIEWER: Honglin Luo, Karla Bretherick, Ayesha Vawda, Sakara Hutspardol, Alberto Delaidelli, Catherine Hogan, David Grynspan, Nevio Cimolai, Will Wong, Kerstin Locher

POSTER SESSION JUDGES: Lik Hang Lee, Inna Sekirov – micro, Anastasia Drobyshova [CWBC], Jeff Terry, Leandro Venturutti, Ramon Klein Geltink, Kerstin Locher, Miguel Imperial, Lien Hoang [VCH], Helene Cote, Yasir Mohamud, BojanaRakic [CWBC], Muhammad Morshed [BCCDC], Junyan Shi [VCH], Georgina Barnabas, Sukhbir Kaur, Tyler Smith [VCH], Andre Mattman [PH], Joshua Dubland [CWBC], Agatha Jassem [BCCDC]

ORAL SESSION JUDGES: Jordan Hamden – grad student, Inna Sekirov – micro, Gerry Krystal, Cheryl Wellington, Leandro Venturutti, Andrew Minchinton, Jacqueline Quandt, Miguel Imperial, Maria Victoria Monsalve

Committee Members 2022
CONFERENCE OUTLINE

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<td>Kevin Kuchinski</td>
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<td>Ardalan Akbari</td>
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Background/objectives: Lung cancer (LC) is the leading cause of cancer-related death worldwide, mainly due to the late diagnosis of disease. Identifying factors that increase one’s susceptibility to lung cancer is therefore imperative for the development of early intervention strategies. While smoking is the major risk factor for LC, genetics also play a critical role. However, the specific genes responsible for increasing this risk are still poorly understood. In effort to identify new genetic drivers of LC, our group used whole exome sequencing to profile a panel of never-smoker patients with lung adenocarcinoma (LUAD), a type of non-small cell LC. Among the most significantly mutated genes was SNF2 Histone Linker PHD RING Helicase (SHPRH), which encodes for an E3 ubiquitin ligase that functions in DNA repair. Interestingly, SHPRH is chromosomally located within a region associated with familial susceptibility to LC. Taken together, we predict that SHPRH may function as a tumor suppressor gene in LUAD. However, while the alteration of SHPRH has been observed in various other cancers, the functional characterization of SHPRH as a tumor suppressor and its role in LUAD has yet to be determined. The objective of this study is to evaluate the effect of altered SHPRH expression on the development and progression of LUAD.

Methods: To determine the clinical relevance of SHPRH, we analyzed publicly available LUAD datasets for SHPRH copy number and expression level status, and assessed for their association to survival outcomes. To functionally characterize the role of SHPRH in LUAD tumorigenesis, a doxycycline-inducible system was used to express wildtype SHPRH in LUAD cell lines with varying SHPRH expression statuses. Upon expression of SHPRH, we performed in vitro and in vivo assays to assess for alterations in tumorigenic potential.

Results: Analysis of the TCGA LUAD cohort (n=230) reveals that SHPRH is mutated or homozygously deleted in 7% of tumors and 52.2% of LUADs demonstrate a single copy loss of SHPRH, which coincides with having significantly less SHPRH expression. Furthermore, ever- and never-smoker LUAD patients with reduced SHPRH expression have significantly worse overall and progression-free survival outcomes. Assessment of colony growth in vitro suggests that re-expression of SHPRH in cell lines with inactivating alterations of SHPRH reduces their anchorage-dependent and -independent growth in a tumor suppressive manner. Additionally, implantation of these cells into the flanks of mice kept on a doxycycline diet shows that SHPRH re-expression significantly reduces tumor burden compared to the control conditions.

Conclusions: This data suggests that the expression of SHPRH may positively affect patient outcomes and may function to reduce the tumorigenic potential of LUAD. However, continued investigation is required to determine the biological relevance of SHPRH in LUAD pathogenesis. Understanding the role of SHPRH may lead to it becoming an important clinical biomarker for identifying individuals with an increased risk of developing LUAD and to help improve disease outcomes.
THE UTILITY OF BLOOD BASED BIOMARKERS IN DETECTING NEUROLOGICAL COMPLICATIONS OF COVID-19 IN CRITICALLY ILL PATIENTS

ABSTRACT

Background/objectives: Many neurological and neuropsychiatric complications have been documented in patients with COVID-19, however, information about the underlying neuropathology is limited. As changes in the central nervous system can be detected using blood based biomarkers, this study aims to investigate the utility of two neurological biomarkers to predict neurological complications and mortality due to COVID-19 in the intensive care unit (ICU). Neurofilament light (NF-L) is a marker of axonal damage and glial fibrillary acidic protein (GFAP) is a marker of astrocytic activation.

Methods: 327 patients from the Vancouver General Hospital ICU were prospectively enrolled. 265 were diagnosed with COVID-19 and 62 served as non-COVID-19 critically-ill controls. COVID-19 patients were excluded if the diagnosis was an incidental secondary finding upon ICU admission (N=14) or if their enrollment was >10 days after ICU admission (N=42). Control patients were excluded if their enrollment was >4 days after ICU admission (N=17) or if their primary diagnosis was non-respiratory (N=11). Plasma samples were collected upon admission and study enrollment, with additional samples collected on day 7 and day 14 from COVID-19 patients. Plasma NF-L and GFAP were quantified using the Quanterix Simoa HD-X analyzer (Quanterix Neurology 4-Plex E assay kit). Group comparisons were performed using a Mann-Whitney test. Trajectory analysis was performed by a Wilcoxon test, or Friedman one-way ANOVA. Area under receiver operating curve (AUROC) analysis was calculated to predict neurological complications and mortality during ICU stay.

Results: Upon ICU admission, NF-L was 32% (55%-12%) lower and GFAP was 24% (51%-3%) lower in those with COVID-19 compared to those without after correcting for age. NF-L increased significantly by days 7 (p<0.0001) and 14 (p<0.0001) of ICU stay in patients with COVID-19, while GFAP did not significantly change over time. AUROC analyses showed that day 7 NF-L and GFAP levels were superior to admission levels in predicting neurological complications during ICU stay. Specifically, AUROC values for NF-L was 0.707 (p=0.0005) at admission and 0.850 (p<0.0001) at day 7, and AUROC values for GFAP was 0.695 (p=0.001) at admission, and 0.805 (p=0.0002) at day 7. Combining both markers improved prediction of neurological complications with an AUROC of 0.730 (p<0.0001) at admission and 0.882 (p<0.0001) at day 7. Conclusively, both markers were able to predict neurological complications or ICU mortality with moderate to strong accuracy.
ORAL PRESENTATION
KEVIN KUCHINSKI
SUPERVISOR: DR. NATALIE PRYSTAJECKY

USING A NOVEL GENOMIC ALGORITHM TO RECONSTRUCT NETWORKS OF SARS-COV-2 TRANSMISSION IN AN ACUTE CARE FACILITY OUTBREAK

AUTHOR(s) Kevin Kuchinski1, Aidan Nikiforuk2, Katy Short3, Susan Roman1,4, John Tyson5, Linda Hoang1,3, Agatha Jassem1,5, Inna Sekirov1,5, Natalie Prystajecky1,5

AFFILIATION(s) 1Department of Pathology and Laboratory Medicine; University of British Columbia; Vancouver, British Columbia; 2School of Population and Public Health; University of British Columbia; Vancouver, British Columbia; 3Infection Prevention and Control; Fraser Health; Surrey, British Columbia; 4Department of Pathology and Laboratory Medicine; Fraser Health; Surrey, British Columbia; 5Public Health Laboratory; British Columbia Centre for Disease Control; Vancouver, British Columbia

ABSTRACT Background/objectives: The COVID-19 pandemic has accelerated the use of genomic sequencing technologies in routine public health practice, especially the burgeoning field of genomic epidemiology. To date, this field has been dominated by phylogenetic paradigms, but these approaches have limitations when analyzing infectious disease transmission in outbreak scenarios. Phylogenetic trees are computationally intensive and time consuming to generate. Furthermore, phylogenetic trees do not convey direction of transmission between closely related genomes, making chains and networks of transmission difficult to interpret. To address these limitations we created a novel genomic algorithm called GenomeTracer. It rapidly reconstructs potential transmissions between cases from mutations identified during variant calling, and it visualizes transmissions as interactive network figures instead of phylogenetic trees, with nodes connected by arrows clearly showing directional chains of transmission. In this study, we evaluated GenomeTracer on an epidemiologically well-defined outbreak of SARS-CoV-2 infections at an acute care facility in British Columbia, Canada.

Methods: An epidemiologically well-defined outbreak of 180 COVID-19 cases was identified at an acute care facility in British Columbia. Samples were sequenced at the British Columbia Centre for Disease Control’s Public Health Laboratory, and variant call data describing mutations in these infections were generated by during routine analysis. GenomeTracer was used to rapidly compare the single nucleotide polymorphisms present in each pair of infections, determine if transmission between these cases was possible, then reconstruct the likeliest chains and networks of transmission. An independent, in-depth investigation of this outbreak was conducted by Health Authority epidemiologists. This provided extensive metadata on these cases, including infection date and the ward/unit on which each case was suspected to have acquired their infection.

Results: Sufficient high-quality genomic data was recovered for 143 of 180 cases (79%). GenomeTracer identified 7 transmission clusters, ranging in size from 2 to 78 cases (median 9 cases). These transmission clusters all formed monophyletic clades on a conventional phylogenetic tree, and conventional SARS-CoV-2 lineages were consistent within these clusters. In the largest transmission cluster, GenomeTracer allowed us to follow the spread of infection through 4 units in the acute care facility. The order in which unit-level outbreaks occurred, as inferred by GenomeTracer, was concordant with infection date information independently collected during the Health Authority’s outbreak investigation. For 18 cases, epidemiological investigation could not conclude which unit the infection had been acquired on, but analysis of adjacent cases in GenomeTracer transmission networks allowed completion of this missing information for 6 cases (33%).

Conclusions: GenomeTracer provides visually informative diagrams for outbreak investigation. These can be used to support existing epidemiological conclusions and infer missing information.
MOLECULAR CHARACTERIZATION OF A CASE OF ADENOID CYSTIC CARCINOMA OF THE ESOPHAGUS

Background/objectives: Adenoid cystic carcinoma (ACC) most commonly arises in the major and minor salivary glands, though other sites of involvement include the breast, trachea, skin, cervix, and esophagus. The clinical course of salivary gland ACC can vary widely: 10-year overall survival is less than 50%, and median survival following metastasis has been estimated at three years; however, approximately 10% of patients can survive up to 10 years with distant disease. Esophageal ACC is aggressive, with 1-year survival of approximately 23%, whereas ACC involving the breast is more indolent in nature. This varied clinical course raises the possibility of site-dependent molecular heterogeneity underlying this disease.

In the salivary gland, 88% of ACCs possess a translocation involving MYB or MYBL1 with NFIB. These translocations are predicted to be oncogenic drivers. Esophageal ACC is significantly more rare than salivary gland ACC, accounting for 0.1% of all esophageal malignancies. Accordingly, there have been to our knowledge no studies that have thoroughly characterized esophageal ACC at the molecular level. We have identified a recent case at Vancouver General Hospital of esophageal ACC diagnosed upon receipt of the surgical resection specimen, which was originally diagnosed as squamous cell carcinoma (SCC) of the esophagus on biopsy.

Methods: Nucleic acids were extracted from formalin-fixed, paraffin-embedded tissues. We used the nCounter® gene fusion panel from Nanostring Technologies to probe a panel of well-characterized oncogenic translocations. mRNA sequencing was used to identify additional possible driver mutations from a panel of oncogenes using the Find-It v3.4 cancer hotspot panel from Contextual Genomics.

Results: Our case of esophageal ACC does not possess the MYB/MYBL1-NFIB fusion gene associated with the majority of salivary gland ACC. Instead, this esophageal ACC exhibited a mutation in TP53.

Conclusions: Esophageal ACC may have an alternate genetic etiology compared to the majority of salivary gland ACCs. Given that esophageal ACCs are often diagnosed as SCC on biopsy, pathologists should be aware of ACC as a possibility in cases of unusual morphology on biopsy.
ABSTRACT

Background/objectives: Bladder cancer is a heterogeneous disease classified into two broad molecular subtype categories, basal and luminal, with critical treatment and prognostic implications. Recent studies have shown the utility of immunohistochemistry in predicting bladder cancer molecular subtypes, with a two-marker approach using GATA3 and CK5/6 showing good reliability. In the current study, we calculated the accuracy of uroplakin II (UPII), a marker of urothelial differentiation, with different scores (0: <1%, 1+: 1-10%, 2+: 10-50%, 3+: >50%) to predict RNA-based luminal versus basal subtypes in a cohort of muscle-invasive bladder cancer that received neoadjuvant chemotherapy followed by radical cystectomy.

Methods: Eighty pre-treatment bladder tumors from transurethral resection (TURBT) of patients with pT2-4aN0M0 bladder cancer who subsequently received neoadjuvant chemotherapy and underwent radical cystectomy were used for analysis. A tissue microarray (TMA) was constructed using 1 mm cores taken from tumor-rich areas of each tumor. Sections from the TMA were stained with UPII (clone BC-21, 1:100 dilution; Biocare, Concord, CA), GATA3 (clone L50-823, 1:100 dilution, Cell Marque, Rocklin, CA), and CK5/6 (D5/16B4 clone, pre-diluted, Dako) using a DAKO autostainer (Agilent, Santa Clara, CA). The slides were reviewed by three pathologists who were blinded to the RNA-based classification of the tumors. The IHC intensity was assigned a score of 0, 1+, 2+, or 3+ based on the proportion of tumour cells staining positive, with 0: <1% of tumor cell staining, 1+: 1-10% tumor cell staining, 2+: 10-50% tumor cell staining, and 3+: >50% tumor cell staining. Previously reported transcriptomic data (23) were used to assign each tumor to one of the six consensus subtypes: Ba/Sq, LumP, LumU, LumNS, NE-like, and Stroma-rich.

The Fisher exact test was used to calculate two-tailed P values. P values less than 0.05 were considered statistically significant. The cancer-specific survival (CSS) and overall survival (OS) for patients with UPiI score 0-2 vs. UPiI score 3+ were compared using the Kaplan-Meier method with the SPSS program (IBM SPSS Statistics; IBM Corp., Armonk, NY).

Results: The 1% cut-off of the UPII stain predicts the luminal subtype with the sensitivity and specificity of 95% and 56%, respectively. With a UPII cut-off of 10%, the sensitivity and specificity were 93% and 81%, respectively, and with a UPII cut-off of 50%, the sensitivity and specificity were 91% and 96%, respectively. The prediction performance of UPII was better than either GATA3 or CK5/6. There was no significant difference in prognoses between UPiI 0-2+ and UPiI 3+ patients in this cohort.

Conclusions: The current study shows that evaluating the staining intensity of UPII can accurately predict basal and luminal subtypes of muscle-invasive bladder cancer.
ABSTRACT

Background/objectives: There is emerging evidence that human papillomavirus (HPV)-independent vulvar squamous cell carcinoma (VSCC) can be prognostically subclassified into 3 groups based on HPV and p53 status: HPV-associated (HPV+), HPV-independent/p53 wild-type (HPV-/p53wt), or HPV-independent/p53 abnormal (HPV-/p53abn). The goal of our study was to assess the accuracy/feasibility of separating VSCC into these 3 groups via combined use of p16 (HPV surrogate marker) and p53 immunohistochemistry (IHC), and highlight the pitfalls/caveats for clinical pathology.

Methods: A tissue microarray containing 360 cases of VSCC, usual vulvar intraepithelial neoplasia (uVIN/HSIL), and differentiated VIN (dVIN), was constructed, and IHC for p16 and p53 was performed. Expression of p16 was interpreted according to Lower Anogenital Squamous Terminology Standardization criteria. Expression of p53 was interpreted according to patterns established by Tessier-Cloutier et al. (2020) and Thompson et al. (2020): two wild-type patterns: (1) scattered, (2) basal-sparing/mid-epithelial; four mutant patterns: (1) basal overexpression, (2) parabasal/diffuse overexpression, (3) absent/null, and (4) cytoplasmic. The term null-like was used when there was uncertainty between the null and basal-sparing patterns. Discordances between p16 and p53 expression were resolved by repeating IHC on whole sections, HPV ISH (in-situ hybridization), and next-generation sequencing (NGS) for TP53.

Results: Out of 84 cases of p16+ VSCC, 80 had concordant HPV-ISH and p53 patterns (95.2%), allowing classification into the HPV+ group. There were four p16+ VSCC that exhibited discordant p53 staining. One case with null pattern on whole section was weakly positive on HPV-ISH and negative for TP53 mutation. Four cases (3.6%) were discordant, being negative for HPV ISH and demonstrating abnormal p53 IHC. Two of four had a confirmed missense TP53 mutation, conferring a grouping into the HPV-/p53abn category. Out of 144 cases of p16-negative VSCC, 143 (99.3%) were easily classifiable: 27 (18.8%) were HPV-/p53wt and 116 (80.6%) were HPV-/p53abn. One discordant case had a basal-sparing p53 pattern and was positive for HPV ISH (HPV+). No discrepancies were identified between VSCC and in-situ lesions. The use of IHC also led to the following revised diagnoses: HSIL -> dVIN (3/43) and dVIN -> HSIL (3/34).

Conclusions: Overall, 223/228 VSCC (97.8%) could be easily classifiable into the 3 prognostic groups (35.5% HPV+, 11.8% HPV-/p53wt, 51.8% HPV-/p53abn). The null-like pattern on TMA could be distinguished from true null pattern by the finding adjacent basal-sparing patterns on whole sections or via HPV ISH. We found that “double positive” p16+/p53abn patterns did not reflect the HPV group, and should be classified as HPV-/p53abn. Rarely, HPV-associated tumours may show wild-type p53 patterns and false-negative p16 (reaffirming the superior accuracy of p16 over HPV ISH for VSCC classification, in line with ICCR guidelines). Notably, performing p16 and p53 IHC helps to reduce over-diagnosis of dVIN, which can be very challenging on standard H&E sections.
ABSTRACT

Background/objectives: CD8+ T cells are essential in adaptive immunity. Upon cognate antigen encounter, naïve CD8+ T cells differentiate into effector T (TE) cells. TE cells perform their immune function by secreting cytotoxic molecules, such as interferon gamma (IFNg) and perforin (Prf). Metabolic reprogramming is crucial to support proliferation and function upon initial T-cell receptor (TCR) activation in CD8+ T cells. TCR activation promotes glucose uptake and metabolism, supplying energy and redox cofactors in CD8+ cells. In previous studies, glucose restriction of TE cells in vitro improved T-cell longevity and anti-tumour function in vivo, and these phenotypes were associated with altered metabolic pathway activity, higher mitochondrial efficiency and a markedly oxidized redox potential. However, the mechanism by which in vitro glucose restriction augmented in vivo function remains poorly understood. Thus, we questioned how glucose restriction impacts metabolic and nutrient-sensing signaling pathways, and enhances T cell function.

Methods: Mouse OT-I CD8+ T cells were isolated and activated with SIINFEKL peptide. Activated TE cells were exposed to control (10 mM) or limited glucose (1 mM). Anti-tumour function and cytokine production of TE cells were measured by co-culturing with OVA-expressing tumour cell line. In vivo studies were conducted by infusing CD8+ TE cells in tumour-bearing mice, and monitoring tumour growth and the frequencies of circulating donor derived T cells.

Results: We observed glucose-restricted CD8+ TE cells markedly increased IFNg and Prf expression at the mRNA and protein level by TCR restimulation. Single cell RNA-seq. and proteomics data identified glucose-restriction-induced stress response gene expression and p38 kinase activity. Strikingly, the upregulated p38 activity was no longer observed when glucose restricted TE cells were refed. p38 activity was also increased by TCR restimulation, and surprisingly we found that p38 inhibitor (p38i) treatment and shRNA-mediated p38 knockdown blunted restimulation-induced IFNg production in glucose-restricted TE cells. These results suggest that p38 is a glucose sensor and mediates the enhanced cytokine production in glucose-restricted T cells. We next questioned which upstream signal(s) is driving p38 kinase activity during glucose restriction. Reactive oxygen species (ROS) can act as a signaling molecule and studies showed that mitochondrial-derived ROS regulates T cell function. We found that the inhibition of mitochondrial complex III-ROS generation with S3QEL2 during glucose restriction reverted the redox state, reduced p38 activity, and blunted the enhanced TCR-induced cytokine production without altering mitochondrial respiration. Importantly, in vitro pre-treatment of glucose-restricted TE cells with S3QEL2, reduced in vivo anti-tumour function induced by glucose restriction.

Conclusions: Our data suggest that glucose restriction induces mitochondrial ROS and augments p38 signaling-induced increases in cytotoxic molecule production and in vivo anti-tumour function. These findings may provide a new mechanistic insight into metabolic regulation of T cell function.
ABSTRACT

Background/objectives: The ability of red blood cells (RBCs) to deform is central to their proper function and viability. During cold storage, a number of biochemical and metabolic changes occur to the RBC, due to development of “storage lesion”, that impact the ability of RBCs to deform. During transfusions, donor RBCs that have lost their deformability are unable to adequately travel through the microvasculature and are cleared more rapidly by the recipient’s splenic system, necessitating additional transfusions, and increasing complications such as iron overload, and in immunocompromised transfusion recipients; host-versus-graft disease (GVHD). GVHD occurs when the transfused RBC unit still has some of the donor’s lymphocytes present. These lymphocytes can enter the recipient’s circulation, engraft in their bone marrow and mount a catastrophic immunologic response. RBC gamma-irradiation inactivates any remaining lymphocytes in the donor RBC unit and is therefore a potentially life-saving procedure. However, it has been suggested that irradiation can also cause rheological, biochemical, and metabolic changes to RBCs that may reduce their post-transfusion survival in circulation. In this study, we evaluate the rheological properties of irradiated RBCs compared to non-radiated controls to better understand how gamma irradiation affects deformability of stored RBC units.

Methods: ABO-matched pairs of packed RBC units (n=16) were collected and manufactured by Canadian Blood Services Blood4Research facility between July and December 2021. Pairs were pooled and divided to obtain 2 identical units. Bags were stored for 14 days at 4°C, at which point, one bag in the pair was irradiated with 30 GY gamma rays. Bags were then stored for additional 14 days. RBC deformability, standard hematological parameters, and hemolysis rate of both control and irradiated RBC units were measured on the day of RBC unit irradiation, followed by 14 days post-irradiation.

To measure RBC deformability, we used the microfluidic ratchet device. Device consists of a sorting matrix of micrometer-scale constrictions, ranging from 7.5 down to 1.5 µm in size. RBCs are sorted based on their ability to deform and transport through each constriction until they reach a limiting constriction size. Sorted cells are then collected into distinct outlets based on their deformability. Cumulative distribution of cells after sorting is described as a rigidity score (RS) of the cell sample and is used to compare RBC deformability changes between units as well as over time, where lower RS corresponds to a more deformable sample.

Results: We compared the RBC deformability between irradiated and control RBC units. Directly after irradiation, we observed a significant reduction in deformability of the irradiated RBC units (RS=3.05) compared to control units (RS=2.66, mean ΔRS=0.39, P=0.0046). At 14 days post-irradiation, there was further loss of deformability in the irradiated units (RS=3.17), while the deformability of the RBC in the non-irradiated control units stayed constant (RS=2.67, ΔRS=0.49, P<0.0001). Additionally, there was significantly increased hemolysis in irradiated RBC units at 14 days post-irradiation compared to control (P<0.0001).

