PATHOLOGY
laboratory medicine
UNIVERSITY OF BRITISH COLUMBIA

Pathology Day

7th June, 2024 – Abstract Book

GUEST SPEAKERS

Keynote Speaker
Dr. John Hart

Title: "Biopsy Diagnosis of Drug Induced Liver Injury in 2024 - The Dawn of the Molecular Era"

Selected PDF
Dr. Alexandre Aubert

Title: "Rheumatoid Arthritis: Does GzmB make the Cut?"

Guest Lecturer
Dr. David Schaeffer

Title: "Pancreatic Cancer – Does Morphology Still Matter?"

Guest Lecturer
Dr. Philipp Lange

Title: "Towards Multimodal High-content Pathology"

Friday June 7
7:30AM-8PM

VGH-Jim Pattison Pavilion South, JPPS 1891 Lecture Theatre
899 West 12th Avenue Vancouver, BC V5Z 1M9

UBC Medical Student & Alumni Centre (MSAC)
2750 Heather St, Vancouver, BC V5Z 4M2

Message from the Chair

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

As I welcome all of you to this annual Pathology Day, I am also reflecting on the amazing accomplishments made by our outstanding research scientists and academic physicians, our excellent graduate students, resident physicians, postdoctoral and clinical fellows. Despite the resource limitation and ever-increasing competitiveness, our department led 97 new research grants totaling over $26 million and participated in collaborative grants worth nearly $488 million in the year 2023. Our faculty published over 700 peer-reviewed articles and delivered thousands of hours of lectures globally. In the past 3 years, the number Canadian research chairs in our department have doubled (from 3 to 6), and our scientists and students have won numerous awards at the local, national, and international venues. Abstracts in this book, will be presented either on platform or as posters, illustrates the great work done by our trainees in the past year.

Pathology Day is a significant event in the department 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 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.

This year, we are honored to have Professor John Hart, Vice Chair of Pathology at the University of Chicago and former President of the United Staes and Canadian Academy of Pathology as our keynote speaker. We are also privileged to have two remarkable individuals from our own department, Dr. David Shaeffer and Dr. Philippe Lange to speak about their research projects and discoveries.

I would like to extend my sincere thanks to the members of the organizing committee for this event, including Dr. Muhammad Morshed, Dr. Catherine Hogan, Dr. Cheryl Wellington, Dr. Ramon Klein Geltink, Dr. Shazia Masud, Dr. Suzanne Vercauteren, Dr. Spencer Martin, Joyce Zhang, Tetiana Povshedna, Rana Minab, Heather Cheadle, Genevive MacMillan, Debbie Bertanjoli, and Snehaben Dabgar as well as all the other individuals whose efforts make the day a success.

Hoping you all have a wonderful Pathology Day!

PATHOLOGY laboratory medicine
UNIVERSITY OF BRITISH COLUMBIA

Path Day 2024 Planning Committee

Dr. Muhammad Morshed
Co-Chair

Dr. Catherine Hogan
Co-Chair

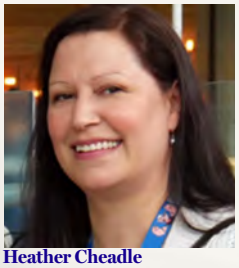
Dr. Cheryl Wellington
Professor

Dr. Ramon Klein Geltink
Assistant Professor

Dr. Shazia Masud
Clinical Assistant Professor

Dr. Suzanne Vercauteren
Clinical Professor

Genevieve MacMillan
Director Human Resources & Administration



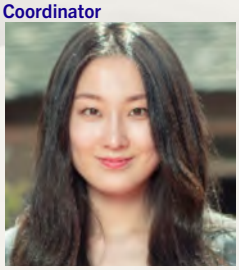
Heather Cheadle
Gratuate Program Coordinator