Conclusions: Gamma irradiation significantly decreases the RBC deformability and increases the hemolysis of cold stored RBC units. Further studies are necessary to explore how the decrease in deformability of the irradiated RBCs correlates to circulation time in transfusion recipients.
AN IN VITRO TRANSFUSION MODEL TO ASSESS COLD-STORED PLATELETS IN TRAUMA

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ABSTRACT Background/objectives: Cold-stored platelets have superior hemostatic effects compared to standard room temperature stored platelets. This finding makes cold-stored platelets an attractive product for actively bleeding patients despite reduced post-transfusion recovery. While in vitro storage characteristics of cold-stored platelets have been thoroughly investigated, there is a significant knowledge gap of their effectiveness in actively bleeding patients. In this study, we use an in vitro transfusion model adapted from the Massive Transfusion Protocol to investigate the function of cold-stored platelets in trauma. Using this model, we assessed the effectiveness of cold-stored platelets to room-temperature stored platelets at restoring hemostatic potential.

Methods: Buffy coat platelets were pooled and split into two identical units. One unit was stored at 22°C under constant agitation (RPs) while the other was stored at 4°C stationary (CPs). RPs or CPs and O+ red blood cells were sampled on day 1,7 and 14 and combined with thawed AB+ plasma in a volume ratio of 1:1:1. Transfusion packages with RPs were labelled warm packages (WTP) while packages with CPs were labelled cold packages (CTP). Whole blood from healthy donors was diluted to 20% hematocrit with saline to simulate hemodiluted patients or mixed with tissue plasminogen activator (8.8 µg/mL) to simulate hyper-fibrinolytic patients. By volume, 70% WTP or CTP was mixed with 30% treated whole blood to simulate transfusion and analyzed by rotational thromboelastometry (ROTEM) with ellagic acid or tissue factor as coagulation initiators. Hemodiluted and hyper-fibrinolytic whole blood alone were also analyzed by ROTEM as controls.

Results: Both transfusion packages restored the clot characteristics of hemodiluted whole blood. Specifically, on day 14, using ellagic acid as coagulation initiator, WTP (69±2mm) and CTP (65±2mm) significantly improved the maximum clot firmness of hemodiluted whole blood (42±5mm) (P<0.0001). There was also a significant reduction in the clot formation time in blood transfused with WTP (63±10s) and CTP (90±7s) compared to hemodiluted whole blood alone (232±27s) (P<0.0001). WTP and CTP also restored the hemostatic potentials of hyper-fibrinolytic whole blood. Of note, on day 14, CTP (68±18%) but not WTP (96±7%) significantly reduced the maximum lysis of non-transfused hyper-fibrinolytic whole blood (91±7%) (P<0.05).

Conclusions: When used in massive transfusion packages, cold-stored platelets can restore the hemostatic potential of trauma patients. Furthermore, they have the potential to significantly improve the hyper-fibrinolytic condition in trauma patients. Together with benefit of significantly reduced bacterial growth during storage, cold-stored platelets can be a superior product for actively bleeding patients.
LYMPH NODE EVALUATION OF COLORECTAL CANCER SPECIMENS: A RETROSPECTIVE SINGLE-CENTER REVIEW

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ABSTRACT  

Background/objectives: Lymph node involvement by metastatic disease is a poor prognostic factor in colorectal cancer. Pathologists play an essential role in accurately determining N stage of colorectal cancer patients. Despite recommendations concerning the minimum number of lymph nodes examined (12), the exact method of pericolonic soft tissue sampling varies between institutions. Previous work has shown that when more extensive sampling does affect N stage, the positive nodes are more likely to be smaller in size. In this study, we sought to compare our institution’s rate of reporting small lymph nodes (<2 mm) that are positive for metastasis, compared to the rate published in studies which have undergone extensive pericolonic soft tissue sampling.

Methods: We performed a retrospective review of all colorectal carcinoma cases with positive lymph nodes in a 6-month period. In these cases, the presence of metastasis and size of nodes with metastatic disease were reviewed microscopically.

Results: 25 cases were identified, which included 67 positive reported lymph nodes. Of these nodes, 20 (30%) were found in lymph nodes less than 2 mm. This proportion is greater than previous reports (3.6% and less than 11.3%). Furthermore, we identified one case where there was an additional positive lymph node not included in the original final diagnosis. Discrepancies that did not affect patient stage, such as total lymph node count, were also identified.

Conclusions: Our study suggests that our institution is likely not routinely missing small positive lymph nodes in colorectal resections with current dissection techniques. Despite the importance of lymph node staging and published guideline, controversies in adequate, accurate assessment of nodes continue to exist. In our short study, there was one patient with upstaged nodal disease after review. Our findings indicate that pathologists should continue to exercise caution and diligence when counting and reporting the number of positive lymph nodes in colorectal resection specimens.
MIXED NEUROENDOCRINE-NON-NEUROENDOCRINE NEOPLASMS OF THE DIGESTIVE SYSTEM: A MINI-REVIEW

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ABSTRACT  

Background/objectives: This mini-review summarizes our current understanding of mixed neuroendocrine-non-neuroendocrine neoplasms (MiNENs) and outlines diagnosis, prognosis, and management of these neoplasms.

Methods: Literature review.

Results: Mixed neuroendocrine neoplasms have been referred to by a long list of names. The latest term, “mixed neuroendocrine-non-neuroendocrine neoplasm”, or MiNEN, captures a wider spectrum of neoplasms than did previous nomenclature. This mini-review summarizes the development of the term MiNEN and reviews current knowledge about the molecular pathogenesis, diagnosis, prognosis, and management of these neoplasms.

Conclusions: MiNENs are clonal neoplasms and their clinical behaviour and management is ultimately determined by the most aggressive component present, which is usually a neuroendocrine carcinoma.
ABSTRACT

Background/objectives: Reconstituted fibrinogen concentrate is considered stable for 8-24 hours based on the product monograph. Given the long half-life of fibrinogen in vivo (3-4 days), we hypothesized that reconstituted sterile fibrinogen protein would remain stable longer than 8-24 hours. Extending the expiry date for reconstituted fibrinogen concentrate could decrease wastage and facilitate reconstitution in advance to minimize turnaround times. We performed a pilot study to define the stability of reconstituted fibrinogen concentrates over time.

Methods: 64 samples of fibryga (Octapharma AG) were reconstituted, frozen, and then thawed and diluted with pooled normal plasma. Diluted fibrinogen samples were stored in the refrigerator (2-8°C) for up to 7 days, with functional fibrinogen concentration measured serially using the automated Clauss method.

Results: Duration of initial freezing had no detrimental effect on functional fibrinogen levels (p = 0.23). Reconstituted fibrinogen samples stored in the refrigerator showed no significant reduction in functional fibrinogen concentration for the entire 7-day study period (p = 0.63).

Conclusions: The ex vivo stability of reconstituted fibrinogen concentrate far exceeds the current expiration limit of 8-24 hours, and instead appears to be stable for up to a week post-reconstitution. With further research, there is the potential to extend the expiry of reconstituted fibrinogen concentrate, which could reduce product wastage and also allow for pre-emptive reconstitution, thereby facilitating reliable and timely delivery of fibrinogen concentrate in clinically urgent scenarios.
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**ABSTRACT**

**Background/objectives:** PRAME (PReferentially expressed Antigen in MElanoma) is a tumor-associated antigen expressed in melanoma as well as sebocytes. The use of PRAME immunohistochemistry (IHC) in the diagnosis of primary cutaneous melanomas (both in situ and invasive) is well-established. Our objective was to validate PRAME IHC for routine use in VGH anatomical pathology service and offer the option of both brown and red chromogen.

**Methods:** We selected surgical pathology specimen blocks from among the archived materials at VGH that we deemed representative of the spectrum of melanocytic neoplasms intended for PRAME IHC, including melanoma metastases to sentinel nodes, various subtypes of primary cutaneous or conjunctival melanomas, as well as a variety of melanocytic nevi. The IHC staining was performed on Dako OMNIS stainer using the Dako EnvisionFlex detection kit per standard protocol. All cases were stained with the standard brown Diaminobenzidine (DAB) chromogen, with a subset of 5 melanoma cases additionally submitted for red chromogen staining.

**Results:** The submitted cases included 5 sentinel nodes with metastatic melanoma, 16 primary cutaneous or conjunctival melanomas, and 15 melanocytic nevi. 5/5 (100%) of the sentinel node metastases were PRAME IHC positive. 14/16 (88%) of the cutaneous or conjunctival melanomas were positive; the negative cases included 1 case of invasive melanoma of lentigo maligna type and 1 case of invasive desmoplastic melanoma. 2 other melanoma cases stained relatively weakly but were easily interpretable. 15/15 (100%) of the melanocytic nevi were PRAME IHC negative. The 5 melanoma cases submitted for red chromogen staining showed an identical pattern of staining to the brown chromogen slides. In all cases where staining was present it was specific to the neoplastic cells, with no aberrant staining of non-melanoma cells.

**Conclusions:** The accuracy of PRAME IHC as performed at VGH is in keeping with that described in published literature. It is validated for use as an ancillary test for the purpose of distinguishing melanoma from melanocytic nevi or detecting lymph node metastases of melanoma.
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ABSTRACT  Background/objectives: Rosette-Forming Glioneuronal Tumor (RGNT) is a rare central nervous system neoplasm, typically associated with the 4th ventricle and FGFR1 alterations. We present a diagnostically challenging case of a cerebellopontine angle Rosette-Forming Glioneuronal Tumor (RGNT) with an FGFR3-TACC3 fusion rearrangement and neuroanatomical location not previously described for this tumor. We review the findings and highlight methylation profiling as an integral component of the diagnostic process.
EPIDEMOLOGY OF CANDIDEMIA IN A CANADIAN TERTIARY PEDIATRIC HOSPITAL: AN 11-YEAR REVIEW

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ABSTRACT

Background/objectives: Candidemia represents a significant cause of morbidity and mortality within the pediatric population. Understanding the local epidemiology of candidemia is essential in aiding clinicians in their empiric therapy choices and implementation of measures to reduce the risk of disease. This study examined the epidemiology over an 11-year period at a Canadian tertiary care pediatric hospital.

Methods: A retrospective chart review was conducted on children with positive blood culture for Candida species between January 1, 2007 and December 31, 2018. Demographic characteristics, Candida species, underlying medical diagnoses, risk factors for candidemia, follow-up investigations and interventions, and outcome data were included in the analysis.

Results: A total of 61 candidemia episodes were included in the final analysis. The most frequent species were C. albicans (54%), Candida parapsilosis (18%), and Candida glabrata (8%). Mixed candidemia was noted in 8% of episodes. The annual incidence rate of candidemia varied, ranging from 3.9 to 9.5 episodes per 10,000 patient admissions. The most common risk factors for candidemia included presence of central venous catheter (95%) and receipt of antibiotics in the last 30 days (92%). Majority of patients received an abdominal ultrasound (89%), ophthalmology consult (86%), and echocardiogram (70%), regardless of age. Line removal was performed in 79% of episodes (n = 58). Evidence of disseminated fungal disease on abdominal imaging was observed in 10% of episodes (all in non-neonates; specifically, 4 receiving immunosuppressants, 1 with gastroschisis, and 1 with a gastrointestinal perforation). The overall 30-day mortality rate was 8%.

Conclusions: The rates of candidemia fluctuated from year to year during our study period. C. albicans was the most commonly isolated species overall. Abdominal imaging should be considered in all patients with relevant risk factors including immunosuppression or underlying gastrointestinal abnormalities.
ORAL PRESENTATION
SAUMADRITAA KAR
SUPERVISOR: DR. BRUCE VERCHERE

PRAMLIINTIDE-EXPRESSING HUMAN-EMBRYONIC STEM CELL-DERIVED BETA CELLS AS A STRATEGY TO IMPROVE ISLET TRANSPLANT FUNCTION

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ABSTRACT
Background/objectives: Type 1 diabetes (T1D) is a devastating autoimmune disease that leads to lifelong insulin dependence and affects over 9 million people worldwide. Transplanting glucose-responsive, insulin-producing β-cells can be a curative therapy; however, the lack of organ donors, need for lifelong immunosuppression, graft failure, and graft rejection hinder widespread clinical implementation. Lack of organ donors may be addressed by inducing human embryonic stem cells (hESCs) to produce mature β-cells, thereby presenting an unlimited source of cells for transplantation.

Islet transplant failure can be exacerbated by the aggregation of islet amyloid polypeptide (IAPP), a peptide hormone co-secreted with insulin from β-cells. Recent studies have found amyloid deposition in islets transplanted into T1D recipients – a pathological feature seen in islets from T2D individuals – which could be a contributor to graft loss and dysfunction. The amino acid sequence in IAPP lends it the propensity to form aggregates that contribute to islet inflammation and β-cell death. Pramlintide is a human IAPP analogue containing proline substitutions in the amyloidogenic region, rendering Pramlintide soluble and non-cytotoxic. We hypothesize that genetically engineered hESC-derived β-cells expressing a non-amyloidogenic form of IAPP such as pramlintide, will lead to human β-cell sources with improved survival and function following transplantation in T1D.

Methods: hESC lines, CRISPR modified and GFP tagged to produce a pramlintide analogue, along with wild-type IAPP (WT) hESCs were differentiated to β-cells suitable for transplant. We confirmed that hESC-derived, pramlintide-expressing, functional insulin-producing cells were generated with gene expression analysis, and insulin secretion. Diabetic immunodeficient mice (n=12) were transplanted with 2.4 million WT cells under the kidney capsule and monitored for graft function 6 weeks post-transplant.

Results: hESCs differentiated into insulin+ (and therefore GFP+) cells were sorted and reaggregated to achieve islet-like clusters. Preliminary transcript analysis indicated expression for markers of β-cell maturation and function in both WT and pramlintide-expressing β cells identical to that of immature human islets. Glucose-stimulated insulin secretion showed a muted response, with an immature functional profile frequently observed using current differentiation protocols. In diabetic mice transplanted with WT β-cells, random blood glucose measured over time signified return to normoglycemia and a detectable C-peptide response at 6-weeks with a glucose tolerance test, compared to mice receiving no transplant.

Conclusions: Our preliminary data suggests pramlintide expression does not impact maturation of hESCs and that WT IAPP β-cells can reverse hyperglycemia in immunodeficient diabetic mice. Future studies will focus on pramlintide detection with specific probes and transplanting pramlintide-expressing β-cells in diabetic mice. We aim to produce a clinically viable cell source for beta cell replacement therapy in diabetes, with the potential to transform the lives of thousands of Canadians living with T1D.
ABSTRACT

Background/objectives: Women living with HIV (WLWH) experience a higher burden of age-related comorbidities and have shorter life expectancy compared to HIV-negative women, which suggests accelerated/accelerated aging. HIV leads to chronic inflammation that may be further exacerbated by persistent viral infections. Healthy aging is also shaped by socio-structural stressors that disproportionately affect WLWH, including economic instability, education, self-medication, and others. The BC CARMA-CHIWOS Collaboration (BCC3) study aims to better understand healthy aging among WLWH by examining molecular, cellular, and social determinants of health. This interim analysis describes prevalence of chronic/latent viral infections, the estimated all-cause mortality risk, and selected social determinants of health in a subset of the BCC3 study participants.

Methods: Prevalence of 9 chronic/latent viral infections was assessed by serology (HIV, hepatitis B and C viruses (HBV, HCV), herpes simplex viruses (HSV-1, HSV-2), Epstein-Barr virus (EBV), human herpesvirus-8 (HHV-8), and cytomegalovirus (CMV)), or self-report (varicella-zoster virus (VZV)). Data about social determinants of health and demographic characteristics were obtained from the BCC3 survey. The Veterans Aging Cohort Study (VACS) index, which estimates 5-year all-cause mortality risk, was calculated based on clinical and demographic parameters.

Results: The age of WLWH (n=72) and controls (n=65) did not differ significantly – median (IQR) 48.3 (41.2 – 57.7) vs 46.6 (27.3 – 55.6), p=0.08. WLWH were more likely to harbour 5/8 viruses studied, namely CMV (76% vs 48%, p<0.001), EBV (97% vs 88%, p=0.047), HSV-2 (68% vs 31%, p<0.001), HCV (32% vs 8%, p<0.001) and HBV (19% vs 5%, p=0.01), but not HHV-8 (4% vs 14%, p=0.07), HSV-1 (74% vs 62%, p=0.15), or VZV (72% vs 82%, p=0.23) compared to controls. WLWH were also more likely to be current smokers (42% vs 27%, p=0.046), but had similar alcohol (53% vs 66%, p=0.12), illicit substance (49% vs 39%, p=0.3), and opiate use (25% vs 14%, p=0.13) compared to controls. However, WLWH were more likely to have experienced homelessness (50% vs 22%, p<0.001), have individual annual income <20,000$ (65% vs 43%, p=0.01) than controls, and less likely to feel extremely safe at home (58% vs 80%, p<0.01), to not have difficulties paying for housing (36% vs 57%, p=0.02), to be currently employed (35% vs 52%, p=0.04), and to be a high-school graduate (69% vs 94%, p<0.001). WLWH had a significantly higher estimated 5-year all-cause mortality risk than controls - median (IQR) 7.8% (3.7 – 18.0) vs 4.2% (3.7 – 8.0), p=0.005.

Conclusions: Despite similar age, WLWH had almost twice the estimated risk of mortality within 5 years compared to controls. This striking difference can be driven by biological variables, such as a higher burden of persistent viruses, or socio-structural challenges, which highlights the importance of studying aging using a holistic approach. With a larger BCC3 sample, identifying the driving factors of accelerated/accelerated aging and mortality risk among WLWH will help close the health gap between WLWH and HIV-negative women, and improve their quality of life.
ORAL PRESENTATION
EMILY KAMMA
SUPERVISOR: DR. JACQUELINE QUANDT

A NOVEL MOUSE MODEL BASED ON A NR1H3 MUTATION LINKED TO PROGRESSIVE MULTIPLE SCLEROSIS IN FAMILIES PRESENTS WITH AN ALTERED PATHOGENIC T CELL PHENOTYPE

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ABSTRACT  

Background/objectives: Multiple sclerosis (MS) is an inflammatory neurodegenerative disease characterized by demyelination and disability following axonal loss. Over 100,000 Canadians live with MS, and 50% have progressive MS that worsens without recovery. Finding treatments to limit progression requires animal models that recapitulate human progressive disease. To address this need, we developed a novel mouse model carrying a nuclear receptor subfamily 1 group H member 3 (Nr1h3) mutation identified as a risk variant in rapidly progressive familial MS. Given NR1H3's important roles in immune modulation, the objective is to determine whether the Nr1h3 mutation predisposes mice to develop a more progressive disease phenotype by shifting the immune profile to one associated with enhanced inflammation and reduced repair.

Methods: Serum in naïve heterozygous (HET) and homozygous (HOM) Nr1h3 mutant vs. wild-type (WT) mice was assessed with enzyme-linked immunosorbent assays for CD5 antigen-like (CD5L), a NR1H3 transcriptional target that modulates T cell and macrophage phenotypes. Naïve serum levels of inflammatory cytokines were screened with Mesoscale assays. Mutant and WT mice were immunized with myelin oligodendroglial glycoprotein (MOG35-55) to induce experimental autoimmune encephalomyelitis (EAE), a preclinical model of MS. Flow cytometry was used to phenotype immune cells and intracellular cytokines at EAE onset (day 10) and peak (day 17). Mesoscale was used to evaluate cell culture supernatants from MOG-reactive spleen cells.

Results: Naïve Nr1h3 mutant mice had reduced serum CD5L levels (WT vs HOM: 3mo p=0.0022, 6mo p=0.0005) and altered serum cytokines consistent with less reparative macrophage phenotypes: increased IL-12 (p=0.034), reduced IL-10 (p=0.013), and reduced IL-30 (p=0.006). In peripheral immune organs, generation of autoreactive effector T cells that produce cytokines such as IFNy, TNFa, and IL-17 are linked to pathology in EAE and MS. Cytokine profiles of autoreactive T cells from the peripheral immune organs in our EAE model at disease onset were largely similar between mutant and WT mice. Subtle differences were observed at peak EAE where splenic T cells challenged with MOG35-55 in HET mice had increased production of inflammatory cytokines (MIP-2, TNFa, p<0.05; IL-17, IL-22, p<0.1). The most striking differences were in the central nervous system at peak EAE. Despite similar numbers of infiltrating T cells observed between HET and WT mice, HET mice had reduced numbers of CD4+ T cells producing IL-10 (p=0.007) and IL-22 (p=0.023). IL-10 and IL-22 are members of the same cytokine family known to dampen neuroinflammation and promote recovery after peak EAE. IL-22 receptor variants are strongly associated with increased risk of MS in humans. This may explain the failure to recover from disability following the primary bout of EAE observed in Nr1h3 mutant mice.

Conclusions: The Nr1h3 mouse model has altered mediators of inflammation and repair relevant in MS. This highlights the association of Nr1h3 dysregulation with progressive MS and helps validate this model for development of novel therapeutics to limit MS progression.
A NEW MIRNA-MODIFIED COXSACKIEVIRUS B3 INHIBITS TRIPLE NEGATIVE BREAST CANCER GROWTH WITH IMPROVED SAFETY PROFILE IN IMMUNOCOMPETENT MICE

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ABSTRACT

Background/objectives: Breast cancer is the most commonly diagnosed malignancy worldwide, accounting for approximately 1.7 million new cases annually and roughly 25% of all adenocarcinomas. Conventional treatments such as radiotherapy and chemotherapy fail to significantly improve overall survival of patients with triple-negative breast cancer (TNBC) (defined as human estrogen receptor negative (ER-)/progesterone receptor negative (PR-)/human epidermal growth factor receptor 2-negative (HER2-)). Oncolytic viruses are a group of genetically engineered viruses with the ability to infect and kill cancer cells while demonstrating little to no impact on healthy tissue. Among different oncolytic viruses, Coxsackievirus type B3 (CVB3) has attracted tremendous attention due to its excellent anti-tumor activity against various cancers. However, potential toxicity of CVB3 administration, particularly cardiotoxicity, restricts its clinical application. One of the strategies to reduce off-target effects of oncolytic viruses is adding targeting sequences of miRNAs that are typically expressed on normal tissues, while their expressions are downregulated in cancer cells into the viral genome. Here, CVB3 was genetically engineered through the addition of miRNA targeting sequences into the viral genome to establish a safe and efficient oncolytic virus that specifically targets breast cancer.

Methods: The miRNA-CVB3 was created by insertion of miR-145, miR-143, miR-1, and miR-216 target sequences (pancreatic and cardiac-specific miRNAs) into the 5' UTR of the CVB3 genome using the plasmid pCVB3/T7. Western blot analysis was performed to evaluate the infectivity of miRNA-CVB3 into different breast cancer cell lines including human TNBC cells, human ER+/PR+/HER2- cells, human ER-/PR-/HER2+ cells, as well as mouse TNBC. MTT assay was conducted to assess the cytotoxic effect of miRNA-CVB3 in all different breast cancer cell lines. Anti-tumor activity of miRNA-CVB3 and its safety were assessed in triple-negative breast tumor (4T1)-bearing Balb/c mice.