Debbie Bertanjoli
Databse Info Systems Manager

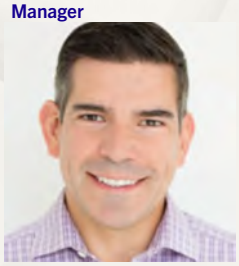
Sneha Dabgar
Executive Assistant

Rana Minab
Grad Student

Tetiana Povshedna
Grad Student



Joyce Zheng
Grad Student



Dr. Spencer Martin
Resident

Judges

Abstract Reviwers	Dr. Angela Fung, Dr. Billie Velapatino, Dr. Cheryl Tomalty, Dr. Eric McGinnis, Dr. Janet Simons, Dr. Joshua Dubland, Dr. Julia Naso, Dr. Karina Rodriguez-Capote, Dr. Maria Victoria Monsalve, Dr. Meng Weng, Dr. Sakara Hutspardol, Dr. Shazia Masud, Dr. Tara Spencer
Oral Session Judges	Corrie Belanger, Dr. Agatha Jassem, Dr. Cheryl Tomalty, Dr. Eric McGinnis, Dr. Julia Naso, Dr.Gerald Krystal
Poster Session Judges	Adriana Airo, Corrie Belanger, Dr. Agatha Jassem, Dr. Cheryl Tomalty, Dr. Chris Fjell, Dr. Cornelia Laule, Dr. Jacqueline Quandt, Dr. Joshua Dubland, Dr. Julia Naso, Dr. Krista Marcon, Dr. Leandro Venturutti, Dr. Shannon Russell, Dr. Shazia Masud, Dr. Tara Spencer, Dr. William E. Schreiber

UBC Pathology Day
June 7, 2024

CONFERENCE OUTLINE

VGH-JPSS 1891 LECTURE THEATRE

7:30am – 8:00am	Light breakfast (Lobby)
8:00am – 8:10am	Welcome and Opening Remark: Dr. Gao
8:10am – 8:40am	Selected Presentation (PDF): Farhia Kabeer **Title**: "Rheumatoid Arthritis: Does GzmB make the Cut?"

ORAL PRESENTATIONS BY STUDENTS & RESIDENTS (VGH-JPSS 1891 LECTURE THEATRE)

8:40am – 8:50am	Hasan Hamze	9:10am – 9:20am	Madeline Lauener
8:50am – 9:00am	Deepak Toor	9:20am – 9:30am	Tali Romero
9:00am – 9:10am	Abdulrahman Almodakha		

9:35am – 10:05am	Power Pitch Talks
10:05am – 10:20am	Break
10:20am – 11:20am	**Keynote Speaker** – Dr. John Hart **Title**: "Biopsy Diagnosis of Drug Induced Liver Injury in 2024 - The Dawn of the Molecular Era

ORAL PRESENTATIONS BY STUDENTS & RESIDENTS (VGH-JPSS 1891 LECTURE THEATRE)

11:20am – 11:30am	Ardalan Akbari	11:50am – 12:00pm	Yu-Yu Lin
11:30am – 11:40am	Derek van Pel	12:00pm – 12:10pm	Fang Fang Li
11:40am – 11:50am	Collin Pryma	12:10pm – 12:20pm	Joyce Zhang

12:35pm – 2:35pm	Poster Presentation & Lunch - **MSAC**

VGH-JPSS 1891 LECTURE THEATRE

2:45pm – 3:15pm	**Keynote Speaker** – Dr. David Schaeffer **Title**: "Pancreatic Cancer – Does Morphology Still Matter?"

ORAL PRESENTATIONS BY STUDENTS & RESIDENTS (VGH-JPSS 1891 LECTURE THEATRE)

3:15pm – 3:25pm	Jamie Lee	3:45pm – 3:55pm	Allen Zhang
3:25pm – 3:35pm	Sirim Kim	3:55pm – 4:05pm	Erin Tanaka
3:35pm – 3:45pm	Genevieve Amaral	4:05pm – 4:15pm	Renying (Loulou) Cai

4:20pm – 4:50pm	**Keynote Speaker** – Dr. Philip Lange **Title**: Towards Multimodal High-content Pathology"
4:50pm – 5:00pm	Thank you from Conference Cochairs
5:15 Onwards	Awards Presentation, Drinks & Canapes, and Networking - **MSAC**

MSAC Address: UBC Medical Student & Alumni Centre (MSAC), 2750 Heather St, Vancouver, BC V5Z 4M2

VGH Address: JPSS 1891 Room, Lecture Theatre, 899 West 12th Avenue Vancouver, BC V5Z 1M9

TABLE OF CONTENTS

ORAL PRESENTATIONS

#		ABSTRACTS
13	**Jamie Lee**(resident)	Simultaneous Lupus Nephritis and Disseminated Tuberculosis with Renal Involvement: To Immunosupress or Not? Jamie Lee 1, Maziar Riazy 1
14	**Sirim Kim**(resident)	Assessing the diagnostic accuracy of p16 immunohistochemistry stain on head and neck fine needle aspirate cytology specimens Sirim Kim1, Lawrence Lee1
15	**Genevieve Amaral**(resident)	Evaluation of the NG-Test® CTX-M MULTI lateral flow immunoassay Geneviève Amaral1, Calvin K.F. Lo1, Jennifer Bilawka2, Gordon Ritchie1,2, Marc G. Romney1,2, Willson Jang2, Christopher Lowe1,2, Nancy Matic1,2, Michael Payne1,2, Aleksandra Stefanovic1,2
16	**Allen Zhang**(resident)	Tracing tissue of origin in squamous cell carcinomas of unknown primary by methylation profiling Allen W. Zhang1, Andrew Galbraith2, Vahid Akbari2,3, Wan Lam1,4, David Huntsman1,5, Steven Jones2,3, Steven Yip1, Julia Naso1
17	**Erin Tanaka**(Grad student)	Mitochondrial Complex II Modulates CD8+ T cell Function Erin Tanaka1,2, Juhee Oh1,2, Liam Johnston2,3, Anne-Sophie Archambault2, Rachel Cederberg3,4, Meredith Clark3,4, Annette Patterson2, Kevin Bennewith1,3,4, Will Bailis5, Ramon Klein Geltink1,2,3
18	**Renying (Loulou) Cai**(Grad student)	The association of hiv, and seven other chronic/latent viral infections with markers of immune aging Renying (Loulou) Cai1,2,7, Nancy Yang1,2, Anthony Hsieh1, Mel Krajden1,6, Melanie Murray3,4,5,7, Helene Cote1,2,3,7

POSTER PRESENTATIONS

#		UNDERGRADUATE STUDENTS
19	**Boaz Li**	Quality controls for spatial gene expression analysis in formalin-fixed paraffin embedded tissues Boaz Li1,2, Samuel Leung1,2, Melody Cheng1,2, Maria Elishaev1,2, Yuancheng Mao1,2, Coco Ng2, Tiffany Chang2, Gurpreet Singhera2, Bobby Grewal3, Basak Sahin2, Chi Lai1,3, Amrit Singh2,4, Ying Wang1,2
20	**Carly Lin**	Viral proteins 3A and 3D synergistically mediate mitochondrial dysfunction in coxsackievirus B3-induced myocarditis Carly Lin1,2, Wendy Hwang1,2, Yasir Mohamud1,2, Honglin Luo1,2
21	**Edward Sobczak**	Placental mitochondrial dna heteroplasmies in women living with hiv and their association with preterm birth Edward Sobczak1, 2, Zeshuo Li1, 2, Hélène C.F. Côté1, 2, 3
22	**Florence Sanjaya**	Effect of Switching to an Integrase Inhibitor on Mitochondrial DNA content in Women with HIV Renying Cai1,2,3, Florence Sanjaya1, Marcela Ardengue Prates Da Silva4,5, Shelly Tognazzini6, Angela Kaida4,6, Melanie CM Murray3,4,5,7,8, Helene CF Cote1,2,3,4,7, on behalf of the Children and Women: Antiretrovirals and Markers of Aging (CARMA; CTN 277) and the British Columbia CARMA-CHIWOS Collaboration (BCC3; CIHR CTN 335)
23	**Ghazal Sokhanran**	Macrocytosis in Mitochondrial DNA Deletion Syndromes Ghazal Sokhanran1, Michelle M Mezei2 3, Farida Almarzooqi4 5, Hilary Vallance 1, Anna Lehman2 6, Gabriella Horvath2 7, Bojana Rakic1, Leslie Zypchen8, Ingrid Blydt-Hansen4, Andre Mattman1 2
24	**Ghazal Sokhanran**	Steps to Improving Glomerular Filtration Rate estimation in British Columbia Ghazal Sokhanran1, Rong Yi2, Mark Ularte2, Andre Mattman1 2
25	**Jennifer Wu**	Clinical validation of the atellica chemistry analyzer for hemoglobin A1C testing Jennifer Wu[1], Vilte Barakauskas[1,2]