Results: Western blot analysis revealed that miRNA-CVB3 can internalize and replicate in all breast cancer cell lines in TNBC cells. Furthermore, MTT assay confirmed that miRNA-CVB3 has substantial cytotoxic effects in these cell lines. Results showed that MCF-7 cells are resistant to miRNA-CVB3. Furthermore, Animal studies revealed that intratumoral injection of miRNA-CVB3 is able to decrease tumor growth in TNBC tumor-bearing mice and increase survival rate compared with the control group and CVB3-treated group. H&E staining results demonstrated that administration of miRNA-CVB3 diminish toxicity caused by CVB3 treatment. In addition, it was shown that administration of both miRNA-CVB3 and WT-CVB3 can elevate immune cells infiltration into the tumor microenvironment.

Conclusions: These results suggest that modification of CVB3 with miRNA targeting sequences can decrease side effects such as pancreatotoxicity that occur after CVB3 treatment. Additionally, miRNA-CVB3 can impede tumor growth in immunocompetent mouse models. Therefore, miRNA-CVB3 might be an efficient treatment for patients suffering from breast cancer.
ABSTRACT

Background/objectives: The management of papillary lesions of the breast diagnosed on core needle biopsy (CNB) is controversial, largely owing to discrepancies in the reported upgrade rates of papillary lesions on excision specimens. Some authors have recommended expectant management with close follow-up in younger women with small non-atypical intraductal papillomas. Therefore, clinical, pathological, and imaging characteristics that are associated with a greater risk of upgrade have become important determinants in guiding treatment decisions following CNB. The aim of this study was to determine the rate of upgrade of all breast papillary lesions diagnosed on CNB from 2017-2019 in the Fraser Health Authority of Greater Vancouver, BC, and determine the factors associated with a statistically significant risk of upgrade.

Methods: This is a retrospective cross-sectional population-based study of 268 biopsy-proven papillary lesions with surgical follow-up. The following parameters were recorded through chart review and review of pathology reports: patient age, lesion size, presence of atypia, clinical presentation, distance from nipple, multiplicity, personal history of breast cancer, and BI-RADS [Breast Imaging–Reporting and Data System] assessment category. Statistical analysis was performed to determine the overall rate of upgrade and association with each factor. All tests were two-tailed, and p < 0.05 was considered statistically significant.

Results: The 268 papillary lesions included 129 (48.1%) benign, 16 (6.0%) atypical, 79 (29.5%) ductal carcinoma in situ (DCIS), and 44 (16.4%) invasive carcinoma cases. Mean age was 61.2 years (range 20 - 96 years). The mean age of patients with benign papillomas was significantly younger (55.2 years) than those with malignant pathology (66.9 years). The upgrade rate in benign papillary lesions to atypia/malignancy was 12/129 (9.3%). Atypia on CNB was significantly associated with a final malignancy diagnosis (p < 0.001), as was peripheral location of the lesion compared to central (p < 0.05). Upgrade to invasive carcinoma was found in 35/125 (28.0%) lesions diagnosed as atypical papillary lesions on biopsy. A final resection diagnosis of invasive carcinoma or encapsulated/solid papillary carcinoma was significantly associated with lesions larger than 10 mm (p < 0.001). BI-RADS category, clinical presentation, multiplicity, and personal history of breast cancer were not significantly associated with a final diagnosis of atypia or malignancy.

Conclusions: The overall risk of malignancy is significantly associated with older age, larger size, and the presence of atypia on CNB. We suggest that close observation without surgery is justified for women <55 years-old with intraductal papillomas without atypia and <10 mm.
CHARACTERIZING THE POPULATION LANDSCAPE OF POST-THERAPY ACUTE MYELOID LEUKEMIA SAMPLES BY MULTIPARAMETER FLOW CYTOMETRY AND HIGH DIMENSIONAL DATA ANALYSIS

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ABSTRACT

Background/objectives: Multiparameter flow cytometry (MFC) is one of the main analytic techniques used for minimal/measurable residual disease (MRD) testing in patients with acute myeloid leukemia (AML). Detecting rare residual AML cells by MFC depends on detailed knowledge of the complex developmental hierarchies of normal myeloid hematopoiesis, yet there are limited resources documenting these patterns in post-therapy marrows. The objective of our study was to characterize hematopoietic population heterogeneity in clinically relevant, post-chemotherapy samples using high-dimensional analysis.

Methods: Bone marrow aspirates from 15 AML patients who were a median 139 days from initial diagnosis were assayed with a 12-colour, three tube AML-MRD panel. All samples were reported as MRD-negative by a separate, clinically validated assay. Uniform Manifold Approximation and Projection (UMAP) was used for dimensionality reduction and visualization. Myeloid and lymphoid subsets were characterized and compared across patients.

Results: In our cohort the median age was 55 and AML with mutated NPM1 was the most common AML subtype (66%). We found that UMAP disentangled the intricate maturation trajectories of myeloid lineages, which facilitated the recognition of different cell subset hierarchies and their associated marker expression patterns. The relative abundance of hematopoietic precursor subsets varied between patients, and we also identified recurrent populations that aren’t well-documented in the current literature.

Conclusions: We report a novel approach for visualizing clinical MFC data from post-therapy AML samples. Our results provide a high-resolution portrait of this real-world bone marrow ecosystem and delineate individual-level heterogeneity.
IDENTIFYING OVARIAN MESONEPHRIC CARCINOMAS, MISCLASSIFIED AS OVARIAN ENDOMETRIOID CARCINOMAS – AN INTEGRATED IMMUNOHISTOCHEMICAL AND MOLECULAR APPROACH

ABSTRACT

Background/objectives: Mesonephric carcinomas (MNC), are associated with mesonephric remnants and typically arise in the lateral wall of the cervix and vagina. Mesonephric-like adenocarcinomas (MLA) are rare tumors arising in the ovary. These tumors are thought to arise from either embryonic mesonephric duct remnants (vestiges of the male reproductive tract), or from Mullerian-derived tumors that have exhibited secondary mesonephric transdifferentiation. Ovarian MLA have been mistaken for endometrioid and clear cell ovarian carcinomas, based on its varied morphologic patterns. MLA have been shown to stain positive for GATA3 and TTF-1 and negative for ER/PR, allowing for accurate histologic classification. From a molecular standpoint, most MLA harbor KRAS and gain in chromosome 1q (with a subset showing concurrent loss of 1p). Two thirds have mutations in chromatin remodeling genes (ARID1A/B or SMARCA4), and they lack PTEN mutations. Due to the rarity of these tumors, the clinical behavior is currently not well studied, although small case series suggest they are aggressive.

The morphologic characteristics of MLA are diverse and there often a wide variety of reported histologic patterns – glandular, solid, papillary, tubulocystic, hobnail, sieve-like, glomeruloid, ductal, retiform, and sex cord. It is important to note that MLA display more than one histologic pattern and can often be misdiagnosed as endometrioid or clear cell carcinoma.

The objective of this study is to identify the number and proportion of MLA that were misclassified as endometrioid in the ovary (ENOC), using an integrated approach of histologic appearance, immunohistochemical staining, and molecular profiles.

Methods: Hematoxylin and eosin (H&E) stained histology slides for cases suspected of being categorized as MLA based on their immunohistochemical (IHC) profile (either GATA3 or TTF-1 positive) using pre-built tissue microarrays (TMAs), were retrieved for further histologic evaluation. These slides were reviewed by three gynecologic pathology subspecialists who were blinded to additional immunohistochemistry and next-generation sequencing data. Based on the histologic features, the pathologists were asked to categorize cases as definite MLA, definitely not MLA, or possible MLA.

Results: A total of 260 cases previously diagnosed as ENOC were examined using TMAs. 42 cases showed GATA3 and/or TTF-1 IHC positivity, and we able to retrieve histologic slides for 26 cases. One case was excluded due to necrosis/non-viable tumor. We identified at least 8 cases of MLA (3.1% of 260 cases). Consensus was reached among all pathologists for 9/25 cases (3 definite MLA, 6 definitely not MLA). ARID1A and PTEN mutation status were also assessed.

Conclusions: Although MLA are rarely encountered in the ovary, they must be considered in the differential diagnosis of endometrioid ovarian carcinomas. A comprehensive overview of gross appearance, multiple histologic subtypes, immunohistochemical staining (positive GATA3 and/or TTF-1, negative ER), and the presence of KRAS mutations are practical features to consider in daily surgical pathology practice.
THE IMPACTS OF A NEW ANTIBODY INVESTIGATION ALGORITHM ON REDUCING FALSE-POSITIVE WARM AUTOANTIBODY DETECTION

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ABSTRACT

Background/objectives: Solid-phase (SP) assays are sensitive methods for antibody screening, though false positives occur as with other automated methods. When the screen is positive, antibody panels determine whether reactions are specific or panreactive. A panreactive panel indicates a warm autoantibody (WAA) when a direct antiglobulin test (DAT) is positive. Our centre transitioned to following positive SP results with more specific methods such as saline indirect anti-globulin test (SIAT) and classifying these as “antibody of undetermined significance” (AUS) if all clinically significant antibodies could be ruled out. This allowed us to differentiate WAA and AUS and reduce the false positivity of WAA detection from SP, as AUS is less associated with clinical hemolysis. Thus, patients with AUS do not require phenotype-matched units or further antibody investigations and transfusion delays can be minimized. We aimed to assess the effect of this new investigation paradigm in September 2019 on immunohematology investigations at our centre.

Methods: Samples from patients investigated for RBC antibodies after a positive screen from September 2017-2021 at our centre were selected for the study. Antibody screening was performed in the SP method (Immucor Galileo NEO and Capture-R Ready-ID, Canada) and confirmed by polyethylene glycol–indirect antiglobulin test (PEG-IAT).

Data extracted include demographics, antibody investigation results (including DAT, presence of AUS, and presence of alloantibodies), and whether transfusion was required for WAA and AUS. Percentages of WAA results were compared before and after implementation, using a chi-square test with a significance level of 0.05.

Results: There were 2,485 antibody investigations performed in 1,925 patients during the study period. A total of 2,237 tests were positive for antibodies including 1,343 alloantibodies (54.0%), 238 unidentified antibodies (9.6%), 77 AUS (3.1%), 123 cold agglutinin (4.9%), 17 drug-related antibodies (0.7%), 94 passive anti-D (3.8%), 266 autoantibodies (10.7%), and 70 combined auto and alloantibodies (3.2%).

After implementing the new antibody investigation algorithm, there was a significant reduction of investigations classified as WAA from 207 (14.1%) to 59 (5.8%), p < 0.001. Of the 189 patients with WAA, 117 (62%) received RBC transfusion.

Of the 77 AUS in 71 patients, 47 (61%) and 30 (39%) were positive and negative for polyspecific DAT respectively; with positive for IgG alone in 40 (85%) and both IgG and C3d in 7 tests (15%). While 59 patients (77%) had AUS identified for the first time, 18 patients (23%) were previously classified as either having unidentified antibodies or WAA. Four patients developed specific RBC alloantibodies with a median time of 91 days (27-144 days post AUS).

Conclusions: A significant reduction in WAA prevalence was observed post-implementation of the new antibody investigation algorithm, leading to less resource use. Some patients developed subsequent RBC alloimmunization, so clinically relevant alloantibodies should be excluded before determining AUS.
ABSTRACT #24

POSTER PRESENTATION - UNDERGRADUATE
LIAM BYRNE
SUPERVISOR: DR. NATALIE PRYSTAJECKY

WASTEWATER SURVEILLANCE FOR MONITORING OF SARS-COV-2 IN METRO VANCOUVER COMMUNITIES, A VITAL TOOL TO COMPLIMENT CLINICAL TESTING

AUTHOR(s) Liam Byrne¹, Melissa Glier², Jennifer Kopetzky³, Tenysha Ross-Van Mierlo⁴, David McVeá⁵, Michael Kuo⁶, Sunny Mak⁵, Natalie Prystajecky¹,⁶

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ABSTRACT

Background/objectives: The COVID-19 pandemic has emphasized the need for innovative testing approaches, including wastewater surveillance, to monitor the prevalence and dynamics of SARS-CoV-2 in the population. While SARS-CoV-2 is detectable in the respiratory tract of infected individuals, it is also shed in the feces of both symptomatic and asymptomatic individuals. Routine diagnostic testing data can be used to estimate community SARS-CoV-2 prevalence but it is biased towards symptomatic individuals and its scope can be constrained by testing guidelines and testing capacity. The reliability of diagnostic testing data to determine community prevalence became evident during the Omicron wave, when diagnostic testing was restricted to target populations and there was increased use of rapid antigen tests. In contrast, measurements of wastewater SARS-CoV-2 concentrations are free from the limitations seen in clinical testing and have been shown, in many regions, to positively correlate with clinical case counts. In this study, methods to detect and quantify SARS-CoV-2 in influent wastewater were developed and SARS-CoV-2 concentrations were compared to the incidence of community COVID-19 cases. The goal of this study was to evaluate the correlation between SARS-CoV-2 concentrations in wastewater and COVID-19 cases to demonstrate the utility of wastewater testing as a viable community surveillance tool.

Methods: A longitudinal prospective study was conducted over the course of 2021 by collecting 24-hour composite influence wastewater samples 1-3x/week from the five wastewater treatment plants located in Metro Vancouver. Wastewater samples were concentrated using 10-kDa molecular weight cut-off centrifugal ultrafiltration devices and nucleic acids were extracted from the resulting viral concentrates. Nucleic acid eluates were tested for the presence of SARS-CoV-2 using real-time Reverse Transcription Quantitative PCR (RT-qPCR). Concentrations of SARS-CoV-2 in wastewater were interpolated using external standard curves. To examine the correlation between SARS-CoV-2 wastewater concentrations and clinical case counts, viral concentrations were first adjusted to wastewater flow and then plotted against the clinical case counts within each wastewater treatment plant catchment area.

Results: Across the time series, 421 of 428 wastewater samples tested positive for SARS-CoV-2. Viral loads in wastewater ranged from 1.54 x 10¹⁰ genomic copies/day to 8.44 x 10¹³ genomic copies/day. The viral load of SARS-CoV-2 showed a positive correlation with clinical case numbers overall, ranging from 0.432 to 0.896 depending on the wastewater treatment plant.

Conclusions: Strong correlations between clinical testing data and SARS-CoV-2 loads suggest that in most Metro Vancouver sewer catchment areas, both surveillance methods yield comparable data. This study demonstrates the ability of wastewater testing to reliably capture and monitor the prevalence of SARS-CoV-2 in communities and serve as a complimentary surveillance tool to clinical testing.
OPTIMIZING DRIED BLOOD SPOT PROCESSING FOR SARS-COV-2 ANTIBODY DETECTION

AUTHOR(s) Ella Chan¹, Bonny So¹,², Brynn McMillan¹,³, Aidan Nikiforuk¹, Tamara Pidduck², Ana Citlali Marquez², Annie Mak², Sofia Bartlett¹,², Muhammad Morshed¹,², Inna Sekirov¹,², Agatha N Jassem¹,²

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ABSTRACT Background/objectives: Blood antibody titre is an important metric of pathogen exposure and immunization. The detection and quantification of specific antibody levels requires serological testing from whole blood, which can be collected by venipuncture or capillary finger prick using proper size lancet commonly known as dried blood spots (DBS). These DBS samples are cost effective, require low sample volume, reduce the risk of infection and are well suited for deployment in remote settings. Moreover, DBS samples are stable at ambient temperatures for up to weeks and can be self-collected, eliminating the need for cold chain transport and trained personnel. Throughout the COVID-19 pandemic, monitoring population level immunity to SARS-CoV-2 has been essential in understanding both naturally acquired and vaccine elicited immunity. Establishment of serosurveillance studies necessitated the optimization of DBS processing and testing protocols to ensure efficient processing and accurate results, which is the main objective of this study.

Methods: Because the finger prick blood is dried on a filter paper card, DBS samples needed to be eluted in a medium that is compatible with the instruments used for serology. We validated a buffer provided by Ortho Clinical Diagnostics that also works on the Meso Scale Discovery (MSD) assay. Diagnostic accuracy was assessed, to ensure that DBS samples were comparable to traditional venipuncture samples. Lastly, to ensure sample quality, long term storage solutions were developed following guidance from a literature review. Overall, these processes created a workflow that covered steps through sample receiving, processing and testing.

Results: DBS optimization resulted in the creation of a standard of practice workflow, which allowed for quality assurance in DBS sample processing and testing. The workflow included steps from sample collection (providing video training and pamphlets for study participants), transport optimization, to elution in a specific buffer that is compatible with both MSD and Ortho machines (dipotassium phosphate buffer with 0.5% sodium azide and 1.5% bovine serum albumin). Storage solutions were also developed to maintain sample quality overtime (-20°C freezer with desiccants, gas impermeable bags and humidity indicators).

Conclusions: The optimization of DBS sample preparation for SARS-COV-2 antibody testing demonstrated that DBS samples is an effective alternative to traditional serological sampling and created efficient and high-quality laboratory testing protocols and workflows. Self-collection of samples and the ease of DBS transportation allows for rural and remote participants to engage in studies that use DBS sample collection. Furthermore, the decreased invasiveness of sample collection provides benefits in pediatric settings.

Future work includes analyzing data from various large-scale provincial SARS-CoV-2 serosurveillance and vaccine efficacy studies that have used DBS to test for the effects of endemic coronavirus antibody titres on the response to natural and vaccine-mediated SARS-CoV-2 immunity.
ABSTRACT

Background/objectives: During the COVID-19 pandemic, hand hygiene has become increasingly important as one of the most effective ways to prevent infections. Although health care worker hand hygiene is a well-established strategy to prevent health care-associated infections, far less attention has been placed on patient and family hand hygiene. Previous audits at BC Children’s & BC Women’s Hospitals found hand hygiene rates to be under 10% among this population. To address this issue, we launched an educational campaign focused on patient and family hand hygiene, with the goal of increasing hand hygiene rates from 10% to 20% in three to six months and to 60% in one year.

Methods: The “Hand Hygiene Heroes” campaign will run from summer 2021 until summer 2023. Phase one involved visual reminders (e.g. posters, stickers) that were designed with input from patient and family partners and disseminated in care areas and online. In phase two, initiatives specific to different areas were developed in partnership with local staff champions. These tailored educational activities met the unique needs of each area. Interventions included front-line staff hand hygiene role models (e.g. performing hand hygiene demonstrations).

Phase three will focus on sustaining quality improvement processes. This will include post-intervention hand hygiene observations to celebrate increased hand hygiene rates in patients and families, and follow-up with champions to evaluate the effectiveness of the area-specific initiatives. Hand hygiene rates will continue to be measured covertly by trained students through direct observation.

Results: Baseline hand hygiene rates from June/July 2021 were 14.3% for patients and families and 54.2% for health care workers. Post-intervention hand hygiene observations will be conducted and analyzed after six months. Currently, we have engaged 9 areas in communications for tailored initiatives, with further outreach ongoing.

Conclusions: Baseline patient and family hand hygiene remains suboptimal; there is significant opportunity to prevent the spread of infections among this population. Competing priorities during the COVID-19 pandemic complicated staff involvement. However, we learned that partnering with areas to develop targeted, safety-focused interventions increased staff enthusiasm. Novel ways to enhance staff engagement and ownership in hospital quality improvement programs can help lead to a long-term culture shift towards greater patient safety.
ABSTRACT

Background/objectives: Cytomegalovirus (CMV) is a human herpes virus whose global seroprevalence ranges from 40-100%. In immunocompromised patients, CMV infection can result in tissue invasive disease, a morbid condition which requires antiviral treatment. This diagnosis requires correlation of clinical findings and pathological findings, such as the identification of CMV viral inclusions on either haematoxylin and eosin (H&E) or immunohistochemical (IHC) stained slides. In this study, we investigate which pathological findings are correlated with a response to antiviral treatment.

Methods: We retrospectively identified pathology samples from Vancouver General Hospital that reported CMV findings (H&E or IHC inclusions) from a three year period (2018-2021). A total of 42 cases were identified. All cases were reviewed for the presence of either H&E or IHC viral inclusions. Patient charts were reviewed to determine treatment and outcome status.

Results: We identified 19 cases which demonstrated H&E viral inclusions. 13 of these cases required antiviral treatment, and 6 did not. We identified 15 cases which only demonstrated viral inclusions on IHC stains. 5 of these cases required antiviral treatment, and 10 did not. 7 cases were identified which showed only indefinite findings on IHC stains, and none of these cases required antiviral treatment.

Conclusions: Our results confirm the need for clinical-pathological correlation in the management of our complex patient population. The presence of viral inclusions on IHC stained slides (alone) was found to be a more sensitive, but less specific, indication for antiviral treatment. Surprisingly, we identified 6 cases which showed convincing viral inclusions on H&E stained slides, but which did not require antiviral treatment. This finding, while contradicting some more traditional views of treating CMV, further confirms the value of clinical-pathological correlation in the management of tissue invasive CMV.
ABSTRACT #28

POSTER PRESENTATION - UNDERGRADUATE
MAHTAB GILL
SUPERVISOR: DR. HELENE COTE

SYSTEMIC EXPOSURE TO MATERNAL HIV/COMBINATION ANTIRETROVIRAL THERAPY AND TOBACCO SMOKING DURING PREGNANCY MODULATES MITOCHONDRIAL DNA CONTENT ACROSS SEVERAL TISSUES IN HIV-EXPOSED BUT UNINFECTED INFANTS

AUTHOR(s)
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ABSTRACT

Background/objectives: Blood mitochondrial DNA (mtDNA) content is a marker of age-related pathologies and can be affected by human immunodeficiency virus (HIV) infection, combination antiretroviral therapy (cART), and tobacco smoking. However, during pregnancy, the cumulative effect of exposure to these factors on infant mtDNA remains unclear. Our aim was to investigate the effect of HIV/cART and maternal smoking during pregnancy on mtDNA content in several infant tissues, among children who are HIV-exposed but uninfected (CHEU) and control children who are HIV-unexposed and uninfected (CHUU). We also aimed to determine whether the effect of HIV/cART and smoking during pregnancy could be observed across all investigated tissues.

Methods: Participants in the study included CHEU and CHUU from 0-3 days of age, who were enrolled in three Canadian cohorts. Children were included in the study if they had at least one of the following five tissue specimens available: peripheral whole blood, cord blood, cord tissue, fetal placenta, and mouth swabs. MtDNA content was evaluated using monochrome multiplex qPCR. Statistical models were adjusted for relevant clinical and sociodemographic factors including infant sex, gestational age, preterm birth, small for gestational age, history of Hepatitis C Virus infection, maternal age at delivery, and maternal ethnicity.

Results: Specimens were assayed from 320 infants, of whom 229 were CHEU and 91 CHUU. Among them, 81 (35%) CHEU and 27 (30%) CHUU were exposed to maternal smoking at any time during pregnancy, and all but one CHEU were exposed to maternal cART. Both maternal HIV-positive status (p = 0.020) and smoking (p < 0.001) were independently associated with higher infant whole blood mtDNA content at birth. However, the effect of maternal smoking was more prominent among CHUU than CHEU. In a similar analysis capturing smoking quantity, the effect of over 100 packs smoked was larger than that of less than 100 packs smoked, suggesting dose dependence. In cord blood, maternal smoking was equally associated with higher mtDNA content (p < 0.001) in both HIV groups, and the increased effect of higher smoking quantity was again observed. Cord tissue mtDNA content was also higher among CHEU than CHUU (p < 0.0001), however no effect of smoking exposure was detected. Neither fetal placenta nor mouth swab mtDNA content were associated with exposure to maternal HIV/cART or smoking. When investigating associations between tissues, whole blood and cord blood mtDNA were the most highly associated (rho=0.59), while mouth swab was moderately associated with blood and cord tissues (rho=0.21-0.24). Fetal placenta was not related to any other tissue analyzed here.