#		UNDERGRADUATE STUDENTS CONT'D
26	**Jennifer Yi**	Molecular Stratification of Vulvar Squamous Cell Carcinoma Jennifer Yi1, Kelly Wei1, Julia Chen1, Emily Thompson2, Ardalan Akbari2, Evan Gibbard3, Hang Yang2, Rachel Winardi4, Lien Hoang1, 2
27	**Kate Fitzsimmons**	Utilization and Impact of Ultra-Stat Idylla EGFR Testing for Non-Small Cell Lung Carcinomas Kate Fitzsimmons,1 Curtis Hughesman,2 Marie D'Amours, 3 Deepu Alex, 2,4,5 Diana N. Ionescu, 5 Barb Melosky,3 Kelly McNeil,2 Cheryl Ho, 2 Stephen Yip 2,4,5,6, and Julia R. Naso 4,6
28	**Kaya Frese**	Antiphospholipid IgM distinguish the clinical phenotypes of multiple sclerosis Kaya Frese1, Pal Patel1, Pierre Becquart1, Robert Carruthers2, Anthony Traboulsee2, Ana-Luiza Sayao2, Alice Schabas2, G.R. Wayne Moore1,4, Irene M. Vavasour3,4, Steve Kalloger1,5, Cornelia Laule1,3,4,6, Jacqueline Quandt1,4
29	**Kristen Danielle Go**	Comparison of Multiplex Immunoassays for the Measurement of Neurological Blood Biomarkers Kristen D. Go1, Jennifer Cooper1, Kidus Achalu1, Tali Romero1, Jason Tabor2, Vanessa Dizonno3, Namali Ratnaweera3, Bianca Marginean3, Farnaz Sahragard3, Chantel Debert2, Thalia S. Field3, Carolyn Emery2, Sophie Stukas1, Cheryl Wellington1,4,5
30	**Longyijie Wei**	Dual targeting of cystathionine gamma-lyase and mTOR pathway in clear cell ovarian cancer Longyijie Wei1,2, , Amal El-Naggar1,2, Yuqin (Lucy) Li1,2, Yuchen Din1,2, Busra Turgu1, and David Huntsman1,2.
31	**Lucy Ogoke**	Comprehensive Comparative Analysis of MOLLI (Magnetic Occult Lesion Localization and Imaging) Seed-Guided Excision Versus Fine-Wire-Guided Excision in Breast Cancer Surgery: a pathologic study Lucy Ogoke 1,2,3 , Saangwook Michael Woo 1 , Wei Xiong 1,2,3 , Weiwei Michael Chen 1,2,3
32	**Mckenna Stuart**	Investigating axonal damage after traumatic brain injury (TBI) using whole-brain machine learning image analysis tools Mckenna Stuart* 1,2, Mehwish Anwer*1,2, Bethany Kondiles*4, Wai Hang Cheng1,2, Jianjia Fan1,2, Luis Dias3,4, Carlos Barron1, Wolfram Tetzlaff4, Fabio Rossi3, Peter A Cripton1,3, Cheryl L Wellington1,2,3
33	**Navtej Kang**	Comparison and characterization of opal fluorescent dyes Navtej Kaur Kang
34	**Nicola Wray**	The biomarker potential of neurofilament light and heavy chains in preclinical models of multiple sclerosis Nicola Wray1, Emily Kamma2, Raneen Abdul-Rahman2, Jacqueline Quandt2
35	**Puneet Kaur Arora**	Immune Cell-Tumor Cell Neighbor Pair Analysis to Guide Personalized Lung Cancer Treatment in Canada Puneet Arora1, Kouther Noureddine1, Fumi Inaba1, Spencer Martin2, Richard Xiang3, Nina Wu1, Martial Guillaud1, Anna McGuire3, Calum MacAulay1
36	**Vincent Labador**	Cytosponge pathology test has high specificity in detecting barrett's esophagus-related neoplasia in patients undergoing surveillance Vincent Labador,Wei Xiong
37	**Zheng Fang Yang**	When classifications of myeloid neoplasms clash Zheng Fang Yang, Ian Bosdet1,2, Eric McGinnis2,3
#		**GRADUATE STUDENTS**
38	**Alexandra Witt**	Development of double mutant clotting factor X as a novel thrombolytic agent Alexandra Witt 1,2,3 , Scott Meixner 2,3 , Ed Pryzdial 1,2,3
39	**Amir Parham Pirhadi Rad**	Quantifying consistency in tissue oxygenation across anatomical landmarks in healthy individuals through near-infrared spectroscopy Amir Parham Pirhadi Rad 1,2 , Mehdi Nourizadeh 1,2 , Dr. Babak Shadgan 1,2 ,3