Conclusions: Together, HIV/cART and smoking have systemic but non-uniform effects on mtDNA content in several infant tissues. Given that mitochondrial abnormalities in prenatal life may be linked to diseases in adulthood, further studies are required to characterize the effect(s) of HIV/cART and smoking during pregnancy on health outcomes later in life.
EVALUATING DUAL SPECIFICITY PHOSPHATASE 6 AS A POTENTIAL THERAPEUTIC TARGET IN LUNG CANCER

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ABSTRACT

Background/objectives: Lung cancer cells have adaptive mechanisms to respond to cytotoxics and targeted therapies. Thus, there is a need for novel therapeutics to be developed. Here, we explore dual specificity phosphatase 6 (DUSP6) as a potential drug target for lung adenocarcinoma. DUSP6 is a negative regulator of extracellular regulated kinase (ERK) signaling. In studies using small molecule inhibitors of DUSP6, it has been shown that epidermal growth factor receptor (EGFR) and Kirsten rat sarcoma (KRAS)-driven lung cancers rely on DUSP6 to prevent ERK hyperactivation and subsequent cell death. DUSP6 inhibition has been reported to cause ERK hyperactivation and cell death, a potential therapeutic strategy for lung cancer. In this study, we aim to assess and validate DUSP6 as a therapeutic target in lung cancer.

Methods: To assess the biological significance of DUSP6, we lentivirally transduced shRNAs targeting DUSP6, under the control of a doxycycline (dox)-inducible promoter, into 5 lung adenocarcinoma cell lines. Cell proliferation and ERK hyperactivation were evaluated upon DUSP6 knockdown via crystal violet staining and western blotting, respectively. To understand the effects of removing ERK pathway suppression in the context of DUSP6 knockdown, we maintained cells in the presence of the MEK inhibitor trametinib and assessed cell viability and ERK signaling at various washout time points. In an orthogonal approach to silence DUSP6, we lentivirally transduced Cas9 and sgRNAs targeting DUSP6 to generate DUSP6 knockouts in 5 mutant KRAS or mutant EGFR-driven cancer cell lines. In these cells, we treated with trametinib to assess effects on proliferation and ERK dephosphorylation.

Results: The silencing of DUSP6 by shRNA or CRISPR-Cas9 was not sufficient to hyperactivate ERK to an appreciable extent in most cell lines, suggesting functional redundancy of ERK phosphatases. DUSP6 deletion inhibited cell proliferation in H23 cells but its knockdown did not affect cell proliferation in most lung adenocarcinoma cell lines. In sgDUSP6 H23, H358, H441 and PANC-1 cells, the rate of ERK dephosphorylation was reduced relative to control sgLacZ cell lines.

Conclusions: DUSP6 inhibition via shRNA did not produce ERK hyperactivation and cell death. This may be due to functional redundancy of other negative regulators such as DUSP4. Additionally, the gradual DUSP6 depletion observed in dox-induced shDUSP6 cells may have allowed sufficient time for cells to compensate to maintain the optimal level of ERK signaling. This may warrant the use of small molecule inhibitors which target multiple DUSPs to achieve ERK hyperactivation. The observation that DUSP6 depletion decreases the rate of ERK dephosphorylation but does not cause ERK hyperactivation suggests that inhibiting additional negative regulators of ERK may be needed to hyperactivate ERK. Overall, these findings do not invalidate DUSP6 as a potential therapeutic target, but instead suggest that additional methods and the identification of DUSP6 co-dependencies are needed to evaluate the therapeutic potential of inhibiting DUSP6.
ABSTRACT #30

POSTER PRESENTATION - UNDERGRADUATE
CATHERINE XU
SUPERVISOR: DR. MEI LIN Z. BISSONNETTE

MOLECULAR PROFILING SUGGESTS SIMILARITIES BETWEEN ACUTE AND CHRONIC-ACTIVE T CELL-MEDIATED REJECTION

AUTHOR(s)
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ABSTRACT

Background/objectives: Two types of rejection can affect the kidney allograft: T cell-mediated (TCMR) and antibody-mediated rejection (ABMR). Acute TCMR (aTCMR) has had clear, stable definitions in the Banff Allograft Classification and requires at least moderate interstitial inflammation and moderate tubulitis for diagnosis of aTCMR type IA or IB. Chronic TCMR has not been well characterized but consists of interstitial fibrosis and tubular atrophy (IFTA). Chronic-active TCMR (caTCMR) is an evolving Banff category implemented in the 2017 classification. This category includes the new histologic metric i-IFTA, which refers to areas of IFTA with inflammation, and caTCMR is defined by at least moderate i-IFTA and at least moderate tubulitis. Patients with caTCMR have been found to have increased graft loss. However, the relationship between aTCMR and caTCMR is not clear, as not all caTCMR patient have history of aTCMR. Additionally, it is not known if caTCMR responds to steroid treatment or if treatment affects graft loss. This study compares RNA expression in renal allografts of aTCMR and caTCMR to controls to assess if they may be genetically related entities.

Methods: The provincial renal pathology archive was searched from 2018-2020 for cases of kidney allograft rejection in allografts 6 months or more post-transplant. The case search included all adult for-cause, pediatric for-cause, and pediatric protocol biopsies. Cases with ABMR, mixed rejection, vascular rejection, or another diagnosis (such as polyomavirus nephropathy) were excluded. 11 aTCMR (types IA and IB) and 11 caTCMR cases were identified. For comparison, 6 time-zero implantation biopsies and 12 IFTA-only cases in native kidneys within the same time frame were selected as controls. Total RNA was extracted from archival formalin-fixed, paraffin embedded kidney biopsy tissue using QIAGEN RNeasy FFPE kit (Toronto, ON), and analyzed using nCounter Human Organ Transplant Panel with a 770 mRNA code set (NanoString, Seattle, WA).

Results: 657 mRNAs of the 770 mRNA panel were expressed in all samples and were included in the analysis. In a cluster heatmap of mRNA expression, the samples clustered into three major biopsy groups: 1. implant, 2. IFTA, and 3. aTCMR and caTCMR. Volcano plots of aTCMR and caTCMR versus IFTA mRNA demonstrated similar highly expressed genes in aTCMR and caTCMR. Many cytokines were commonly expressed in aTCMR and caTCMR, including CXCL10, which has proposed use as a urinary biomarker of aTCMR.

Conclusions: Our study suggests that caTCMR is genetically similar to aTCMR due to significant overlaps in their mRNA expression profiles. This finding supports that caTCMR is likely a progression of aTCMR, and raises the possibility that patients with caTCMR may respond to and benefit from treatment with steroids, similar to aTCMR. Additionally, caTCMR and aTCMR showed high expression of distinct genes that may mediate active rejection, including CXCL10. Urinary CXCL10 has been proposed as biomarker to indicate aTCMR, and its high expression in caTCMR raises the possibility of its utility in caTCMR.
POSTER PRESENTATION - UNDERGRADUATE
LYANNE ZHANG
SUPERVISOR: DR. YASIR MOHAMUD

THE ROLE OF CYCLIC GMP-AMP SYNTHASE-STIMULATOR OF INTERFERON GENE PATHWAY IN COXSACKIEVIRUS B3 INFECTION

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ABSTRACT Background/objectives: Coxsackievirus B3 (CVB3), belonging to the genus Enterovirus (EV) of the family Picornaviridae, are small, non-enveloped, single-stranded positive RNA viruses. CVB3 is the most prevalent pathogen causing viral myocarditis, which is the most common cause of heart failure and sudden death in infants, adolescents, and young adults. Despite significant efforts, no clinically validated treatment for this condition is available. Our body relies on the innate immune system to fight against invading viruses. However, CVB3 has been shown to inactivate various immune signalling pathways that canonically suppress RNA viral infection. On the other hand, the cyclic GMP-AMP synthase (cGAS)-stimulator of interferon genes (STING) DNA sensing pathway senses cytosolic pathogenic DNA or mislocalized self-DNA to initiate downstream immune responses. Although a large body of research has focused on the interplay between DNA viruses and DNA sensors, emerging evidence highlights an antiviral role for the cGAS-STING pathway against RNA viral infection.

Methods: In this study, we used gene silencing and overexpression of the cGAS-STING pathway in combination with CVB3 infection to evaluate the respective roles. The role of cGAS-STING was also studied with various chemical activators (cGAMP, diABZI, poly dA:dT) and inhibitors (H-151, RU.521). Viral replication and viral protein production were assessed by TCID50 and western blotting respectively. ELISA was also used to measure cGAS activation following CVB3 infection.

Results: We showed that, upon activation, the cGAS-STING pathway showed robust suppression of CVB3 replication, and silencing of cGAS and STING rescues viral replication, confirming the potential of cGAS-STING against RNA viral infection. Mechanistically, we demonstrate that CVB3 infection disrupts mitochondria integrity and promotes mtDNA mislocalization. Nevertheless, we showed that CVB3 infection of HeLa cells does not activate STING and the downstream immune response, suggesting that CVB3 develops strategies to target this pathway for immune evasion.

Conclusions: In summary, our study suggests CVB3 evades the anti-viral function of cGAS-STING to facilitate disease progression. The mechanism by which this is accomplished is currently under investigation.
ABSTRACT

Background/objectives: Multiple sclerosis (MS) is a chronic, inflammatory, and neurodegenerative disease affecting the central nervous system. Disability is caused by inflammatory demyelination and axonal loss, highlighting the need for approaches that protect axonal components to promote recovery. Neuronal PAS-domain containing protein 4 (NPAS4) is a neuronal transcription factor activated by calcium transients with key roles in development and survival, primarily via its dimerization with aryl hydrocarbon receptor nuclear translocator 2 (ARNT2) to produce neuroprotective factors. We showed Npas4 increases preclinically as the earliest immune mediated CNS insults occur in experimental autoimmune encephalomyelitis, a mouse model of MS. Surprisingly, Npas4 deletion protected against disability in this model. Since high axonal calcium levels caused by immune cell infiltrates precede axonal loss in neuroinflammatory lesions early in EAE and later in cortical regions associated with MS progression, we sought to establish an in vitro model to assess regulation of NPAS4 expression by inflammatory mediators and associated impacts on neuronal viability and function.

Methods: NPAS4 and ARNT2 expression in brain cortical and subcortical regions were examined using immunohistochemistry in NPAS4 wild type mice. To model this in vitro, primary neuron-enriched cortical cultures were established from embryonic day 16-17 mice. NPAS4 expression levels at 3, 7, 10 and 14-days in vitro (DIV) were assessed in untreated cells versus excitatory stimuli.

Results: NPAS4 expression levels in the CNS of young adult mice are generally low. Expression in vivo is restricted to neuronal cells, with highest levels in the somatosensory, motor, and other cortical areas as well as the olfactory bulb, sporadic cells in the hippocampus, and cerebellar cortex or hindbrain, with negligible staining throughout other grey matter regions. In contrast, ARNT2 was expressed in all regions of the CNS at moderate to high levels, and was particularly high in glia. However, colocalization with NPAS4 expressing neurons was only observed in cortical regions. Our in vitro cortical cell model displayed increasing dendritic/axonal complexity over time, with less than 15% glial contamination. Mirroring the in vivo findings, NPAS4 expression was low to moderate in a small subset of neuronal cells in DIV3-10 cultures. By DIV14, NPAS4 expressing neurons were nearly negligible. Excitatory stimuli such as KCl induced NPAS4 expression in >80% of neurons across all cultures, nearly doubling NPAS4's mean fluorescent intensity compared to untreated cells, while 4-aminopyridine induced expression in approximately half of the cells. ARNT2 was detected in the majority of cells, but coexpression with NPAS4 in neurons was rarely observed.

Conclusions: Enriched neuronal cortical cultures model NPAS4 and ARNT2 expression patterns observed in vivo. Through our use of in vitro and in vivo models and techniques, we aim to characterize the functional relevance of Npas4 and its partners to neurodegenerative processes in MS in hopes of developing therapies that limit axonal damage and loss and promote recovery.
POSTER PRESENTATION - GRADUATE  
VRITI BHAGAT  
SUPERVISOR: DR. BRUCE VERCHERE  

THE IMPACT OF INHIBITING PROHORMONE CONVERTASES ON HUMAN PROIAPP PROCESSING

AUTHOR(s)  
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ABSTRACT  
Background/objectives: The beta-cell prohormones, proinsulin and proIAPP, are processed to their mature biologically active forms, insulin and islet amyloid polypeptide (IAPP), in the beta-cell secretory pathway. This process involves the prohormone convertases PC1/3 and PC2, carboxypeptidase E (CPE), and peptidyl-glycine alpha-amidating mono-oxygenase (PAM). Both type 1 and type 2 diabetes patients exhibit persistent secretion of proinsulin and proIAPP forms, indicating that processing of beta-cell prohormones is impaired in diabetes. The current understanding of the mechanism of beta-cell prohormone processing has mainly been derived from rodent studies. These studies have shown that both Pc1/3 and Pc2 mediate proinsulin and proIAPP processing in mice; however, recent data suggest important differences may exist in proinsulin processing in human versus rodent beta cells. Specifically, a recent study suggested that PC1/3 is essential for proinsulin cleavage in human beta cells and that PC2 plays little to no role in human beta-cell prohormone processing. Our studies in mice have demonstrated that while Pc1/3 is sufficient for complete proinsulin processing, it is more efficient with Pc2 present. Additionally, we have shown that Pc2 is necessary for complete proIAPP processing in mice. We hypothesize that as in mice, PC2 plays an essential role in processing of proIAPP in human beta cells, and knockdown of PC2 will result in accumulation of the processing intermediate, proIAPP₁⁻⁴₈.

Methods: To elucidate the role of PC2 in human beta-cell prohormone processing, here we propose to manipulate PC2 activity in primary human beta cells using enzyme inhibitors or CRISPR-based genome editing to knockdown PC2 expression. The levels of proIAPP₁⁻₆₇, proIAPP₁⁻⁴₈, and mature IAPP will be detected by western blot and enzyme-linked immunosorbent assay (ELISA). Amyloid formation will be assessed by fluorescence imaging of islet sections stained with thioflavin S.

Results: The findings of this proposed study will address knowledge gaps in the field on whether PC2 is critical for proIAPP processing in humans.

Conclusions: This study will enable better understanding of the role of PC2 in human beta-cell proIAPP processing, and determine whether inhibition or knockdown of PC2 will result in amyloid formation. The results from this study may have implications for identifying new targets for therapeutics for the treatment of type 1 and type 2 diabetes.
ABSTRACT

Background/objectives: Ovarian carcinoma has the highest mortality of all female reproductive cancers and current treatment has become histotype-specific. Pathologists diagnose five common histotypes by microscopic examination, however, histotype determination is not straightforward, with only moderate interobserver agreement between general pathologists (Cohen's kappa 0.54-0.67). We hypothesized that machine learning (ML)-based image classification models may be able to recognize ovarian carcinoma histotype sufficiently well that they could aid pathologists in diagnosis.

Methods: We trained four different artificial intelligence (AI) algorithms based on deep convolutional neural networks to automatically classify hematoxylin and eosin-stained whole slide images. Performance was assessed through cross-validation on the training set (948 slides corresponding to 485 patients), and on an independent test set of 60 patients from another institution. The utility of color normalization and partially balancing the training dataset across the histotypes was also explored.

Results: The best performing model achieved a diagnostic concordance of 81.38% (Cohen's kappa of 0.7378) in our training set, and 80.97% concordance (Cohen's kappa 0.7547) on the external dataset. Eight cases misclassified by ML in the external set were reviewed by two subspecialty pathologists blinded to the diagnoses, molecular and immunophenotype data, and ML-based predictions. Interestingly, in 5 of 8 cases from the external dataset, the expert review pathologists rendered diagnoses, based on blind review of the whole section slides classified by AI, that were in agreement with AI rather than the integrated reference diagnosis.

Conclusions: We demonstrate a deep learning strategy for ovarian carcinoma histotype classification based only on histological features that is generalizable even on an externally stained test set. The performance is at a level that it could be implemented into practice, for validation. This approach holds potential as an adjunct for informing histotype diagnosis and in supporting histotype-specific ovarian cancer treatment.
ABSTRACT #35

SEVERAL HIV INTEGRASE INHIBITORS AFFECT MITOCHONDRIAL HEALTH AND DECREASE PROLIFERATION IN PERIPHERAL BLOOD MONONUCLEATED CELLS

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ABSTRACT

Background/objectives: HIV requires lifelong treatment, and with over 37 million people living with HIV, it is imperative to fully characterize the safety of antiretrovirals (ARVs). Some older HIV ARVs can damage mitochondria and affect their function. The relatively new drug class of HIV integrase strand transfer inhibitors (InSTI) are popular among people living with HIV for its high tolerability, few side-effects, and low pill burden. However, less is known about their mitochondrial toxicities compared to older ARVs. The InSTI dolutegravir has been associated with weight gain in adults, which may reflect changes in cellular metabolism governed by mitochondria. Mitochondrial toxicity of recently approved InSTIs bictegravir, elvitegravir/cobicistat, and cabotegravir remain unclear. Our aim was to characterize the effects of InSTI exposure in cultured immune cells on mitochondrial health, cellular activation, and proliferation.

Methods: PBMCs from healthy volunteers were activated with anti-CD3/CD28 for 6 days while exposed to InSTIs at 1xCmax (peak plasma concentration) in 0.1% DMSO. Mitochondrial intermembrane potential (MMP), reactive oxygen species (mtROS), and mass (mtmass), along with cellular proliferation, apoptosis, differentiation, and activation were determined by flow cytometry. Significance was determined using paired t-tests.

Results: Compared to DMSO (n=9 biological replicates), bictegravir exposure had the most pronounced effect, with greatly decreased mtmass (p<0.001), mtROS (p<0.001), MMP (p<0.001) and arrested proliferation (p<0.001). Elvitegravir/cobicistat also decreased MMP (p<0.001) and proliferation (p<0.001). In contrast, dolutegravir and cabotegravir both increased MMP (p<0.045), while raltegravir had no effect on any parameters. In a pilot experiment, bictegravir exposure appeared to elevate early and middle but decrease late cellular activation markers compared to controls and other InSTI treatments.

Conclusions: These data clearly show that InSTIs can affect mitochondria in PBMCs. Furthermore, the effects of bictegravir ex vivo suggest a potential underlying metabolic mechanism which could hinder immune responses. It is imperative to investigate the effect of InSTIs as certain toxicities may not be apparent nor revealed by clinical trials but may exert long-term immunological and health consequences.
DISTRIBUTION OF MITOCHONDRIAL DNA MUTATIONS IN LYMPHOCYTE SUBSETS OF PEOPLE LIVING WITH HIV

ABSTRACT

Background/objectives: People living with HIV experience accelerated aging compared to the general population. Although the mechanisms are unclear, increased oxidative and immune stress caused by HIV are potential factors. Mitochondrial DNA (mtDNA) is prone to elevated mutation rates due to the mitochondrial environment being highly oxidative, as it is the site of oxidative phosphorylation, and the fact that mitochondrial polymerase gamma (POLG) is error-prone. MtDNA mutations are associated with aging and age-related diseases, though it is unclear which is the driving force behind the other. Studies of mtDNA mutations are often limited to high frequency (>1-2%) variants, and few have aimed to identify or quantify rare mutations. As lymphocyte subset composition changes with age and with immune activation, the contribution of distinct subsets is unknown. In this novel study, we investigate low frequency mtDNA mutations, that could be induced by HIV infection or its treatment, in subsets of sorted lymphocytes of people living with HIV and uninfected controls.

Methods: We isolated CD4+ T cells, proliferative and senescent CD8 T cells (CD28+ and CD28- respectively), as well as B cells (CD19+) from 49 people living with HIV+ and 40 HIV-negative controls, using fluorescence-activated cell sorting. We then used unique molecular identifier (UMI) next-generation sequencing assay to interrogate a 264bp region of the mitochondrial D-loop, a highly variable and non-coding regulatory region of mtDNA. This sequencing was performed on the four subsets as well as whole blood.

Results: We identified rare mutations present at frequencies as low as 0.05%, and 3 regions containing multiple potential mutational hotspots observed across all subsets. All four subsets showed highly concordant mutation distributions with each other and with whole blood. However, total mutation burden differed between subsets (p=0.022), with B cells showing elevated mutation burden compared to T cells. In people living with HIV, we observed a trend toward increased mutations present above 2% compared to HIV-negative controls.

Conclusions: Our novel findings offer the first glimpse into the mtDNA mutation burden of lymphocyte subsets and validate the use of whole blood in further studies. Our data suggest a potential association between HIV and cell-specific mtDNA mutations, and the identification of specific mtDNA regions that may be more affected, highlighting the need for further investigation.
POSTER PRESENTATION - GRADUATE
HONOR CHEUNG
SUPERVISOR: DR. CHERYL WELLINGTON

USING LIGHT SHEET MICROSCOPY TO INVESTIGATE THE ROLE OF APO-LIPOPROTEIN E4 IN TRAUMATIC VASCULAR INJURY

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ABSTRACT Background/objectives: Apolipoprotein E4 (APOE4) is the major genetic risk factor for sporadic Alzheimer’s Disease (AD). Even in the absence of AD diagnosis, the APOE4 allele has emerged as a risk factor for small vessel disease and vascular cognitive impairment. The role of vascular injury sustained via traumatic brain injury (TBI) has been well established, where multiple secondary pathways triggered upon traumatic vascular injury contribute to neuronal damage and subsequent cognitive decline. We are using the Closed-Head Impact Model of Engineered Rotational Acceleration (CHIMERA) to study APOE isoform effects on traumatic vascular injury. As CHIMERA is a non-surgical model that produces diffuse brain injury, we have developed tissue clearing methods to assess whole-brain traumatic vascular injury. We aim to test the hypothesis that APOE4 contributes to greater vascular damage compared to APOE3 controls in targeted replacement mice with and without CHIMERA TBI.

Methods: Mice expressing human APOE4 and APOE3 (Cure Alzheimer Fund) are bred in-house and randomized to TBI or sham groups at 6-7 months of age. TBI animals receive a 3.0J impact at 6-7 months of age; sham controls receive all procedures except for impact. To enable 3D mapping of cortical and subcortical vascular damage after TBI, mice are transcardially perfused with Alexa Fluor 594 conjugated wheat germ agglutin (WGA) and brain tissue is subsequently cleared using the SHIELD passive clearing protocol and imaged using light sheet microscopy.

Results: Pilot experiments with APOE3 mice revealed dramatically altered vascular anatomy 1 day after TBI, including hematoma and a greatly disorganised cortical vascular network compared to sham controls. We have established an analytical pipeline for 3D quantification of vascular density, branching and tortuosity. We are currently expanding our colony to examine the effect of APOE genotype on traumatic vascular injury outcomes at acute and chronic timepoints.