#		GRADUATE STUDENTS CONT'D
40	**Ali Khajegili Mirabadi**	Beyond words: evaluating pathology large vision models with large language models Ali Khajegili Mirabadi1, Katherine Rich1, Hossein Farahani1,2, Ali Bashashati1,2
41	**Bob Lin**	Investigating the Phosphatidylinositol 4-phosphate Pathway of Coxsackievirus B3-Induced Non-Canonical Autophagy Bob Lin1,2, Yasir Mohamud1,2, Honglin Luo1,2
42	**Cyril Helbling**	Navigating Challenges in the Development of Seed Amplification Assay for Synucleinopathies Cyril Helbling1, Mari L. DeMarco1,2
43	**Fabiola Wu Wu**	Different metabolomic profiles in adult compared to pediatric chronic graft-versus-host Disease Fabiola Wu Wu1,3, Tashi Rastogi1, Bernard Ng2, Liam Johnston3, Sayeh Abdossamadi1, Amina Karimina1, Madeline Lauener1, Elena Ostroumov1, Barnaby Malong1, Dong Jun Zheng1, Kirk R. Schultz1
44	**Forouh Kalantari**	Human endometrial organoid model implicates G6PD-dependant metabolism as a potential targetable pathway in ARID1A mutant gynecological cancers including clear cell ovarian cancer Forouh Kalantari, Dawn Cochrane, Amal El Naggar, Christopher Hughes, Rodrigo Vallejos, Maxwell Douglas, Christine Chow, Bmlsc, Gian Negri, Gregg Morin, David Huntsman.
45	**Fumi Inaba**	Large-scale DNA Organization Identifies Aggressive Prostate Cancer in Low and Intermediate-Risk Patients Treated with Radical Prostatectomy Fumi Inaba1, Zhaoyang Chen1, Anita Carraro1, Paul Gallagher1, Miha Pukl2, Mira Keyes3, Calum MacAulay1, Martial Guillaud1
46	**Jennifer Cooper**	Validating the utility of plasma biomarkers to diagnose autopsy confirmed Alzheimer's disease Jennifer Cooper1,2, Sophie Stukas1,2, Robin Hsiung2, Cheryl Wellington1,2,3,4
47	**Guadalein Tanunliong**	Anti-SARS-CoV-2 IgA and IgG antibodies in the classification of recent COVID-19 among pregnant individuals Guadalein Tanunliong1, Ana Citlali Marquez2, Hind Sbihi1,2, Tamara Pidduck2, Fang Fang Li1, Danielle Luk2, Mel Krajden1,2, Agatha Jassem1,2, Deborah Money1,3, Inna Sekirov1,2
48	**John Perrier**	Coagulation initiated by tissue factor on a coronavirus John Perrier1,2, Henry West1,2, Michael Sutherland1,2,3, Ed Pryzdial1,2,3
49	**Julliet Zama**	The Impact of hepatitis C virus (HCV) clearance on markers of immune aging and inflammation among women living with and without human immunodeficiency virus (HIV) over time. Julliet K. Zama1, 2, 3, Izabella Gadawski1, Hélène C. F. Côté1, 2, 3
50	**Katlyn Richardson**	Granzyme k induces keratinocyte proliferation and inflammation: implications for psoriasis pathogenesis Katlyn C. Richardson1,2,3, Alexandre Aubert1,2,3, Christopher Turner1,2,3, Layla Nabai1,2,3, Sho Hiroyasu1,2,3, Megan Pawluk1,2,3, Karen Jung1,2,3, Angela Burleigh4, Richard Crawford2,4, David Granville1,2,3
51	**Kidus Achalu**	Comparison of Plasma Proteomic Profiles of Adolescent Athletes Pre- and Post-Concussion Achalu, Kidus1,2, Cooper Jennifer1,2, Stukas Sophie1,2, Agbay Andrew1,2, Ghodsi Mohammad1,2, Tabor, Jason5,6,8, Debert Chantel 6,7,8,11, Fraser Douglas D10, Emery, Carolyn A5,6,7,8,9, Wellington Cheryl1,2,3,4
52	**Lauren Deneault**	The role of cysteine residues in the binding of novel therapeutics for prostate cancer L Deneault1,, A Tien 1, CA Banuelos 1, T Tam 1, RJ Andersen 2, MD Sadar 1
53	**Liam Byrne**	Extracting antibiotic resistance genes from wastewater Liam Byrne1,2, Natalie Prystajecky1,2
54	**Mayur Mallya**	Deep learning-based foundation models predict ovarian bevacizumab response using histopathology images Mayur Mallya, Ali Mirabadi, Hossein Farahani, Ali Bashashati