Conclusions: Our preliminary findings establish tissue clearing as an efficient tool for the assessment of vascular injury sustained after TBI.
IDENTIFICATION OF A NEW ENDOMETRIAL CANCER SUBGROUP WITH INFERIOR OUTCOME USING ARTIFICIAL INTELLIGENCE-BASED HISTOPATHOLOGY IMAGE ANALYSIS

AUTHOR(s)  
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ABSTRACT  
Background/objectives: Endometrial cancer (EC), also referred as uterine carcinoma, is the most common gynecologic malignancy in the developed world. In contrast to histopathological assessment, molecular categorization of EC provides a reproducible classification system with significant prognostic value. The Proactive Molecular risk classifier for Endometrial cancer (ProMisE) was introduced by our team as a practical, cost-efficient, and therapeutically useful molecular classifier, replacing the complex genomic methods. ProMisE stratifies EC into four subtypes: (i) POLE mutant (POLEmut), (ii) mismatch repair deficient (MMRd), (iii) p53 abnormal (p53abn) by immunohistochemistry, and (iv) NSMP (No Specific Molecular Profile), which lacks any of the distinguishing features of the other three subtypes. Despite the fact that ProMisE has provided significant prognostic value, there are variations in clinical outcomes within its four subtypes. This is especially evident in the largest ProMisE subtype, NSMP (which accounts for about half of all ECs), where a fraction of patients encounter a very aggressive disease course, similar to the behaviour of patients diagnosed with p53abn.

Methods: We considered the problem of refining the EC NSMP subtype using ubiquitous histopathology images, an accessible and often mandatory routine in cancer diagnosis. We hypothesized that examination of the digital hematoxylin and eosin (H&E)-stained images of NSMP could possibly discern clinical outcome outliers. Therefore, we designed an image analysis framework, utilizing artificial intelligence (AI), to detect NSMP patients with comparable histological characteristics to the p53abn subtype. We employed a discovery cohort of 182 and an independent validation cohort of 195 NSMP ECs.

Results: Our AI-based image analysis framework, determined that 21 (11.5%) of the 182 NSMP patients had analogous histomorphological features to p53abn patients. We call these patients ‘p53abn-like’ NSMPs. In contrast to the rest of the NSMP cases, these cases had noticeably inferior disease-specific survival (DSS) (10-year DSS 58.9% vs. 93.1% (p<3.44e-8)) and progression-free survival (PFS) (10-year PFS 55.1% vs. 91.4% (p<3.76e-6)). These findings were replicated in the validation cohort, by categorizing 21 (10.7%) of the 195 patients as ‘p53abn-like’ cases with 10-year DSS of 51.3 vs. 82% (p<5.28e-5) and PFS of 56.6 vs. 89.3% (p<2.15e-4).

Conclusions: With exploiting an AI-based method, we have expanded the NSMP subtype into two subgroups: ‘p53abn-like’ NSMPs, resembling morphological features of p53abn, and the rest of the NSMP cases. With following the same trend in two independent cohorts, ‘p53abn-like’ NSMPs display comparable clinical behaviour to p53abn where they have markedly unfavourable outcomes in comparison with the remainder of the NSMP cases. These findings highlight the prospects of AI screening as an additional stratification tool within the ProMisE molecular classifier.
Background/objectives: Biofluid markers specific for TDP-43 pathology are highly desired given the challenge to distinguish (ante-mortem) frontotemporal lobar degeneration with TDP-43 pathology (FTLD-TDP) from phenotypically related disorders. TDP-43 post-translational modifications, like C-terminal fragments, are regarded as disease-specific TDP-43 proteoforms; however, the exact structure of these proteoforms remains unclear. This lack of clarity is due in part to the use of instrumentation and techniques with low structural resolution and the study of small sample sizes. With this in mind, we performed high resolution mass spectrometry (HRMS) analysis of brain tissue from cases with and without TDP-43 proteinopathy to identify TDP-43 proteoforms unique to FTLD-TDP.

Methods: HRMS was used to determine TDP-43 proteoform composition in insoluble frontal lobe brain tissue from immunohistochemically confirmed FTLD-TDP (n=13), related dementias without TDP-43 deposits (n=10) and neuropathologically unaffected controls (n=3). Brain tissue was fractioned by gel electrophoresis, with HRMS analysis performed on molecular weight regions corresponding to <28 kDa (low molecular weight TDP; L-TDP), 28-38 kDa (mid molecular weight TDP; M-TDP), and 38-55 kDa (intact TDP; I-TDP).

Results: HRMS analysis revealed TDP-43 sequence coverage was greatest for I-TDP, followed by L-TDP and then M-TDP, in all samples tested. TDP-43 peptides from L-TDP were more frequently detected in the FTLD-TDP cases compared to both sets of controls, whereas no frequency differences were observed for I-TDP and M-TDP. Quantitative analysis revealed peptide concentrations from M- and L-TDP were significantly increased in FTLD-TDP cases compared to controls. Using the concentration of L-TDP, we were able to differentiate FTLD-TDP cases from related dementias and unaffected controls with 78% sensitivity and 100% specificity. Further, in vivo cleavage sites of TDP-43 were identified, which were unique to FTLD-TDP cases and supported by the recently published cryo-electron microscopy structure of TDP-43 fibrils.

Conclusions: This is the largest reported proteomics study to date of histology-confirmed FTLD-TDP. This is also the first study to include a large number and range of control tissues, and to provide supporting evidence for identified in vivo cleavage sites. Clarity and consensus on the sequence of TDP-43 disease-specific proteoforms will be helpful in advancing biomarker and drug discovery efforts for TDP-43 proteinopathies.
ABSTRACT

**Background/objectives:** Type 1 diabetes mellitus (T1D) is a devastating disease in which the β-cells in the pancreas produce little to no insulin due to their autoimmune destruction. The gut microbiome has emerged as a potential driver of autoimmunity against the beta-cell in T1D. A recent clinical study showed that in new-onset T1D individuals, autologous fecal microbiota transplantation (FMT), preserved β-cell function for over 12 months (de Groot et al, 2020). To gain insight into the mechanism underlying this protective effect, we will assess the impact of human FMT in the non-obese diabetic (NOD) mouse model. We hypothesize that FMT from human will lead to durably improved beta-cell function and less insulitis in NOD mice.

**Methods:** In order to determine whether diabetes incidence in NOD mice is affected by FMT from individuals with T1D, feces from human T1D recipients of FMT, including both responders (those whose diabetes was delayed) and non-responders, will be transplanted into 6-week old NOD mice pre-treated with antibiotics. Blood glucose will be monitored and following development of diabetes or at 21 weeks of age the microbiota and pancreas of these mice will be assessed by metagenomic analysis and histology, respectively. Beta-cell function will be followed by glucose tolerance test and proinsulin: C-peptide ratio. Quantification of beta cell mass and other inflammatory cell infiltrations within the pancreatic islets will be done by immunostaining.

**Results:** Prior to commencing NOD mice experiments, we measured serum C-peptide and proinsulin levels from T1D individuals using enzyme immunoassays at different timepoints before, during and after FMT. The proinsulin to C-peptide ratio (PI:CP), as a measure of β-cell dysfunction, revealed that even before FMT, T1D individuals who responded well to FMT treatment had significantly lower PI:CP ratios (p<0.05). These data suggest that PI:CP ratios could be a marker of how well persons with T1D will respond to FMT.

**Conclusions:** As we continue this study, we aim to further understand the effects of FMT on pancreatic tissues, revealing at the molecular level how FMTs lead to improved β-cell function. A better understanding of the role of the microbiome in T1D pathogenesis could lead to the development of microbiome-based therapies for T1D.
Background/objectives: Clear Cell Ovarian Carcinoma (CCOC) is an uncommon ovarian carcinoma which accounts for about 12% of all ovarian cancers in North America. CCOC has the worst prognosis if it is diagnosed at an advanced stage, and it is inherently resistant to chemotherapy. ARID1A, a subunit of SWI/SNF chromatin remodeling complex, is frequently mutated in CCOC. Our lab has discovered inactivating mutations of ARID1A in about 50% of CCOC, often together with a PIK3CA activating mutation. CCOC is strongly associated with endometriosis which is functional endometrial tissue outside of the uterine cavity. ARID1A mutation occur with high prevalence in precursor lesions adjacent to CCOC suggesting this mutation to be an early event in tumor development. PIK3CA mutation has been identified as an early event in CCOC as well. While loss of Arid1a alone is not sufficient for tumorigenesis in mice, the addition of a Pik3ca H1047R activating mutation initiates tumour development in the ovary in a mouse model of CCOC. In this project I use organoid culture of human endometrium to model early initiation events of cancer progression. Organoid culture is a specialized 3D cell culture system that favours the stimulation of progenitor/stem cells to differentiate. The CCOC field would benefit from additional model system to understand the initiation events that contribute to transformation. This project will focus on understanding how mutations and extracellular environment converge to initiate oncogenic transformation and promote tumour progression.

Methods: I derive organoid cultures from dissociated primary normal human endometrium tissue in which ARID1A is knocked down using CRISPR-Cas9, and mutant PIK3CA is introduced using lentiviral transductions. I dissociate the organoids and perform single cell RNA and ATAC sequencing using the 10X genomics platform on the organoids. The transcriptomes and chromatin accessibility compared between uninfected, single or double mutant conditions to elucidate the mechanisms underlying oncogenic transformation.

Results: Double mutant human organoids demonstrate phenotypic differences compared to the non transduced ones. The mutant organoids are 3 times larger than the uninfected organoids and at later passage, the organoids manifest CCOC histopathology, including hobnail cells in H&E staining, and positive Periodic acid–Schiff (PAS) staining (a clinical CCOC stain). Single cell gene expression profiles from three experiments showed downregulation of tumor suppressor cellular retinol binding protein (CRBP1) (FDR= 2.0199812400260, log FC= -2.8072610279928) which correlates with ATAC-seq data. These data are further validated by Western blot in CCOC cell lines and by IHC in endometrial organoids.

Conclusions: Understanding early initiation events may lead to early detection of CCOC, which would increase survivorship. The preliminary results demonstrated the importance of vitamin A signaling pathway in mutant cells. Recent publications have shown how this signaling pathway can affect immune cells development and immunotherapy in cancer. We will validate this effect more in our system.
ABSTRACT

Background/objectives: Acute lymphoblastic leukemia (ALL), a bone marrow malignancy caused by the uncontrolled growth of precursor T- or B-lymphoblasts, is the most common pediatric cancer in Canada. Although pediatric ALL is highly treatable due to advances in risk-directed chemotherapy, some patients will develop chemoresistance and the prognosis for relapsed disease remains poor. Recently, the lectin co-chaperone proteins calreticulin (CALR) and PDIA3 are increasingly implicated in various human cancer biological processes, along with their main role of facilitating protein folding in the endoplasmic reticulum (ER). However, the role of ER lectin chaperones in the context of ALL remains scarcely discussed. Work in our laboratory have demonstrated that cytosolic CALR is associated with STAT3 activation in Jurkat T-lymphoblasts, suggesting these lectin chaperones may promote oncogenic signaling. Therefore, this study aims to investigate the potential prognostic significance of the lectin chaperones CALR and PDIA3 by evaluating their mRNA expression profile in pediatric ALL patients.

Methods: The clinically annotated datasets used in this study were obtained from the National Cancer Institute Therapeutically Applicable Research to Generate Effective Treatments (TARGET) program. Two pediatric patient cohorts consisting of 264 T-ALL and 177 relapsed high-risk B-ALL were used in the analyses. Tumour samples from bone marrow or peripheral blood were collected at diagnosis and RNA-sequencing was performed on tumour cells within the TARGET study. Gene expression levels were stratified into high and low groups based on the median. The presence of minimal residual diseases (MRD) on day 29 is used as an indicator of poor treatment response. Kaplan-Meier survival analyses were carried out for the B-ALL cohort.

Results: In T-ALL (n=264), CALR (p<0.0001) and PDIA3 (p=0.02) expression were differentially expressed across the different molecular subgroups. Notably, CALR expression was elevated significantly in the LMO2/LYL1 molecular subgroup. CALR (p=0.002) and PDIA3 (p=0.04) expression were significantly higher in T-ALL patients with MRD. Contingency analyses showed that T-ALL patients with high CALR are at a higher risk of developing MRD (Relative Risk=1.556, p=0.0098). In B-ALL, CALR expression inversely correlates with 5-year overall survival rate (p=0.028, n=177), but not PDIA3.

Conclusions: LMO2, a key hematopoietic transcriptional regulator, is known to interact with STAT3 protein. High CALR in the LMO2/LYL1 subgroup implicates a potential oncogenic mechanism whereby increased levels of LMO2 and CALR may act cooperatively to activate STAT3 signaling in T-ALL. Also, T-ALL patients with high CALR are associated with increased risk of developing MRD, suggesting these patients are at a higher risk of adverse outcome and receiving more aggressive treatment regimens. Also, high CALR is correlated with poor survival in B-ALL. Thus, analyzing CALR expression of diagnostic ALL tumour samples may provide insight on clinical treatment response.
ABSTRACT

Background/objectives: Chronic graft-versus-host-disease (cGvHD) is a major cause of morbidity and mortality after Hematopoietic Stem Cell Transplantation (HSCT). In 3 large human HSCT patient cohorts (approximately 500 patients) we identified increased numbers of a CD56bright, Granzyme B-, perforin- natural killer (NK) cell population associated with the absence of cGvHD. We hypothesized that this cell population, consistent with previously described regulatory NK cells (NKregs), is important in the induction of operational immune tolerance after HSCT. Our objective was to effectively isolate and characterize the NKreg cells according to phenotype and function to define the NKreg population associated with a lack of cGvHD.

Methods: We utilized HSCT patient blood samples (n=12) to identify the unique transcriptome of CD56bright NK cells associated with immune tolerance using nanoString. To both phenotype the NK cells and determine the optimal cell surface markers for sorting NKreg cells, based on genes determined to be associated with immune tolerance (nanoString study), healthy donor PBMC samples were stained for identification of the proteins CD56, CD3, CD16, perforin, Granzyme B, Granzyme K, GPR183, CD127, CD62L, and CXCR3. To investigate the suppressive capacity of NKreg cells against allogeneic CD4+ or CD8+ T cells, NKreg cells (and the CD56dim NK cell and Treg cell controls) were isolated and co-cultured with CD3/CD28 activated CD4+ or CD8+ T cells. To determine if the suppression occurs through killing, the FITC Annexin V apoptosis detection kit was utilized. Additionally, the cytolytic ability of the NK cells was verified using a standard NK cell versus K562 cell killing assay. To determine if the suppression is contact dependent, the suppression assay procedures were followed using a 96-well transwell plate. Further, the soluble NKp44, NKp46, and GPR183 blockers were added to the suppression assay to determine the receptor dependence.

Results: NKreg cells phenotypically associated with cGvHD suppression can be sorted with CD56 and CD16 cell surface antibodies (>95% purity). These NKreg cells express Granzyme K, GPR183R, IL-7R, CXCR3, and CD62L with a lack of Granzyme B and perforin expression. The NKreg cells engaged in a lack of cytotoxicity towards the CD4+ T cells and K562 cells, compared to the CD56dim NK cells which lysed both targets. Further, the NKreg cells suppressed CD4+ (but not CD8+) T cell proliferation comparable to Treg cells through a cell-to-cell contact dependent mechanism, which was not reliant upon the NKp44, NKp46, or GPR183 receptors.

Conclusions: Our studies have phenotypically and functionally defined the NKreg cell population associated with a lack of cGvHD and differentiated these cells from the classic, cytolytic NK cells. We have also shown the CD56brightCD16- NKreg cells to have a more selective, but comparable suppressive capacity to Tregs, a cell subset that has been well studied in the context of GvHD and cell therapy. With further investigation we may decipher the mechanism of NKreg suppression and optimize the expansion of NKreg cells for use as a cell therapy for cGvHD.
ABSTRACT

**Abstract**

Background/objectives: Since their first emergence in early 2020, variants of concern (VOC) have presented as a major public health challenge in the management of the COVID-19 pandemic, with reported observations of increased vaccine/immune escape and transmissibility. To support the public health response, VOC characterization has largely depended on whole-genome sequencing (WGS) to identify single nucleotide polymorphisms (SNPs), insertions, and deletions. However, WGS requires several laboratory workflows, large computational power and analytical expertise resulting in long turn-around times, presenting as a challenge where rapid responses are needed. Oligonucleotide ligation assays (OLA) pose as potential alternatives to WGS, detecting select mutations with high sensitivity within a single workday and can be deployed in various settings. Here, we have validated the utility of an OLA capable of simultaneous detection of five mutations (deletion (del) 69-70, N501Y, D614G, T859N, and K417N) for identification of Alpha, Beta, and Omicron lineages, benchmarking its performance against WGS.

Methods: To assess the OLA assay, a validation panel of 97 (Alpha = 20, Beta = 4, Omicron = 6, other lineages = 67) SARS-CoV-2-positive nasopharyngeal swabs were obtained from routine COVID-19 testing at the BC Centre for Disease Control, with lineage previously assigned by WGS using Pangolin. Following RNA extraction, primers flanking the spike gene were used for amplification. Hybridizing probes specific for wildtype or variant were bound to the amplicons and ligated to biotinylated oligonucleotide probes. Probe-bound amplicons were then captured by complementary oligonucleotides immobilized at the bottom of a well and detected using a streptavidin-conjugated reporter. Lineages and variants were called based on mutation combinations: Alpha = del 69-70, N501Y, D614G; Beta/Omicron (BA.2) = N501Y, D614G, K417N; Omicron (BA.1) = del 69-70, N501Y, D614G, K417N.

Results: 27 specimens have been analyzed in triplicate on the OLA to date (Alpha = 7, Beta = 4, Omicron (BA.1) = 4, Omicron (BA.2) = 2, other lineages = 10), with an overall observed agreement of 85.2% (23/27) and kappa statistic of 0.77 compared with WGS. Two Omicron (BA.1) specimens were misclassified as being of another lineage due to failure to detect del 69-70 and K417N, and two Alpha specimens were misclassified as being of another lineage due to failure to detect N501Y. Observed agreement for mutation calls between replicates ranged from 80% to 86.7% for all specimens assessed.

Conclusions: OLAs are capable of lineage assignment based on hallmark mutations in short turn-around times without the need for computational power and sequencing equipment. Our analyses thus far have found that OLAs have good agreement with respect to the gold standard of WGS currently employed by clinical care in addition to good intra-assay reproducibility, suggesting the potential utility of these assays in the acute and rural settings for variant characterization. Future experiments will evaluate assay performance in specimens with varying viral loads as well as the effect of storage condition and specimen quality.
EXPOSURE TO HIV/COMBINATION ANTIRETROVIRAL THERAPY AND SMOKING DURING PREGNANCY SYSTEMICALLY MODULATES MITOCHONDRIAL DNA CONTENT AND TELOMERE LENGTH ACROSS VARIOUS TISSUES AMONG HIV-EXPOSED BUT UNINFECTED


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ABSTRACT Background/objectives: Combination antiretroviral therapy (cART) during pregnancy effectively prevents vertical transmission of HIV. We previously demonstrated that infant blood mitochondrial DNA (mtDNA) and telomere length (TL), both considered immune aging biomarkers, are affected by exposure to maternal HIV/cART and tobacco smoking during pregnancy. To determine whether these are systematic effects, we investigated these two biomarkers in several infant tissues from children who are HIV-exposed but uninfected (CHEU) and children HIV-unexposed uninfected (CHUU) controls.

Methods: MtDNA content and TL were measured using qPCR in whole blood, cord blood, cord tissue, and mouth swabs collected at birth. Their associations with exposure to maternal HIV/cART and smoking were investigated, while adjusting for confounders.

Results: The study sample included 229 CHEU and 91 CHUU. In whole blood and cord blood, maternal smoking was associated with shorter TL among CHEU but longer TL in CHUU. In both tissues, maternal smoking was also linked to increased mtDNA content in both groups, whereas HIV/cART exposure had the same effect in whole blood only. Although HIV/cART exposure was associated with higher mtDNA content and shorter TL in cord tissue, smoking had no effect on either biomarker. Lastly, longer mouth swab TL was associated with maternal smoking in both groups, but mtDNA content remained unaffected.

Conclusions: Both biomarkers are affected by these exposures, and in multiple tissues. However, for TL, exposure to HIV/cART modulates the effect of maternal smoking in blood tissues. How these biomarkers may predict clinical outcomes should be investigated in future studies.
ABSTRACT

Background/objectives: The worldwide outbreak of the novel Coronavirus (COVID-19) has highlighted the need for a rapid screening system for individuals infected by respiratory diseases such as COVID-19 and acute viral pneumonia. Early diagnosis of respiratory disease, particularly in high-risk individuals such as those in long-term care facilities, can improve the effectiveness of treatment options. Current screening methods are subjective and rely on monitoring patient symptoms and vital signs such as body temperature. Near-infrared spectroscopy (NIRS) is an optical technology capable of providing continuous and real-time monitoring of tissue hemodynamics and oxygenation. A multi-modal biosensor containing a NIRS sensor has the potential to detect and monitor reduced tissue oxygenation and abnormal respiration patterns commonly seen in individuals affected by respiratory disease. The objective of this study was to examine the feasibility of using a wearable NIRS sensor to collect respiratory signals and distinguish between normal and simulated pathological breathing.

Methods: Twenty young, healthy adults were recruited for participation in the study. Participants were monitored with a NIRS sensor affixed over the sternum while undergoing five separate breathing conditions to simulate pathological breathing. Participants began with three minutes of regular breathing to establish a baseline. This was followed by five minutes of imposed difficult breathing using a respiratory trainer to increase the difficulty of inspiration and expiration (loaded phase). Following a five-minute recovery period of regular breathing, participants began five minutes of imposed rapid and shallow breathing (rapid phase). During the rapid phase, participants used a metronome to increase their respiratory rate to 25 breathes per minute, approximately double the normal resting respiratory rate. The study concluded with five more minutes of regular breathing.

Results: Five relevant, respiratory features were extracted from the acquired NIRS signals: breathing depth, respiratory rhythm, respiratory effort index, hemoglobin difference concentration and tissue saturation index. Statistical analysis showed that the breathing depth, respiratory effort index and hemoglobin difference in the loaded and rapid breathing phases were significantly different than their baseline values.

Conclusions: This study demonstrates that a wearable NIRS sensor can capture different respiratory patterns and that selected features have the potential to differentiate between normal and pathological breathing. Future studies can expand upon this project by identifying additional NIRS features that can quickly and accurately identify individuals with respiratory disease.
MOLECULAR DETECTION OF THEILERIA SPECIES IN RESPONSE TO THEILERIOSIS IN PAKISTAN IN BOVINE POPULATION

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ABSTRACT

Background/objectives: Theileria is a tick-borne hemoprotozoan parasite, transmitted by tick genus Hyalomma, causes a disease named tropical theileriosis in bovine populations. This study is to develop five real-time PCR reactions to target Pan Theileria and four other Theileria species found in ruminants from Khyber Pakhtunkhwa region of Pakistan.

Methods: DNA were extracted from whole blood from Theileria symptomatic animals using the Phenol-Chloroform method at Quaid-i-Azam University, Pakistan. The primers and probes designed to target hypervariable V4 region in the 18S rRNA gene allow us to detect all Theileria spp., Theileria annulata, Theileria parva, Theileria lestoquardi, and Theileria ovis. Synthetic gBlock™ Gene Fragments and clinical specimens were used for analytical and clinical validation for its specificity, sensitivity, PCR efficiency, precision, ruggedness, and accuracy. PCR Positive Theileria samples were then confirmed using Sanger sequencing on 18S rRNA gene.