#		GRADUATE STUDENTS CONT'D
55	**Mehdi Nouri Zadeh**	Potential application of near-infrared spectroscopy in monitoring muscle spasticity; a feasibility study in healthy individuals Mehdi Nouri Zadeh1,2, Yekta Saremi 1, Stefan Lazarevik 1, Babak Shadgan 1,2
56	**Michael Lane**	Granzyme b: a novel mechanism for dermal-epidermal junction degradation in stevens-johnson syndrome/toxic epidermal necroylsis Michael Lane1,2,3, Faith Liu1,2,3, Alexandre Aubert1,2,3, Valerio Russo1,2,3, Touraj Khosravi4, Karen Jung1,2,3,Hongyan Zhao1,2,3, Layla Nabai1,2,3, Richard Crawford2,4, Elizabeth Phillips5, and David J. Granville1,2,3
57	**Mohammad Ghodsi**	Preliminary overview of neurological blood-based biomarkers in survivors of acute intimate partner violence Mohammad Ghodsi1, Shambhu Adhikari2, Hannah Varto3, Jennifer Ehirchiou3, Jennifer Cooper1, Megan Harper1, Karen Mason4, Noah D. Silverberg1, Sandy Shultz5, Paul van Donkelaar2, Cheryl Wellington1
58	**Parisa Golesorkhi**	Development of an anti-adhesive and antibacterial coating for platelet storage bags to improve quality of platelets during storage Parisa Golesorkhi
59	**Nikolay Alabi**	Novel deep learning-based diagnosis of micropapillary carcinoma in bladder cancer whole-slide pathology images Graham Archibald1* , Maryam Asadi1* , Nikolay Alabi1* , Ali Khajegili Mirabadi1, Alberto Contreras-Sanz2, Walid Eshumani2 , Hossein Farahani1 , Peter Black2** , Gang Wang3** , and Ali Bashashati1,3**
60	**Peyman Malek Mohammadi Nouri**	immune protection of chimeric antigen receptor T cells via direct enzymatic polysialylation to improve persistence for cell therapy Peyman Malek Mohammadi Nouri1,2, Haiming Luo2,3, Vivian Fung4,5, Lyann Sim3, Majid Mojibian4,5, Megan K. Levings4,5,6, Stephen G. Withers3, Jayachandran N. Kizhakkedathu1,2,3,6
61	**Razieh Sadat Banijamali**	Mesenchymal stem cell-derived extracellular vesicles: a new strategy for delivering oncolytic coxsackievirus B3 in triple-negative breast cancer treatment Razieh Sadat Banijamali 1,2>, Amirhossein Bahreyni 1,2, Yasir Mohamud 1,2, Honglin Luo 1,2*
62	**Rebecca Ho**	Targeting metabolic reprogramming in ARID1A/B dual-deficient dedifferentiated endometrial carcinoma Rebecca Ho 1,2, Eunice Li 2, Bengul Gokbayrak 2, Shary Chen 1,2, Ran Tao2
63	**Ryan Chan**	Histopathology of posttraumatic syringomyelia in a porcine model of spinal cord injury Ryan Chan1 , Jing Wang1, Shenani Basnayake1, Sigrun Jarlsdottir1, Femke Streijger1, Juliana Mitchell1, Ali Zaidi1, Brian K. Kwon1,2
64	**Taylor Da Silva**	Investigating the role of platelet factor 4 in asthma Taylor Da Silva1,2, Lynn Huang1, Ahmed Kabil1,3,4, Michael Hughes1,3,4, Kelly McNagny1,3,4, Tillie Hackett5, Dana Devine1,2, Hugh Kim1,6,7
65	**Tetiana Povshedna**	Self-reported hiv viral load is reliable and not affected by adverse lived experiences of women living with hiv in british columbia Tetiana Povshedna1,2,3, Shayda A Swann4,5,6, Marcela A.P. Silva4,5, Shelly Tognazzini7, Melanie Lee7, Angela Kaida4,7, Melanie CM Murray1,3,4,5,6, Helene CF Cote1,2,3,4, on behalf of the British Columbia CARMA-CHIWOS Collaboration (BCC3; CIHR CTN 335)
66	**Wan Hei CHENG**	Characterizing inflammation in coronary artery disease Wan Hei Cheng, Yuancheng Mao, Samuel Leung, Coco Ng, Gurpreet Singhera, Basak Sahin, Amrit Singh, Chi Lai, Ying Wang
67	**Vivian Zhu**	Evaluating the impact of an Nr1h3 risk variant identified in multiple sclerosis families on neuroinflammation in preclinical models Vivian Zhu1 , Emily Kamma1, Pierre Becquart1, Jacqueline Quandt1
68	**Yuchen Ding**	Exploring the therapeutic effectiveness of EO3001 in the treatment of clear cell ovarian cancers with ARID1A mutations Yuchen Ding1,2, Lucy Yuqin Li1,2, Joyce Yuhan Zhang1,2, Grace Longyijie Wei1,2, Farhia Kabeer1,2, Forouh Kalantari1,2, Jeffrey Bacha3, Dennis Brown3, Sarath Kanekal3, Neil Sankar3, Michael Lizardo2, Amal M EL-Naggar1,2, David Huntsman1,2