Results: For analytical validation, serial dilution of gBlock were used to assess the linearity and sensitivity. Regression coefficient (R²) of five reactions recorded between 0.9637 and 0.9913 showed a great linearity. Analytical sensitivity ranged from 10 to 100 copies per reaction. The coefficient of variation was ≤ 5% on repeat specimens demonstrated good test precision. Total 21 samples tested for the clinical validation, 15 were positive in Pan Theileria, also amplified on the speciation reaction and confirmed by sequencing. The rest of 6 specimens were negative for Pan Theileria, Speciation, and sequencing. That brought us 100% sensitivity and 100% specificity for our clinical test.

Conclusions: The real-time PCR assay described in this study is found to be a reliable method for screening Theileria infection in animal population. It also provides a quick identification for four species of Theileria without sequence confirmation. The sequence data from 15 positive specimens indicated they are closely related to each other; however, more specimens are needed to understand the phylogenetic and epidemiological relationship among the isolates from any specific and or geographical locations.
ABSTRACT

Background/objectives: The ABO blood group specificity is determined by the terminal monosaccharides, N-acetylgalactosamine on A and galactose on B RBCs. Enzymatic removal of these sugars would uncover universally acceptable H-antigen (O group RBC). Enzymatic conversion of A RBCs was lagged behind due to lack of very active, specific glycosidases and much more complex nature of A antigen. In this research, two novel glycosidases with improved kinetic properties and high specificities for A antigen are studied to efficiently convert A RBCs to universal donor O RBCs with improved conversion conditions and their immunological compatibility.

Methods: Whole blood samples are collected from consenting A donors (with approval from the University of British Columbia clinical research ethics board), washed and diluted to 10 % hematocrit in PBS buffer (pH7.4). Then washed RBCs were treated with newly developed glycosidases for 1 h at 37 °C. The efficiency of the newly developed glycosidases and the characterization of enzyme converted O (ECO)-RBCs were evaluated by gel-column based micro typing system (MTS) cards against A antigen and flow cytometry using anti A and H antibodies. Safety and compatibility of ECO-RBCs were assessed by blood group serology studies. In brief, ECO-RBCs were incubated in Rh-matched sera collected from diverse donors and any serum reactivity against ECO-RBCs was assessed using anti IgG MTS cards and flow cytometry using anti C3d (complement protein) antibody. To further confirm the cross-matching of ECO-RBCs, monocyte monolayer assay (MMA), a clinically used method, was performed using monocytes collected from B and O blood donors to assess the phagocytic activity against ECO-RBCs pre-incubated with serum samples. Phagocytic activity against ECO-RBCs was calculated using the monocyte index (MI).

Results: Anti A MTS cards and flow cytometry analysis of antigen A and H expression, after enzymatic treatment, confirmed successful conversion of A blood group to universal O group. Our studies showed no complement protein (C3d) deposition on ECO-RBCs pre-incubated with sera demonstrating the compatibility. Serology studies showed that ECO-RBCs are compatible with most of sera (~50 donors) studied under transfusion conditions; serum reactivity was shown by some O and B sera. MI value of ≤ 5 % was obtained for ECO-RBCs indicating minimal risk of an acute hemolytic reaction associated with ECO-RBC transfusion.

Conclusions: We have developed novel glycosidases that efficiently remove immunodominant A antigen from RBC membrane with high specificity to create universal blood donor cells at neutral pH and at enzyme concentrations that are much lower than previously reported. Based on preliminary results, ECO-RBC has the potential to greatly advance the field of transfusion and increase the donor blood supply.
MULTI-OMIC CHARACTERIZATION OF PEDIATRIC ACUTE LYMPHOBLASTIC LEUKEMIA (ALL) MICROENVIRONMENT

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ABSTRACT  Background/objectives: The tumour microenvironment is important in the maintenance of cancer at various stages. While much progress has been made in understanding the leukemia microenvironment; thus far, these studies largely focused on the role of stromal cells or immune cells. The fluid portion of the microenvironment is often overlooked and studies that explore it have been scant. To date, a comprehensive exploration of ALL-relevant interstitial fluids has not been conducted. Examination of the fluid portion is warranted, however, as it establishes an interface upon which leukemic and stromal cells can reciprocally interact.

Methods: To investigate the ALL-relevant microenvironment and describe its changes during chemotherapy, we collected bone marrow interstitial fluid (BMIF) and peripheral blood plasma (PBP) from 8 pediatric B-cell acute lymphoblastic leukaemia (B-ALL) patients. Each patient sample was collected at diagnosis (Dx) and after induction phase of chemotherapy (D29). Patient samples were processed using mass spectrometry-based strategies to obtain quantitative proteomic, terminomic, metabolic, and lipidomic profiles.

Results: Our multi-omic analysis found that the BMIF and PBP contribute to the tumourigenesis of B-ALL differently. Particularly, the BMIF was enriched for extracellular vesicle-associated proteins. Furthermore, either compartment also went through different changes through chemotherapy. In the PBP, immune regulatory proteins decreased over the course of chemotherapy and restoration of lipid homeostasis can be observed. After induction therapy, the BMIF has a post-apoptotic and immunosuppressive microenvironment.
INDUCING ANTIGEN-SPECIFIC IMMUNE TOLERANCE IN A MURINE MODEL OF TYPE 1 DIABETES

Background/objectives: Type 1 diabetes (T1D) is an autoimmune disease driven by self-reactive immune cells that destroy insulin-secreting pancreatic beta cells, resulting in lifelong dependency on exogenous insulin injections. T1D onset can be marginally delayed using broad immunosuppression, however, these effects are transient, and these patients are at increased risk of unwanted adverse effects. Alternatively, antigen-specific immunotherapy presents a more attractive option through selective targeting of the autoimmune processes responsible for T1D development. Specifically, antigen presenting cells can be modulated using nanomedicine to present a specific target antigen, such as insulin, to surrounding lymphocytes while expressing low levels of costimulatory molecules. This leads to the suppression of effector T cell functions and induces immune tolerance. Given that there is a loss of immunological self-tolerance to specific antigens in T1D, this type of strategy which restores immune tolerance is a promising therapy for those at risk for or diagnosed with T1D. Using lipid nanoparticles formulated with mRNA encoding immunodominant T1D antigens, we aim to investigate whether T1D onset can be delayed or prevented in a murine model of T1D through the induction of antigen-specific immune tolerance.

Methods: Lipid nanoparticles, formulated and prepared by Integrated Nanotherapeutics Inc., will be injected intravenously in non-obese diabetic (NOD) mice at 3 and 5 weeks of age. Mice will be injected with either saline, lipid nanoparticles loaded with T1D-relevant mRNA, or lipid nanoparticles loaded with irrelevant mRNA. Blood glucose will be monitored weekly to assess diabetes progression, indicated by readings ≥ 14.0mmol/l on two consecutive days. Once mice reach diabetes onset or 30 weeks of age, we will collect their pancreas, secondary lymphoid organs, and blood for further analysis. T cell phenotype and frequency will be determined using flow cytometry, and antigen-specificity will be confirmed by tetramer assays and mixed lymphocyte reactions. Single-cell RNA sequencing will be used to identify and analyze T cell and dendritic cell subsets.

Results: I predict that, relative to mice receiving saline or irrelevant mRNA-loaded lipid nanoparticles, the time until diabetes onset will be increased in mice injected with lipid nanoparticles loaded with mRNA encoding T1D autoantigens. Antigen-specific CD4+ T cells isolated from mice immunized with mRNA encoding T1D antigens will be expanded into an antigen-specific T regulatory phenotype and will consist of proportionately fewer Th1, Th2, and/or Th17 T cell subsets.

Conclusions: Successful delay of diabetes onset, in the absence of hypersensitivity reactions, using lipid nanoparticle delivery of mRNA-encoded T1D immunodominant antigens will support the tandem utility of tolerogenic dendritic cells and nanomedicine as a candidate T1D therapy. Findings from this research may contribute to the prevention of T1D in at-risk individuals and the development of a long-term therapeutic strategy if used in combination with beta-cell replacement therapy.
POSTER PRESENTATION - GRADUATE
KHUSHBU PATEL
SUPERVISOR: DR. MARI DEMARCO

PERSONAL DECISION-MAKING POST-ALZHEIMER'S DISEASE BIOMARKER TESTING: FINDINGS FROM THE IMPACT-AD BC STUDY

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ABSTRACT

Background/objectives: While the use of cerebrospinal fluid (CSF) analysis for Alzheimer’s disease (AD) biomarkers (i.e., amyloid-beta peptides and tau proteoforms) has been well-documented to improve diagnostic accuracy, far less is known about the direct impact of this testing on the individuals undergoing testing, and their family or friends (‘care partners’). We aimed to address this gap in knowledge by examining the perspectives of patients who underwent AD CSF biomarker testing as part of routine care, and a care partner.

Methods: Within the ‘Investigating the Impact of Alzheimer’s Disease Diagnostics in British Columbia’ (IMPACT-AD BC, www.impactAD.org) study (NCT05002699), we conducted semi-structured telephone interviews with patients that meet the appropriate use criteria for AD CSF biomarker testing and separate interviews with their care partner. A subset of patients (n=34) and care partners (n=31) were interviewed post-CSF biomarker disclosure and again about 5 months (median: 4.9 [4.2-5.3]) after the initial interview. Thematic content analysis was performed to understand the impact of AD CSF biomarker testing on health behaviors, financial and care planning decisions, and resources and support needs.

Results: Most patients (94%) rated their decision to undergo testing as “easy”, with the remainder noting the decision was neither easy nor difficult. After result disclosure, a few patients (8%) reported feelings of concern, but the majority (80%) reported overall positive feelings from having more information about their brain health, certainty around their diagnosis, and the ability to plan ahead. Regarding future actions, many expressed an intention to adopt or continue with healthy behaviors (42%). From the care partners’ perspective, many expressed relief at having more diagnostic certainty post-disclosure. Care partners also relayed an increased awareness of future caregiving responsibilities and the need or desire to connect with community resources to help navigate this new role following result disclosure.

Conclusions: In summary, individuals undergoing AD CSF biomarker testing and their care partners found that the testing provided information needed to help them make wellbeing decisions and plan their future. Our findings will provide the patient and care partner insight needed in the development of effective resources for biomarker disclosure, and subsequent care planning and wellbeing support.
ABSTRACT

Background/objectives: Radiation dermatitis (RD) appears in up to 95% of patients receiving radiation therapy for cancer treatment. Symptoms of RD include erythema, pruritus, pain, scaling, and ulceration with increased chance of infection. Severe symptoms can often delay or prevent additional courses of radiation treatments. Unfortunately, current treatments are not effective. Granzyme B (GzmB) was originally characterized as a pro-apoptotic serine protease secreted by cytotoxic lymphocytes to promote target cell apoptosis. It is now recognized that GzmB can be expressed and secreted by numerous immune as well as non-immune cell populations, including macrophages. Extracellular GzmB is elevated in a number of skin disorders, including atopic dermatitis, autoimmune blistering and scarring. Extracellular GzmB proteolytically cleaves numerous substrates that contributes to reduced epithelial barrier function, cleavage of basement membrane proteins, impaired matrix remodeling and scarring. E-cadherin is a cell-cell junction protein which is has been shown to be cleaved by GzmB. GzmB is abundant in skin lesions of humans with RD. Based on these findings, we hypothesized that GzmB contributes to increased severity and delayed healing of RD through the cleavage of cell-cell junction proteins resulting in impairment of epidermal barrier function and increased inflammation.

Methods: GzmB expression and E-cadherin levels were assessed using immunohistochemical analysis of biopsies taken from patients exhibiting RD. Immunofluorescence microscopy was used to determine cell source(s) in human RD biopsies. The role of GzmB was investigated in an established murine model of RD, comparing GzmB knockout (GzmB-KO) to wild type (WT) mice. RD was induced in mice by applying a single 40 gy dose of radiation to the upper back. RD severity was assessed and scored by independent investigators that were blinded to the treatments. RD severity was quantified based on a scoring system adapted from Douglas and Fowler (1976) and described in Holler et al. (2009). Tissue samples were collected on days 4 and 14 and examined by histology and enzyme-linked immunosorbent assay (ELISA) for pro-inflammatory markers and GzmB.

Results: Elevated GzmB and reduced E-cadherin was observed in RD human skin tissues compared to healthy human skin tissues. CD68 immunopositivity suggested that macrophages are an important source of GzmB in RD. GzmB-KO mice (N=14) exhibited a significant decrease in RD severity compared to WT mice (N=14) at day 4 (p=0.044), day 6 (p=0.016), day 8 (p=0.008), day 10 (p=0.012), and day 12 (p=0.014). A significant reduction in skin redness (erythema), scaling, and scabbed wounds 4-12 days post-radiation. Future studies will examine the utility of a topical GzmB inhibitor.

Conclusions: GzmB is abundant in human RD. GzmB may contribute to RD severity through E-cadherin cleavage and the subsequent loss of epithelial barrier function. GzmB may be a novel therapeutic target for the treatment of RD.
POSTER PRESENTATION - GRADUATE
KATLYN RICHARDSON
SUPERVISOR: DR. DAVID GRANVILLE

DETECTION OF NOVEL PATHOLOGICAL MECHANISMS FOR GRANZYME K IN PSORIASIS

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ABSTRACT

Background/objectives: Psoriasis is a skin disease that currently affects over one million Canadians. It is characterized by skin inflammation and increased epidermal proliferation forming thick, scaly plaques. Unfortunately, current therapies are not completely effective and often present with side effects. As such, a deeper understanding of the pathological mechanisms of psoriasis is necessary. Previously, we have demonstrated the protease Granzyme K (GzmK) to be elevated in human psoriasis skin and contribute to disease development in a murine model of psoriasis. However, the mechanism(s) by which GzmK alters skin pathology in psoriasis is unknown. We hypothesized that GzmK contributes to the development of psoriasis through the cleavage of inflammatory proteins.

Methods: The role of GzmK was investigated in an established imiquimod (IMQ)-induced murine model of psoriasis, comparing wild-type (WT) to GzmK knockout (K-KO) mice (n=6 per genotype). IMQ-induced psoriasis severity was assessed macroscopically for onset and severity of erythema (redness) and plaque formation using a modified Psoriasis Area and Severity Index (PASI). IMQ-induced psoriasis tissue extracts were used to discover novel GzmK substrates using High-efficiency Undecanal-based N Termini EnRichment (HUNTER), a mass spectrometry-based method. Through this method, peptides from protein N-termini were enriched and quantified, identifying proteolytic differences between IMQ-treated WT and K-KO mice. Quantitative analyses of mass spectrometry data (using MaxQuant) were used to detect high-confidence candidate substrates/proteins of GzmK versus background cleavage products. We are currently using this workflow in vitro with keratinocytes treated with or without GzmK to evaluate GzmK activity relevant to human skin.

Results: IMQ-treated K-KO mice exhibited an approximate 60% decrease in psoriasis severity (erythema and plaque formation) compared to WT mice (PASI scores of 4.25 vs 7.25 p≤0.001 at day 7). Preliminary work demonstrates the number of peptides derived the N-termini of proteins is significantly increased in IMQ-treated WT mice compared to GzmK-KO mice. This result supports previous work showcasing GzmK as an important mediator of psoriasis development in this disease model. Cleavage assays will validate which of the putative GzmK substrates are directly cleaved by GzmK. We will identify which direct GzmK substrates are implicated in the pro-inflammatory response during psoriasis. Finally, to confirm the link to these pro-inflammatory proteins/substrates in human skin, we are incubating keratinocytes with or without GzmK.

Conclusions: GzmK contributes to psoriasis development. The identification of novel GzmK substrates will further elucidate the complex pathological mechanisms of psoriasis. Inhibition of GzmK may represent a novel therapeutic approach for treating psoriasis.
ABSTRACT

Background/objectives: Adoptive T cell therapy (ACT) is a targeted cancer treatment that involves transferring antigen-specific CD8+ T cells to patients, and its therapeutic efficacy depends on the ability of CD8+ T cells to produce pro-inflammatory molecules. Despite the promising results in leukemia, the effectiveness of T cell therapies remains limited for solid tumours, in part because of the nutrient limitations within the tumour microenvironment (TME). Specifically, the lack of glucose within the TME impairs T cell function by dampening glycolysis and limiting inflammatory cytokine (IFNg) production. Interestingly, one way to overcome the hostile TME is to subject CD8+ T cells to transient glucose restriction (TGR) in vitro before infusion. When TGR CD8+ T cells are restimulated under saturating glucose levels, they show enhanced glucose transport and IFNg production, while also acquiring complete tumour clearance ability in vivo. Upon activation, CD8+ T cells upregulate glucose transporter Glut1 to increase glucose import. When glucose is depleted in culture, fully-activated CD8+ T cells further upregulate Glut1, while expression of the more efficient Glut3 remains unchanged. This suggests that Glut1 may play a critical role in enhancing the ability of TGR T cells to compete for glucose within the TME. Although glucose metabolism has been linked to IFNg production, the contribution of increased Glut1 to IFNg production in TGR CD8+ T cells remains unknown. Therefore, we aimed to investigate the role of Glut1 in enhancing the effector functions seen in TGR CD8+ T cells.

Methods: CD8+ T cells isolated from Glut1f/f/OTI mouse splenocytes were activated and transduced with a CRE- and GFP-expressing retrovirus to produce fully-activated Glut1 KO CD8+ T cells. These cells were then sorted using fluorescence-activated cell sorting and cultured in either 10mM or 1mM (TGR) glucose media. Given the strong links between glucose metabolism and IFNg, we used a Seahorse assay to assess the metabolic pathways dependent on Glut1. We also assessed effector function by co-culturing the CD8+ T cells with a B16-OVA cell line, which was stained for IFNg and quantified using flow cytometry.

Results: Our data shows that Glut1 KO CD8+ T cells increase basal mitochondrial metabolism without changing basal glucose metabolism. Upon inhibition of mitochondrial ATP production, Glut1 KO T cells could not compensate by upregulating glucose metabolism (glycolytic reserve), despite the sustained expression of Glut3. TGR Glut1 KO CD8+ T cells co-cultured with B16-OVA also had reduced IFNg production when compared to TGR control CD8+ T cells.

Conclusions: Together, these results show that Glut1 is needed to maintain the glycolytic reserve and increased IFNg production seen in TGR CD8+ T cells. These findings suggest that Glut1 may play a critical role in mediating the acquired complete tumour clearance ability of TGR T cells in vivo. Future experiments will involve elucidating the underlying mechanism by which Glut1 enhances TGR T cell function, with the hope of uncovering ways to improve and broaden the application of CD8+ T cell therapy for different types of cancer.
POSTER PRESENTATION - GRADUATE
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TOXICITY OF HIV ANTIRETROVIRALS IN HUMAN EMBRYONIC STEM CELL MODELS

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ABSTRACT

Background/objectives: Women living with HIV give birth to ~1.1 million infants each year, with 80% of them exposed to antiretrovirals (ARVs) in utero. The use of ARVs during pregnancy has successfully reduced vertical transmission rates from 25% to <2% worldwide, but their safety has not been fully elucidated in the context of pregnancy. A recent study reported an early signal for increased neural tube defects in infants exposed from conception to dolutegravir (DTG), a commonly used HIV integrase inhibitor (InSTI). This suggests that exposure during early fetal development may be detrimental. Our objective was to characterize the dose-dependent effects of four HIV integrase inhibitors in two human embryonic stem cell (hESC) lines with respect to markers of pluripotency, early differentiation, and cellular health.

Methods: H9 and CA1S hESCs (n=6 and n=3 independent experiments, respectively) were exposed to DTG, cabotegravir (CAB), bictegravir (BIC), or raltegravir (RAL) at doses ranging from 0.01X to 1X peak plasma drug concentrations (Cmax) or DMSO diluent control. After three days, cells were harvested and assessed via flow cytometry for cell viability, apoptosis, and two markers of pluripotency, specifically SSEA-3 (lost early in differentiation) and TRA-1-60 (a later marker). Cells treated with 0.5X Cmax were further examined for markers of differentiation towards the three germ layers via RT-qPCR. Measures were compared between InSTIs and DMSO by paired t-tests.

Results: H9 hESCs exposed to DTG, CAB, and BIC at ≥0.5X Cmax exhibited ≥2-fold decreased proliferation (p≤0.04). Exposure to DTG or BIC at 1X Cmax severely reduced viability (p<0.001) and increased apoptosis (p≤0.001). Similar cell toxicity trends were seen in CA1S hESCs exposed to ≥0.5X Cmax DTG, CAB, and BIC. H9 hESCs exposed to ≥0.5X Cmax DTG, CAB, or BIC showed decreased SSEA-3 (≥20%, ps≤0.02) and TRA-1-60 (≥20%, ps≤0.03) expression and increased early mesendoderm lineage gene expression. CA1S hESCs exposed to ≥0.5X Cmax DTG or CAB showed ≥75% decrease in SSEA-3, but no effect on TRA-1-60 or differentiation marker gene expression. In both hESC lines, RAL did not induce any cytotoxicity or differentiation, regardless of dose exposure.

Conclusions: Even at sub-clinical concentrations, some InSTIs induce cytotoxicity and differentiation in hESCs. Given their common use by women of reproductive age, it is imperative to elucidate their long-term safety in the context of pregnancy and embryonic development. Our data also indicate that RAL shows a safer profile in this model, a reassuring finding that warrants further investigation.
ABSTRACT

Background/objectives: Metabolic processes continue to emerge as key regulators of immune cell differentiation and function, uncovering potential therapeutic targets for immune-related diseases. The metabolic enzyme xanthine oxidoreductase (XOR) catalyzes the final two steps of purine catabolism and has been implicated in such diseases including diabetes, obesity, and cancer. XOR exists in two interconvertible isoforms which catalyze the same reaction but produce unique byproducts: xanthine dehydrogenase (XDH) produces NADH from NAD⁺ while xanthine oxidase (XO) produces reactive oxygen species (ROS) from oxygen. Both NAD⁺/NADH ratios and ROS are known modulators of macrophage metabolism, influencing function. Our preliminary data suggest that XOR activity influences mitochondrial metabolism during pro-inflammatory macrophage activation. These cells typically exhibit substantial loss of mitochondrial energy metabolism and this metabolic reprogramming is known to be critical for their phenotype. Our objective is to elucidate the role of XOR in modulating pro-inflammatory macrophage metabolism and function in hopes of identifying novel therapeutic targets for inflammatory diseases.

Methods: Bone marrow was isolated from 8-12-week-old WT C57BL/6 mice and cultured in M-CSF for 7 days to generate mature macrophages, followed by polarization to pro- or anti-inflammatory subsets overnight in the presence or absence of one of two XOR inhibitors, allopurinol or febuxostat. Glucose and mitochondrial metabolism were measured using the Seahorse XF assay. Protein expression of mitochondrial electron transport chain (ETC) complex subunits and relative phosphorylation of AMPK, a cellular energy-sensing kinase were measured via Western blot. Relative activities of the two XOR enzyme isoforms were measured by fluorescence assay.

Results: Inhibition of XOR by either inhibitor preserved mitochondrial metabolism in pro-inflammatory macrophages, increasing ATP-linked respiration and decreasing non-mitochondrial oxygen consumption. There was a concomitant increase in expression of specific subunits for mitochondrial ETC complexes I-IV, in XOR-inhibited cells. Direct activation of the energy sensing kinase AMPK, and inhibition of mitochondrial ETC complex I with phenformin resulted in a striking decrease in protein levels of XOR. Compared to non-polarized and anti-inflammatory subsets, pro-inflammatory macrophages showed decreased relative XO:XDH activity.