#		GRADUATE STUDENTS CONT'D
69	**Yuqin Li**	Cystathionine gamma-lyase as a therapeutic target for clear cell ovarian cancer Yuqin Li1,2, Amal El-Naggar1,2, Yuchen Ding1,2, Grace Wei1,2, Artem Cherkasov1,3, Jason Smith3, David Huntsman1,2
70	**Zhihan Wang**	Unraveling the Genetic Factors Influencing Susceptibility and Resistance to Viral Myocarditis Zhihan Wang 1 , 2, Yasir Mohamud 1 , 2, Amrit Singh 1 , 3, Honglin Luo 1 , 2
#		**PDF/RA/STAFF SCIENTIST**
71	**Kevin Shopsowitz** (resident)	Global hemostatic monitoring with ROTEM for managing coagulopathy in ECMO patients: a retrospective study. Kevin E Shopsowitz1, Robert Guo2, Geoffrey Chan3, Jian Mi3, Tyler Smith1,3,4, Kristine Roland1,3, Jacqueline Trudeau5, Hussein D Kanji6, Sakara Hutspardol1,3
72	**Anne-Sophie Archambault** (PDF)	Purine catabolism is important in the secretion of IL-1beta in macrophages Anne-Sophie Archambault*1,2, Lauar de Brito Monteiro*1,3, Lucas F. Starchuk1,2, Yvonne Pang1,4, Armando Alcazar5, Joshua Dubland1,2,6, Bojana Rakic1,2,6, Annette E. Patterson1,2, Juhee Oh1,2, Emilia Perraso1,2, Susan Menzies1,4, Laura Sly1,4, C. Bruce Verchere1,3, Ramon I. Klein Geltink1,2,7.
73	**Guangze Zhao** (PDF)	CVB3-induced m6A modification of RNA enhances viral replication via suppression of YTHDF-mediated stress granule formation Guangze Zhao1,2, Mary Zhang1,2, Yankuan T. Chen2, Sana hakeshmiri2, Fione Yip2, Decheng Yang*1,2
74	**Zhenwei Ma**(PDF)	Multifunctional hydrogel bandage device for head and neck cancer management Zhenwei Ma, Wena Shi, Hui Xue, Tanwei Destin Du, Victor Ling, Yuzhuo Wang, Zu-hua Gao
75	**Ingrid Elisia (Staff)**	A low carbohydrate diet high in soy protein and fish oil reduces azoxymethane/dextran sodium sulfate-induced colorectal cancer in balb/c mice: role of metabolism, inflammation and the microbiome Ingrid Elisia1, Sara Kowalski1, Michelle Yeung1, Gerald Krystal1
76	**Ingrid Elisia** (Staff)	Fishoil enhances the efficacy of a ketogenic diet in lowering tobacco carcinogen-induced lung cancer Ingrid Elisia1, Michelle Yeung1, Sara Kowalski1, Jason Tee1, Gerald Krystal1
77	**Jaswinder Khattra** (Clinical Scientist)	Evaluation of TB Xpert® MTB/Rif Ultra assay on formalin-fixed paraffin-embedded tissues for Mycobacterium tuberculosis diagnosis Calvin Ka-Fung Lo1, Dale Purych2, Inna Sekirov1,3, Jaswinder Khattra2, Trevor J Hird3, Shazia Masud1, 2

#1

ORAL PRESENTATION

Dr. Alexandre Aubert

Supervisior: Dr. David J. Granville

Title: Rheumatoid Arthritis: Does GzmB make the cut?