Conclusions: XOR activity, potentially the XDH isoform, appears to drive progressive loss of mitochondrial metabolism during polarization of pro-inflammatory macrophages, where XOR inhibition preserves this, potentially resulting in incomplete polarization. Our data also suggests crosstalk between purine catabolism and the AMPK energy-sensing pathway. The precise role of the two XOR isoform activities in the regulation of mitochondrial metabolism remains to be elucidated. Given the importance of mitochondrial metabolic reprogramming in macrophage polarization, a better understanding of the XOR-mediated mitochondrial changes could provide novel immunotherapeutic targets.
SINGLE-CELL TRANSCRIPTOMIC ANALYSIS TO ASCERTAIN HOW CANCER CELLS TALK TO SURROUNDING IMMUNE ENVIRONMENTS

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ABSTRACT

Background/objectives: How do cancer cells communicate with neighbouring cells? During tumour progression, cancer cells exploit mechanisms of the immune system to facilitate survival, proliferation, and metastasis. Understanding how cancer cells communicate with adjacent immune cells is a crucial question in cancer therapeutics but has not been clearly answered to date. It is conceivable that cancer cells would try to communicate with surrounding cells to evade immune surveillance and promote tumour growth. Improved knowledge of tumour-immune interaction patterns can translate into potent cancer immunotherapy targets. The advancement in single-cell RNA sequencing (scRNA-seq) has enabled transcription profiling of millions of cancer cells feasible, providing necessary data sets to characterize and dissect millions of cell-cell communication patterns in an unbiased manner.

Methods: We hypothesized cells communicating with other cells generally share pathways and transcriptional signatures; hence, pairs of interacting cells are expected to be closely located within a low-dimensional cellular topic space. However, such statistically-predicted cell-cell interactions must be validated and interpreted using the knowledgebase of ligand-receptor interaction profiles. We directly attempted to find evidence of cancer-to-immune cell pairs communicating through known ligand-receptor interactions. Using a publicly-available pan-cancer atlas of tumour-infiltrating T cells profiled across 21 cancer types and 316 donors, constituting one hundred eighty thousand cells, we annotated cell states/topics using an unsupervised machine learning method. In the cell topic space, we prioritized cell pairs interacting with a high probability and characterized the mechanisms of interactions in terms of coordinated ligand-receptor activities.

Results: We clustered millions of ligand-receptor interaction patterns observed among cell-cell pairs into tens of unique interaction modules, recapitulating common TCR-mediated immune-immune, tumour-immune interactions. Unlike existing inter-cell-type correlation analysis followed by predefined single-cell clusters, our ML framework can investigate actual cell-cell interaction profiles with a higher resolution in an unbiased manner. We expect our method to enable researchers to identify novel cell types/states only transiently occurring in cancer-related processes. Moreover, we provide a molecular basis of why certain cell pairs interact prevalently in a tissue-specific context, seeking to provide a guideline for designing novel or repurposing immunotherapies.

Conclusions: Conventional single-cell analysis methods, relying on clustering and data visualization, may fail to reach a necessary resolution of cell-cell communications in cancer biology. We propose a novel computational approach to characterize inter-cellular communications in single-cell RNA-seq analysis. We investigated tissue-specific tumour-microenvironmental contexts across millions of cell pairs.
ABSTRACT

Background/objectives: CD8+ T cells are a type of immune cells important for the defense against infections and tumours. CD8+ T cells are regulated by many metabolic pathways that control their activation, differentiation, and production of pro-inflammatory cytokines, for which glucose metabolism is essential. How these cells balance the use of glucose to sustain sufficient ATP and intermediary metabolism for energy and biosynthesis respectively, remains incompletely understood. Triosephosphate Isomerase (TPI) is a highly conserved and critical glycolytic enzyme for the interconversion of dihydroxyacetone phosphate (DHAP), involved in lipid metabolism, and glyceraldehyde 3-phosphate (GAP), used in glycolysis to produce NADH, ATP, and pyruvate for oxidative phosphorylation. We hypothesize that TPI plays a key role in the regulation of carbon allocation in CD8+ T cells, especially in glucose restricted CD8+ T cells, which display significantly increased in vivo survival and anti-tumour function.

Methods: To assess if TPI expression and activity are regulated, we in vitro activated ovalbumin-specific CD8+ T cells for 72 hours. The cells were then subjected to control (10mM glucose) or glucose-restricted media (1mM glucose), or media containing IL-15 to induce memory cell differentiation. TPI protein expression was analyzed by Western blot analysis, and enzyme activity was determined by a colorimetric TPI enzyme activity kit. To determine TPI-mediated effects on CD8+ T cell cytokine production and anti-tumour function, we co-cultured fully activated CD8+ T cells transduced with a control or TPI-targeting retroviral shRNA construct with ovalbumin-expressing tumour cells and determined production of IFN-gamma with flow cytometry.

Results: TPI protein expression was gradually upregulated over 72 hours following CD8+ T cell activation, and glucose restriction further increased TPI protein expression, although TPI activity appeared to decrease. Strikingly, the production of IFN-gamma was significantly increased in cells after TPI knockdown, despite the importance of the enzyme for glycolysis, a key metabolic pathway for pro-inflammatory cytokine production.

Conclusions: These data suggest that TPI might play a role in the activation, expansion, function, and response to nutrient restriction conditions in CD8+ T cells. Given the stark increase in anti-tumour function by T cells exposed to limiting glucose concentrations in vitro, metabolic conditioning of T cells for cancer therapy is a promising avenue. Our preliminary data suggests that this could be in part mediated by altered TPI activity. Therefore altering TPI activity in CD8+ T cells could present a new avenue to increase efficacy of cellular therapy in patients with cancer.
Background/objectives: Pregnant women affected by COVID-19 are at increased risk for adverse perinatal outcomes, such as preterm birth. Along with changes in the maternal physiology and immune system during pregnancy, immune memory from prior exposures to 4 common cold human coronaviruses (HCoV: 229E, NL63, HKU1, OC43) may also contribute to the disparity in COVID-19 severity and outcomes. We broadly hypothesize that pre-existing HCoV antibodies can modulate the serological response to SARS-CoV-2 infections, and consequently affect pregnancy outcomes. Here, our initial objective was to describe the antibody responses to SARS-CoV-2 and HCoV among pregnant women with COVID-19, before and after SARS-CoV-2 infection.

Methods: Our overall nested case-control study design (N=555) included paired residual sera from pregnant women across BC who underwent routine 1st trimester prenatal screening (P-sera) and 3rd trimester delivery testing (D-sera) between Dec 2019 and Oct 2021. Analysis for this objective focused on all cases (N=185), defined as women with a positive SARS-CoV-2 polymerase chain reaction (PCR) test between their P-sera and D-sera collection. A multiplex electrochemiluminescent immunoassay was used to quantify serum antibody levels against the Spike protein of the 4 HCoVs, and the Nucleocapsid, Receptor Binding Domain, and Spike proteins of SARS-CoV-2. Preliminary statistical analysis among 185 cases was performed using Mann-Whitney U Test, Wilcoxon Signed-Rank Test, and multiple linear regression.

Results: Comparisons between P-sera (pre-infection) and D-sera (post-infection) demonstrated that 79% (147/185) developed SARS-CoV-2 antibodies post-infection (seroconverters), while 18% (34/185) did not (non-seroconverters), and 2.2% (4/185) were seropositive throughout. 9/34 non-seroconverters had D-sera collected >30 days post-PCR-positivity, exhibiting a 4.9% (9/185) non-seroconversion rate among pregnant women with COVID-19. Between seroconverters and non-seroconverters, no differences were observed in HCoV antibodies pre-infection, while higher levels of OC43 (P<0.001) and HKU1 (P<0.001) antibodies were found in seroconverters post-infection. Paired analysis between longitudinal pre- vs. post-infection sera showed higher pre-infection levels of NL63 antibodies among non-seroconverters (P=0.049) compared to post-infection, while seroconverters demonstrated elevated post-infection HKU1 (P=0.004) and OC43 (P<0.001), and lower post-infection 229E (P<0.001) and NL63 (P<0.001) antibodies compared to pre-infection levels. Multiple regression models revealed that lower NL63 antibodies pre-infection is predictive of a larger increase in SARS-CoV-2 spike antibody levels post-infection (Est=-0.63, P=0.009, R2 =0.06).

Conclusions: We found that pregnant women who developed SARS-CoV-2 antibodies post-infection had varying HCoV antibody levels compared to those who did not seroconvert, both pre- and post-infection. Further analysis will be performed by linking our results to vaccination status, clinical characteristics, and pregnancy outcomes. Findings can be used to find immunological correlates of SARS-CoV-2 protection and adverse COVID-19 outcomes.
**ABSTRACT**

**Background:** According to the World Health Organization, about 50% of the world’s population lives in regions where dengue virus (DENV) is endemic. Approximately 400 million people are infected yearly, of which nearly half are asymptomatic despite high virus titers. Annually, about 25,000 deaths are reported from symptomatic patients exhibiting severe hemorrhage and vascular leakage. The most common clinical manifestation observed in all DENV infections is the reduction of platelet (PLT) number (thrombocytopenia). However, the mechanism by which DENV induces thrombocytopenia is not well understood. DENV is an RNA virus that is able to reproduce in various host cells including PLTs, which are enucleated blood constituents. Our lab demonstrated that DENV can bind and enter PLTs, and utilize their simple cellular machinery to replicate and assemble infectious virus progeny. Similarly, other studies have shown the PLT precursor, megakaryocytes (Megs) to be the preferentially infected cells in the bone marrow that have the ability to produce infectious DENV. Furthermore, physiological agonists such as thrombin enhance DENV binding to PLTs. Highly immunogenic DENV-encoded NS1 protein was specifically shown to be synthesized by DENV-infected PLTs. NS1 is the only membrane-associated DENV protein and it may contribute to the antibody-mediated clearance of Megs and PLTs. Here we investigate DENV-induced translation in PLTs and follow the surface expression of NS1 in infected Megs and PLTs.

**Objectives:**

**Goal 1:** Distinguish surface and secreted DENV NS1 on infected PLTs and Megs. **Goal 2:** Determine the DENV-dependent PLT translatome.

**Methods:** DENV was propagated in Vero (African green monkey kidney epithelial cells) and purified by differential density sedimentation. PLT quality and purity was measured by flow cytometric analysis. PLTs were treated with DENV, physiological agonist (thrombin), or both. Protein synthesis was followed by using a biotinylated translation inhibitor, puromycin, and identified by LC-MS/MS. Parallel experiments were western blot analyses were conducted for confirmation. Using flow cytometry, NS1 surface expression was followed in Meg-01 cells infected with DENV in the presence of a potent differentiation initiator Phorbol 12-myristate 13-acetate (PMA).

**Results:** Using LC-MS/MS, we identified proteins involved in different PLT functions such as metabolism, mitochondrial activity, RNA-binding, cytoskeletal organization, and cell signaling in DENV-infected PLTs. For WB validation, we selected five after further comparing the statistical significance of the relative abundance parameters of the peptide identification confidence and functional interest of the proteins. Preliminary studies show global synthesis and secretion of NS1 in PLTs by flow and WB. Furthermore, cell-associated NS1 and cell-surface expression was demonstrated by WB and flow analysis of infected Meg-01 cells.

**Significance:** Identification of PLT proteins found to be induced by DENV and the detection of cell surface exposure of NS1 will define an antiviral strategy to attenuate DENV infection and mitigate clinical symptoms of thrombocytopenia.
ABSTRACT #61

POLYCATIONIC POLYMER MEDIATED INHIBITION OF THE PROCOAGULANT AND ANTIFIBRINOLYTIC PROPERTIES OF NUCLEIC ACIDS

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ABSTRACT  Background/objectives: Nucleic acids (polyanionic molecules) including cell-free DNA, RNA, Neutrophil Extracellular Traps (NETs) are indicated to be the mediators of pathological thrombosis. They are shown to promote thrombosis post-chemotherapy treatment and in sepsis. Besides, these polyanionic molecules also show antifibrinolytic potential and alter clot structure. Targeting these mediators instead of key coagulation enzymes could prevent thrombosis associated with them. Furthermore, this strategy could improve the outcomes of thrombolysis where nucleic acids are involved. Our objective is to screen a library of polycationic polymers and identify safe and effective candidates that target polyanionic nucleic acids and reverse their accelerated blood clotting potential in vitro.

Methods: Inhibition of thrombin generation triggered by poly I:C (a surrogate for nucleic acids) was measured using Stago Thrombinoscope. FXII inhibition studies in plasma were carried out by monitoring S2302 substrate activity. FITC labeled Poly I:C was used to study the binding of nucleic acid to the Alexa-546 labeled fibrin clot. Plasma clot lysis assay was performed in presence of thrombin CaCl2 and tPA.

Results: We identified candidates with optimal neutralization potential for nucleic acids using plasma clotting and thrombin generation assays. PNBI inhibited thrombin generation triggered by nucleic acids We also found PNBI inhibit nucleic acid mediated FXII activation in plasma. We found that the PNBI's can inhibit the binding of FITC labeled poly I:C to the fibrin clot. From the clot lysis studies, we found that they reverse the antifibrinolytic potential of nucleic acids. Fluorescently labeled PNBI's are seen to bind NETs.

Conclusions: Our results demonstrate that PNBI's can reverse the procoagulant potential of nucleic acids making them a suitable antithrombotic agent. These inhibitors also bind to NETs suggesting their potential to prevent DIC in sepsis. PNBI's also enhance the effectiveness of tPA-mediated clot lysis attributed to nucleic acids.
CHARACTERIZATION OF HUMAN ISLET-RESIDENT MACROPHAGE TRANSCRIPTOMES USING SINGLE CELL SEQUENCING

Background/objectives: Diabetes is one of the top ten leading causes of death in Canada, with 11.5 million Canadians currently living with some form of diabetes today. Recent research shows an increase of macrophage involvement initiating immune responses in diabetes. Previous findings in the Verchere Lab support the hypothesis that islet macrophages in mice actively regulate b-cell mass and islet function and thus, are potential targets for diabetes therapy. Other studies in mice suggest that the islet macrophages may be a rich source of regenerative factors whose expression is induced in response to beta-cell death or in other states of islet remodeling, including obesity. However, mouse islets have different composition of endocrine cells and architecture compared to human islet cell clusters. In addition, the heterogenous role of tissue-resident macrophages in human pancreatic islet cell clusters is not yet defined. To evaluate this, we are using single cell RNA sequencing (scRNAseq) of human islets to construct a dataset of islet cell types from donors of different clinical parameters: obese, T2D, and healthy controls. Our objective is to characterize the transcriptome of tissue-resident macrophages and their influence on other islet cell types.

Methods: Obese (body mass index >30), T2D, and age and sex-matched healthy control human donor islets are obtained on a rolling basis from the University of Alberta Human Islet Core. 1500-2500 islets from donors are then picked based on purity then dispersed into a single-cell suspension. Next, the cells are divided using magnetically activated cell sorting (MACS) based on a pan-leukocyte CD45 marker to isolate immune cells. After the CD45+ immune cells and remaining CD45- cells are isolated, sample libraries for both cohorts are prepared according to 10X Genomics V3 Chemistry protocol. Two libraries per donor are generated (CD45+ islet immune cells and non-immune cells) to obtain robust read-depth of macrophage populations. The libraries are then sequenced using a NextSeq 500 until about eight hundred million reads are obtained. The sequencing output reads are then aligned and preprocessed using CellRanger software before downstream analysis using the Seurat V4 package in R. After labeling cell types based on marker genes, differentially expressed gene lists will be generated from the same cell types between samples of different clinical parameters such as diabetic status and type, body mass index, sex, and age. In addition, other computational analyses including but not limited to pathway analysis and trajectory analysis will be performed. In silico findings of interest from this research will be further validated in vitro.

Results: This research will provide novel insight to the influence tissue resident macrophages have on hormone-producing islet cell types in humans, while also creating a robust scRNAseq dataset for secondary analyses.

Conclusions: Characterizing the role of islet-resident macrophages in human pancreases may yield implications for identifying new targets for future therapeutics for the treatment of diabetes.
MODIFIED CLOTTING FACTOR X: TOWARD A SAFER CLOT BUSTER

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ABSTRACT

Background/objectives: Heart attack and stroke are the leading causes of death worldwide and usually involve a deadly blood clot, which blocks the flow of blood. These blood clots are currently being treated by a recombinant (r) form of tissue plasminogen activator (tPA), the enzyme that would normally initiate the process of clot-busting and restore blood flow. However, when used as a medicine, rtPA must be given at very high doses, and can cause life-threatening bleeding because of its enzyme function. The Pryzdial lab believes that the treatment of blood clots would therefore be improved by developing a non-enzymatic clot-busting drug to replace rtPA.

Methods: The Pryzdial lab has shown that clotting factor (F) X can be modified to become a non-enzymatic tPA accelerator that enhances clot busting. In pre-clinical studies, this new compound called Xai-K was shown to be just as effective as rtPA at restoring blood flow in both benchtop and animal models of blood clots, while being safer than rtPA. As Xai-K is derived from human plasma sources, there is a risk for disease transmission. The current work uses the same in vitro and in vivo methods to evaluate the safety and efficacy of mutated rFX as an alternative. This includes plasmin cleavage profiling, chromogenic assays, plasma lysis assays, and surgical models of murine carotid artery clots to evaluate efficacy. Visualization of intracranial bleeding through Evan’s blue and biomarker assays encompass the safety assessments.

Results: Preliminary data show that a mutated rFX, with one mutation designed to prevent its usual clotting factor role and another to stabilize the clot-busting drug, may be a Xai-K and rtPA alternative. Plasmin lysis assays have suggested that the mutations have been successful, and that the protein does not get cleaved into a clot-generating form. Clot lysis assays show the first evidence that the double mutant can be used to bust blood clots in vitro. Further characterization of the new protein and its function in vivo are required.

Conclusions: No major advances to clot-busting therapy have been made for three decades. This project aims to change that unacceptable progress and provide a safer option to current therapy, filling a major gap in patient care. Pre-clinical studies indicate that the double mutant rFX may be used as an alternative to Xai-K, overcoming the production issues and maintaining high clot-busting efficacy. Future work aims to compare rFX to both rtPA and Xai-K in vivo to evaluate its safety and efficacy using the established mouse models of blood clots.
INVESTIGATING THE PATHOLOGY OF DIFFUSE AXONAL INJURY IN TRAUMATIC BRAIN INJURY MURINE MODELS WITH LOW-IMPACT REPETITIVE TBI AND HIGH-IMPACT INTERFACED SINGLE TBI

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ABSTRACT  

Background/objectives: Widely deemed as a “silent epidemic”, TBI constitutes the largest cause of disability and death amongst traumatic injuries. TBI pathophysiology is complex with high variability depending on the traumatic nature. In falls or motor accidents, the brain experiences rapid acceleration, deceleration and rotational force that leads to skull deformation and contusions. The sheer impact force can inflict primary insults on neurons, glial cells and the neurovasculature acutely, followed by a delayed secondary pathological cascade of inflammation, hypoxia and excitotoxicity. The understanding of TBI pathophysiology is largely limited by the infeasibility of human brain biopsy. Hence, animal models such as CHIMERA (Closed-Head Impact Model of Engineered Rotational Acceleration) play a critical role in elucidating TBI pathophysiology. Our previous work suggests that low-impact repetitive TBI and high-impact interfaced single TBI yield distinct gray and white matter injuries with varying temporal profiles. This study aims to delineate diffuse axonal injury in the two injury modalities and characterize them using a combination of bench-side histology and clinically translatable tools including magnetic resonance imaging (MRI) and blood biomarkers. This study can provide insight into the different white matter pathology of TBI and may inform clinical decisions.

Methods: Low-impact repetitive TBI and high-impact interfaced single TBI will be performed on transgenic Thy1-YFP mice which express yellow fluorescent protein (YFP) in sensory and motor neurons. Longitudinally, TBI mice will be subjected to MRI at various time points (6 hours, 2 days, 7 days, 14 days and 30 days) using a 9.4 Tesla preclinical MR scanner. Using MRI sequences including diffusion tensor imaging, neurite orientation dispersion and density imaging (NODDI) and inhomogeneous Magnetization Transfer (ihMT), neuroimaging abnormalities associated with white matter damage will be investigated.

Saphenous blood collection will be performed longitudinally at the same timepoints of MRI study. Plasma will be extracted from the blood, in which axonal injury biomarkers glial fibrillary acidic protein (GFAP) and neurofilament light chain (NF-L) will be analyzed using Quanterix’s Single molecule array.

At each time point, some of the TBI cohort will be dropped out of the longitudinal study and harvested for brain tissue. Brain tissue will then be subjected to histological assessment, 3D tissue clearing and immunohistochemistry including silver staining and immunolabeling for neuroinflammatory markers such as GFAP and Transmembrane protein 119 (Tmem119). Axonal injury morphology such as axonal varicosity and retraction blubs will also be examined in 3D using light sheet microscopy of Thy1-YFP expression in sensory and motor neurons.

Conclusions: CHIMERA is a highly translatable TBI model that recapitulates human pathology. Our future work on CHIMERA using YFP mice will allow delineation of spatial and temporal evolution of axonal injury after TBI and its association with blood biomarkers.
Background/objectives: Select proteins, including chemokines which are regulated by post-translational protein modifications (PTMs), in particular by proteolysis, play key roles in the emergence of promising tools for difficult-to-treat cancers. Proteolysis generates two novel fragments (proteoforms) with new (neo) N and C termini. Neo-N and C termini can be used to identify and monitor the fragments using N termini enrichment proteomics methods. Proteoforms can have different locations, functions, and interaction partners compared to full-length proteins (for instance, losing the necessary part of the protein for their interaction). Neo-N termini can also impact proteoform characteristics directly (for example, stability and protein interaction). Chemokine forms that have been truncated (shortened) by proteolysis display changed biological activity (activation or deactivation), altered receptor affinity, and altered levels of receptor activation. For example, after proteolytic removal of the N terminal arginine amino acid, CXCL12 can still bind to CXCR4, but no longer activate it, so it effectively becomes an antagonist by blocking access to the receptor for other activators. N termini enrichment methods like HUNTER (High-Efficiency Undecanal-based N-termini Enrichment) enable comprehensive identification of truncated proteoforms but our ability to study their function has not kept up. There are currently no high-throughput methods to study the effect of proteolysis on the interaction of signaling molecules (chemokines) with their cell surface receptors. I will address this gap by establishing a platform to engineer full-length and cleaved chemokines and other newly identified truncated signaling proteins. This will allow me to study the interaction of full-length and truncated chemokines with their receptors and the triggered cellular response. Objective: To establish an efficient tag-free expression system of full-length and cleaved proteoforms and study their binding to cell surface proteins and induced downstream activity. This project is focused on the engineering of recombinant chemokines as a proof of principle for mapping proteolytic proteoform-specific protein interactions at the cell surface.

Methods: I will evaluate multiple expression platforms including tag-free systems like the split-intein approach for their ability to obtain active chemokines. I will use interaction and phosphoproteomics to monitor function and use (HUNTER) N termini Enrichment and topdown mass spectrometry from Chick Chorioallantoic Membrane (CAM) models to identify new proteoforms.

Expected Results: I expect our results to find new truncated proteoforms in cancer and demonstrate how the truncation affects the interaction with cells, other proteins, and cellular receptors. I will gain fundamental insights into the proteolytic regulation of cell-cell signaling.

Significance: The work may further inspire new therapeutic approaches based on the targeted inhibition of the formation of specific cleaved forms.
ABSTRACT

Background/objectives: Over 38 million people are currently living with HIV. Despite effective antiretroviral therapies that have prevented transmission and increased lifespan, people living with HIV experience faster cellular and immunological aging relative to their HIV-negative peers. This may be influenced by co-infection with other chronic/latent viruses such as cytomegalovirus (CMV), Epstein-Barr virus (EBV), human herpes virus 8 (HHV-8), herpes simplex virus 1 and 2 (HSV-1/HSV-2), hepatitis C virus (HCV), and hepatitis B virus (HBV). All of these viruses are individually known to be associated with markers of aging or age-associated diseases, but little is known about the effect of having multiple viruses. Our aim was to characterize the number and type of chronic/latent viral infections in a cohort of people living with or without HIV, and determine any association with HIV status.

Methods: HIV-positive (n=103) and HIV-negative (n=101) female CARMA cohort participants were selected for this analysis, with n~15 per decade of age [0-60+]. Infection status for CMV, EBV, HHV-8, HSV-1, and HSV-2 was determined serologically; HIV, HCV, and HBV were self-reported. Associations between total number of viruses, HIV status, and age were assessed using Mann-Whitney and Spearman’s correlation.

Results: Among the HIV-positive and HIV-negative participants, having a greater number of viruses was univariately associated with older age (rho=0.38, p<0.0001), and with HIV-positive status (p=0.0014). Additionally, in a subsequent analysis containing 173 HIV-negative and 161 HIV-positive participants of both sexes, there was a higher prevalence of 6 of the 7 viruses amongst the HIV-positive group compared to the HIV-negative. This includes CMV (Δ= +28%), EBV (Δ= +14%), HSV-1 (Δ= +11%), HSV-2 (Δ= +22%), HCV (Δ= +7%), and HBV (Δ= +4%).

Conclusions: These data suggest that individuals living with HIV experience a higher prevalence of chronic/latent viral infections that could exert a heavier burden on their immune system. Further multivariable analyses are required to ascertain if the association remains after adjustment for variables such as age. Determining whether a higher prevalence of chronic/latent viruses is associated with immune aging gives insight into the value of treating and/or preventing viral infections to improve health outcomes.
DELTAETM: AN EMBEDDED TOPIC MODELLING FOR DISENTANGLING TRANSCRIPTIONAL DYNAMICS IN DIFFERENT CELLULAR CONTEXTS

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ABSTRACT

Background/objectives: Single-cell RNA-seq technology has been successfully applied to profile regulatory-genomic changes in studying many human diseases mechanisms. A typical single-cell study implicitly assumes observed transcript levels as a static value, considering that every cell is fixed at a particular steady-state. Recently, researchers have developed a complementary method to measure gene expression dynamics (the speed of RNA splicing) by taking the difference between the spliced and unspliced counts in scRNA-seq profiles. The RNA velocity approach attempts to estimate a full transcriptional dynamic of splicing kinetics, assuming a complete process of RNA transcription, splicing, and degradation is observed in the data. However, most single-cell datasets, especially those collected from patient-derived cancer samples, only span over several snapshots of full developmental, evolutionary or disease progression processes. Such a shortened period in a study design may not provide enough information for full-scale dynamic modelling based on a system of ordinary differential equations (ODEs).

Methods: To obtain a more robust estimation of RNA velocity for cancer studies, we propose a new modelling framework, Delta-ETM, short for Dynamically-Encoded Latent Transcriptomic Analysis by Embedded Topic Modelling. Delta-ETM combines two ideas: (1) latent topic analysis that will guide unsupervised machine learning for discovering new dynamic cell states, (2) application of first-order approximation to learn robust relationships between the spliced and unspliced counts instead of estimating a full trajectory of ODE models. For a latent topic model, we view each cell as a document and each gene as a word to make model parameters directly interpretable while keeping the Bayesian model’s capability to impute missing information. The simplified dynamic model also permits an intuitive interpretation of spliced-unspliced differences as multiplicative «delta» parameters in the model.

Results: We applied our Delta-ETM approach to comprehensive single-cell datasets on pancreatic ductal adenocarcinoma (PDAC), one of the most challenging cancer types with a poor prognosis. In the latent space, we identified tumour-specific and stage-specific topics marked by a unique set of genes differentially expressed. We found PDAC-related genes (GNAS, MALAT1) to have different dynamics in different topics. We also found Delta-ETM further dissected sub-topics that were clumped together in traditional clustering methods implicating novel gene modules and cell states that are dynamically controlled along with the cancer progressions.

Conclusions: Delta-ETM learns latent topics that inform new dynamic cell states, provides a more robust estimation of RNA-velocity for cancer studies and provides clustering with higher resolutions.
UNRAVELLING MOLECULAR AND BIOCHEMICAL SIGNATURES UNDERLYING TRAUMATIC BRAIN INJURY PATHOPHYSIOLOGY USING LIGHT SHEET MICROSCOPY AND SPATIAL TRANSCRIPTOMICS

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ABSTRACT Background/objectives: Traumatic Brain Injury (TBI) is a leading cause of global death and disability. Many long term TBI survivors suffer from neuropsychological impairments, adding further to the overall societal burden of TBI. TBI induces white matter injury, inflammation, vascular damage and altered neuronal activity. Animal models that reproduce distinct TBI subtypes are therefore very valuable, however, lack of translational biomarkers and temporal molecular signatures limit their relevance to human TBI. The Closed Head Impact Model of Engineered Rotational Acceleration (CHIMERA) is a non-surgical model of impact-acceleration injury that mimics the biomechanics and pathophysiology of human TBI. The primary objective of this study is to identify the spatial and temporal biochemical and molecular changes in the diffusely injured brain after CHIMERA TBI using whole-brain tissue clearing, light sheet microscopy (LSM) and spatial transcriptomics in transgenic mice.

Methods: cFosTRAP mice, which stably express neuronal cFos upon tamoxifen exposure, were randomly assigned to TBI or sham groups. TBI-induced acute neurological deficits were assessed by loss of righting reflex (LRR) and neurological severity score (NSS). Brain tissue was harvested 6h post-procedure and cleared by the SHIELD technique prior to LSM. For spatial transcriptomics, 10-μm sections from sham and TBI brains were mounted onto 10x Visium slides. Immunohistochemical readouts were established to map expression profiles to glia and neurons. Sections underwent cDNA synthesis and library construction, followed by Illumina NextSeq500 sequencing. Reads were mapped and quantified via SpaceRanger and Seurat R package.

Results: TBI mice exhibited prolonged LRR and higher NSS compared to shams. A LSM analysis pipeline, including stitching of volumetric images, 3D rendering, cell segmentation and registration with Allen Mouse Brain Atlas, was established using ARIVIS and BrainQuant3D software. Whole-brain changes and region-specific changes in cFos+ cell counts in sham and TBI brains are ongoing. We obtained transcriptomic information for 55-μm circular regions tiling entire brain sections (2 sections/mouse; 4992 sequenced regions/section; ~40,000 total RNA-seq datasets). We found strong replicate reproducibility for TBI and sham animals. The initial dataset of >1B reads yielded cell clusters with dysregulated genes previously identified in TBI as well as novel differentially expressed genes that will be validated using in situ hybridization.

Conclusions: We established LSM and spatial transcriptomics as whole brain methods to improve analysis of diffuse brain injury mechanisms and identify potential therapeutic targets. Further investigations into the injury sub-types and associated changes in outcome will allow us to recapitulate TBI pathology and identify new targets for development of diagnostic and prognostic markers.
ABSTRACT

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Background/objectives: Traumatic brain injury (TBI) is a major cause of death and disability worldwide. In humans, a moderate-severe TBI may increase risk of neurodegeneration, including Alzheimer's disease. To study the long-term consequences of TBI, we previously designed an animal model of impact-acceleration TBI called Closed Head Injury Model of Engineered Rotational Acceleration (CHIMERA). We induced a single high energy impact TBI to rTg4510 mice, a mouse model of tauopathy, to determine if CHIMERA TBI exacerbates tau pathology.

Methods: Male rTg4510 mice were impacted at 4.0 J at 4 months of age, using CHIMERA with a high energy impact interface. Sham controls received all procedures except for impact. Brain tissues were harvested at 2 months post-injury and analysed using immunohistochemistry and Western blotting for multiple tau epitopes, neuroinflammation, and axonal injury. Blood samples were collected biweekly for biomarker analyses.

Results: Impact energy of 4.0J induced moderate-severe injury with significant mortality (7/15; 47%) immediately after TBI. Compared to sham controls, surviving TBI mice showed significantly increased duration of loss of righting reflex. Histological analyses showed that at 2-mo post-injury, surviving mice displayed significant microgliosis (Iba1) and axonal injury (Neurosilver). Western blotting analysis showed that TBI mice had a reduced ratio of p-GSK-3β (S9) to GSK-3β, suggesting greater tau kinase activity. Plasma total tau in TBI animals showed a trend towards accelerated tau elevation by 2 weeks. In terms of brain tauopathy, surviving TBI animals showed a divergent response. 3 out of 8 surviving TBI animals had very low level of tau protein in brain lysates (lower than shams) and were thus analyzed separately from the other TBI mice that maintained the expected level of tau. Compared to sham mice, TBI mice with normal tau levels had increased p-tau (PHF1 and AT8), increased accumulation of autophagolysosome (P62 and Cathepsin D) and decreased hippocampal size. These findings were not observed in TBI mice with very low total tau levels.

Conclusions: Our preliminary findings suggest that TBI may lead to chronic white matter injury and alteration in GSK-3β activity. Intriguingly, the post-injury total tau response is divergent, with increased p-tau and accumulation of autolysosome observed only in TBI mice that maintain tau levels. The mechanism underlying reduced tau levels remains to be determined.
ABSTRACT

Background/objectives: Clear cell ovarian cancer (CCC) is the 2nd most common ovarian cancer and is histologically and clinically distinct from other subtypes. Late stage CCC have a worse prognosis than other histotypes as they are inherently resistant to standard platinum/taxane chemotherapy. CCC usually arises directly from endometriosis. Our recent findings show that cystathionine gamma-lyase (CTH), a key enzyme in the transsulfuration pathway, is highly expressed in CCC, but not other ovarian cancer subtypes. Whether and how the transsulfuration pathway, notably CTH, enables CCC to adapt to the hostile microenvironment of an endometriotic cyst and ultimately to promote metastasis remain unanswered.

Methods: We generated CTH knockout (KO) cells using CRISPR/Cas9. We assessed effects of CTH loss in vitro -under ambient and stress conditions- and in vivo on cell viability, cell proliferation, ROS levels, migration, invasion, and metastasis. Further, we used an organoid model system to assess the impact of CTH loss in primary endometrial cells on organoid growth and response to stress conditions.

Results: CTH expression is enhanced under stress conditions, notably hypoxia, and this is critical for optimal HIF1α response. CTH loss significantly enhances cell death and reactive oxygen species accumulation suggesting a critical role in conferring cell fitness. Cell migration and metastasis in vitro and in vivo are significantly inhibited by CTH loss. CTH KO in primary endometrial cells impaired organoid growth subjected to hypoxia or ovarian endometrioma cyst content.

Conclusions: Our findings suggest that CTH is a primary stress adaptation factor contributing to hypoxia response, a key feature of CCC. Further, CTH is essential for CCC progression and metastasis. Data from the organoid modeling system suggests that CTH has a central role in initiation and progression of CCC. Therefore, targeting CTH might represent a novel therapeutic opportunity to patients with CCC.
SAMPLE TYPES AND COVID-19 POSITIVITY OF PATIENTS PRESENTING AT THE CHILDREN’S AND WOMEN’S HOSPITAL COVID-19 CLINIC IN VANCOUVER, BRITISH COLUMBIA

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ABSTRACT

Background/objectives: Rapid and accurate identification of individuals with coronavirus disease 2019 (COVID-19) in the community is essential to limit the spread and inform public health decision making. The aim of this study is to evaluate the characteristics of samples collected from patients presenting at the BC Children’s and Women’s Hospital (C&W) COVID-19 clinic.

Methods: This retrospective study took place at the C&W COVID-19 clinic between March 18, 2020 and March 18, 2021. The study examined the correlation of COVID-19 positivity, determined by cycle threshold (Ct) value, with timing of symptom onset. In addition, we examined the acceptability of saline gargles and nasopharyngeal flocked swabs (NPFS) in the overall population, and in children 4-17 years old. Saline gargle collection for patients ≥ 12 years was implemented on August 28, 2020, but a full implementation for patients ≥ 4 years occurred on September 17, 2020.

Results: A total of 21,502 samples (16,912 patients) were included in this study; out of which, 3.53% samples were positive (759 samples from 758 patients). Saline gargle was collected from 34.2% of patients presenting to the clinic while 62.3% had NPFS collected. A total of 586 positive samples had Ct values recorded. Saline gargle samples have a 3.96 (95% CI: 2.92, 5.01) higher average Ct value than NPFS for all gene targets. The Ct values of the SARS-CoV-2 gene targets showed an upward trend with an increased number of days since symptom onset for both NPFS and saline gargle sample types. There is a 0.53 (95% CI: 0.34, 0.72) overall Ct increase with each one-day increase from symptom onset.

After full saline gargle implementation in September 2020, 60.3% of the population chose it over NPFS. There were a total of 1261 encounters where reasons for NPFS collection over saline gargle were documented, including age < 4 years old (54.1%), eating/drinking one hour before testing (18.0%), patient preference for NPFS (16.4%), failure to gargle (7.3%), and brushed teeth one hour before the test (2.6%).

Conclusions: Higher Ct value was associated with more elapsed days since symptom onset. Both NPFS and saline gargle samples were widely used in the collection centre. Saline gargles were chosen more frequently than NPFS after full implementation. NPFS were mainly used when saline gargle tests could not be performed due to younger age, eating, drinking or failure to gargle.
ABSTRACT

Background/objectives: In 2010, an association between frontotemporal dementia (FTD) and transmembrane protein 106B (TMEM106B) was established, whereby genetic variation at the TMEM106B locus was found to be a risk factor for FTD. Further genetic and biochemical evidence has since implicated TMEM106B in several neurodegenerative disorders. In 2021, our group and another independently discovered TMEM106B amyloidogenic filaments in the brain tissue of individuals with FTD and related dementias. Given the growing associations of TMEM106B with neurodegenerative proteinopathies, our aim was to investigate the structural differences that occur in dementia with the goal of increasing our understanding this protein’s role in the pathological cascade and to assist in the development of new analytical tools targeting TMEM106B.

Methods: Initial examination of TMEM106B structure was performed via both theoretical and experimental methodologies. Physicochemical properties and available structural details (e.g., post-translational modifications) were investigated via bioinformatic tools and -omic databases. To assess the ability of commercial anti-TMEM106B antibodies to bind to TMEM106B proteoforms, western blots were performed. The molecular weight, primary structure, and concentration of TMEM106B proteoforms in brain tissue were analyzed by a combination of gel electrophoresis and high-resolution mass spectrometry, including analysis of human brain tissue from immunohistochemically confirmed cases with a variety of proteinopathies (n=23) and neuropathologically unaffected controls (n=3).

Results: The predominant normal physiological form of TMEM106B is a 274-residue transmembrane protein, whose molecular weight ranges from 31-33 kDa (depending on post-translational modifications); however, only the C-terminal domain is implicated in amyloid formation in neurodegeneration. Characterization of available anti-TMEM106B antibodies revealed they had little specificity for physiological or disease related TMEM106B proteoforms. By proteomic analysis, TMEM106B was abundantly found not only at the expected molecular weight but also in lower molecular weight fractions consistent with the presence of truncated sequences and, more specifically, sequences containing C-terminal region of TMEM106B.

Conclusions: Normal physiological versus pathology associated TMEM106B proteoforms have striking structurally differences, most notably related to primary sequence structure. Our cumulative structural data to date, including cryo-EM, immunometric and proteomic studies, highlight the need for anti-TMEM106B antibodies specifically targeting pathology associated proteoforms. Such antibodies would greatly facilitate research in the detection and characterization of TMEM106B-related pathology.
GRANZYME B SECRETING MAST CELLS IN CUTANEOUS LEISHMANIASIS

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ABSTRACT Background/objectives: Cutaneous leishmaniasis (CL) is an infectious disease caused by leishmania species with diverse clinical presentation leading to disfiguring scars usually in exposed parts of the body. The histopathological findings in CL are variable depending on the clinical phenotype, however, common findings in acute lesions are infiltration of lymphocytes, plasma cells and macrophages filled with leishmania amastigotes in reticular dermis and tuberculoid granuloma with very few parasites in chronic cases. The currently available therapeutic options for CL either have limited efficacy or serious side effects. Understanding the contribution of immune cells to pathogen clearance and tissue damage are important for developing new therapeutic strategies. Granzyme B (GzmB), a serine protease produced and stored along with perforin in granules of cytotoxic T cells and natural killer (NK) cells, has been found to be increased in CL and implicated in tissue injury, however, the underlying mechanism(s) has not been elucidated yet. Emerging evidence has revealed the expression and secretion of GzmB, without perforin, by other immune and non-immune cells, and the non-cytolytic role of extracellular GzmB in inflammatory and autoimmune conditions as well as wound healing has been well documented in recent years. In the present study, we looked at other possible sources of GzmB in cutaneous lesions of L. major infection and the relationship between GzmB and pathological changes was assessed.

Methods: Eleven paraffin embedded skin samples obtained from patients with confirmed diagnosis of CL were sectioned and H&E staining was performed for all samples to determine the pathological changes seen in epidermis and dermis. Double immunofluorescent staining for GzmB, markers of CD8+ T cells, NK cells, neutrophils, eosinophils, macrophages, mast cells, and E-cadherin, a cell-cell junction protein, were performed when possible, otherwise, sequential sections were stained. The number of cells positive for both GzmB and each cell marker and the intensity of E-cadherin staining were determined.

Results: The majority of GzmB expressing cells in skin samples were positive for mast cell tryptase. Few GzmB positive macrophages, eosinophils, and neutrophils were also detected. Interestingly, positive cells for NK cell marker were not positive for GzmB. The intensity of E-cadherin was reduced in areas with increased GzmB and was compatible with spongiosis seen in H&E staining of the same sample.

Conclusions: Our results show that mast cells are an important source of GzmB in cutaneous lesions caused by L. major, and the extracellular GzmB secreted by mast cells might be responsible for degradation of E-cadherin and subsequent epidermal changes seen in CL.
ABSTRACT

Background/objectives: Human MDR3 defects cause multiple devastating diseases including a form of fatal childhood cholestasis (PFIC3), hepatocellular carcinoma (HCC), and cholesterol gallstones. The driver of these conditions is bile duct damage due to enhanced bile acid toxicity in the absence of biliary phospholipids, characterized by elevated alkaline phosphatase (ALP), bilirubin, liver damage, and fibrosis around the bile ducts and portal triads. The Mdr2-/- mouse is a model for human MDR3 null mutations and widely used for studies of related liver diseases. However, many features of Mdr2-/- mice remain poorly described, leading to ambiguous, and potentially incorrect, interpretations of animal studies. The current study is to understand the Mdr2-/- mouse model in terms of histological progression, and the most suitable choice of sex and treatment windows for testing therapeutic agents. We also evaluated the potential efficacy of a tetrahydroxylated bile acid, THBA, in this model.

Methods: We compared the gross appearance, liver function tests, and histological features of Mdr2-/- mice of both sexes at 3, 5, 8, 12, 20, 40 and 58 weeks of age, compared to age-matched wild-type mice of both sexes. We treated Mdr2-/- mice of both sexes in some critical windows at 3-6, 3-12, 5-8, 5-12, and 8-12 weeks of age and examined their progression and treatment effects.

Results: ALP, a key liver enzyme indicator for bile duct damage in rodents, is normally high in juvenile wild-type mice, due to bone development, and then gradually reduces to its typical adult levels by 12 weeks of age, in both sexes. In Mdr2-/- mice, however, the males exhibited a steady decrease of ALP from 3 to 20 weeks of age while females at the same ages showed unchanged, and then slightly elevated, ALP, indicating more severe liver damage. Gallstone development was also more severe in female Mdr2-/- mice. The trend of pathological histological features was correlated with the change of ALP in these age groups. From 3 to 20 weeks, we observed increasing portal tract inflammation and ductular proliferation with periductal edema, which progressed to periductal fibrosis. Noticeably, prominent, transient, florid hepatocellular mitosis at 8 weeks of age was observed, but disappeared after 12 weeks. At 40 weeks, the ductular proliferation started to disappear but at the same time hepatocyte dysplasia, which started to occur at 20 weeks, became more diffused. At 58 weeks, early HCC had developed.

When we fed the Mdr2-/- mice with THBA in different treatment windows, we found that female mice treated between 8 and 12 weeks produced the more significant effect as characterized by ALP levels and histological features, including ductular inflammation and fibrosis in the portal triads.

Conclusions: The current study suggests that female Mdr2-/- mice at 8 to 12 of age weeks are most suitable for testing the treatment effects of a therapeutic agent such as THBA, as reflected in its effect on progressive changes to serum ALP, bile duct inflammation, and fibrosis in the portal triads.
NEUROFILAMENT LIGHT AND GLIAL FIBRILLARY ACIDIC PROTEIN ARE BIOMARKERS OF ACUTE SPINAL CORD INJURY

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ABSTRACT Background/objectives: Introduction: The severity of paralysis after traumatic spinal cord injury (SCI) is assessed by a standard neurologic examination that is dependent on patient participation and assessor experience and training. In the acute, emergency setting, this subjective examination may not accurately reflect injury severity or be predictive of outcome. Serum biomarkers could serve an important role in objectively characterizing the severity of injury and precisely predicting neurologic recovery.

Objective: Characterize the response of serum neurofilament light (NF-L) and glial fibrillary acidic protein (GFAP) as it relates to the time and severity of SCI and determine their ability to predict neurologic recovery.

Methods: 118 patients with acute traumatic SCI were enrolled in a prospective multi-centre, observational trial. Paired serial serum and CSF samples were collected over the first five days post-injury. NF-L and GFAP were quantified using SimoaTM technology. Neurologic assessments were performed to define the ASIA Impairment Scale (AIS) grade and motor score (MS) at presentation and at 6-months post-injury.

Results: Serum NF-L and GFAP increased as a function of severity and distinguished among baseline AIS grades. Both biomarkers predicted 6-month: AIS grade improvement in AIS A patients with 80% accuracy; and, whether a patient would be classified as “motor complete” (AIS A/B) or “motor incomplete” (AIS C/D) with 90% accuracy, agnostic of baseline AIS grade. Serum biomarkers significantly improved upon prediction models using clinical assessments in both settings. Serum GFAP and NF-L levels also predicted which cervical patients would gain more than 8 points of motor recovery at 6-months.

Conclusions: This is the largest study to date to demonstrate the potential of acute serum levels of GFAP and NF-L in SCI. Blood biomarkers can inform on the biology of the injury to better delineate the spectrum of injury severity, and thus recovery potential. As objective markers of injury severity, blood biomarkers will have utility in patient stratification and prognostication in clinical trials of acute SCI, in addition to helping clinicians in communicating prognosis to patients in the early stages of injury.
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