AUTHOR(s)

Alexandre Aubert1, Jenna Goeres1, Katlyn C. Richardson1, Lorenz Nierves2,3, Karen Jung1, Amy Liu1, Martin Kao1, Nabangshu Das1,2,3, Gertraud Orend4, Roman Krawetz5, Philipp F. Lange2,3, Alastair S. Younger6, Jonathan Chan7, David J. Granville1

AFFILIATION(s)

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ABSTRACT

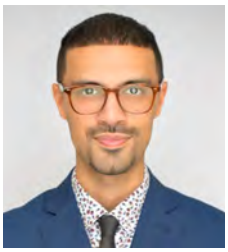
Background/objectives: Rheumatoid arthritis (RA) is one of the most common autoimmune disorders in developed countries, affecting around 1% of the global population with a female:male ratio of 3:1. It is characterized by joint swelling and stiffness, pain, as well as fatigue that can lead to severe disabilities and increased mortality. On a biological level, RA is caused by a sustained inflammation and hyperplasia of the joint synovium that can lead to cartilage degradation, bone erosion, as well as autoantigen generation. Though better known for its role in cytotoxic lymphocyte-mediated apoptosis, Granzyme B (GzmB) is a serine protease that is secreted and accumulates in the extracellular space in inflamed tissues. Retaining most of its proteolytic activity, extracellular GzmB contributes to disease progression through the cleavage of cell surface receptors, cell-cell junction, as well as extracellular matrix (ECM) components. While several reports indicate that GzmB levels are elevated in synovial fluids of RA patients and correlated to disease severity, little is known about its precise role in RA. Preliminary results obtained in our lab by N-terminal degradomics suggest that Tenascin-C (TNC) – a large ECM glycoprotein and a source of autoantigenic peptides in RA – is a novel substrate for GzmB. Consequently, we hypothesize that GzmB cleaves TNC in patients with RA.

Methods and results: In silico modeling using the GrabCas software – a bioinformatics tool that identifies aspase-like protease cleavage sites in protein sequences – predicted the presence of 10 potential GzmB cleavage sites within the sequence of TNC. Using two sources of recombinant human TNC, we confirmed its cleavage by human GzmB in vitro. GzmB cleavage of TNC, impeded by a GzmB inhibitor, generated three TNC fragments (~130, 70, and 25 kDa) consistent with the cleavage sites predicted in silico. Analysis of the generated peptides by mass-spectrometry also confirmed one of the identified cleavage sites, consistent with the release of the 25 kDa fragment. To investigate the relevance of this cleavage in vivo, we collected synovial fluids from RA patients (n=7), osteoarthritic patients (n=4), as well as healthy controls (n=5). By ELISA, we identified a significant elevation of GzmB and TNC levels in the synovial fluids from RA patients compared to healthy controls, as well as positive correlation between the levels of the protease and the glycoprotein (r=0.453, p=0.03). In line with our in vitro results and in silico prediction, immunoblotting analyses revealed the presence of two TNC fragments (70 and 25 kDa) in the synovial fluid of patients with RA, supporting our hypothesis that GzmB mediates cleavage of TNC in RA patients.

Conclusion/significance: By cleaving TNC, GzmB may contribute to the pathobiology of RA through the generation of new autoantigens.

#2

ORAL PRESENTATION



Hasan Hamze

Supervisior: Dr. Nancy Matic

Title: Culture positivity and antimicrobial susceptibility profile of Helicobacter pylori isolates from 2009-2023 in Vancouver, Canada.

AUTHOR(s)

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2 Department of Pathology and Laboratory Medicine, St. Paul's Hospital, Providence Health Care, Vancouver, BC

ABSTRACT

Background/objectives: Helicobacter pylori is a highly fastidious Gram-negative bacterium implicated in gastrointestinal diseases, including gastritis and peptic ulcers. There is limited antimicrobial susceptibility data on this organism in Canada. Patients failing empiric antibiotic therapy may undergo gastric biopsies, which can be submitted for microaerophilic culture and antimicrobial susceptibility testing (AST). Gastric biopsy samples from across Vancouver are referred to St. Paul's microbiology laboratory for AST. We retrospectively analyzed data from the past 14 years to determine the AST profile and factors associated with H. pylori culture positivity.

Methods: Gastric biopsies received for culture were reviewed from July 2009 to February 2023. Specimen transport time, Gram smear, direct urease test, culture result, and AST profile were analyzed (IBM SPSS Statistics 2023). Using gradient strip methodology and EUCAST breakpoints, AST was performed for amoxicillin, clarithromycin, metronidazole, levofloxacin, and tetracycline.

Results: A total of 579 biopsy samples were received for H. pylori culture, of which 228 (39.4%) were culture-positive. The average transport time to the laboratory was 2.7 hours for culture-positive samples, and 3.6 hours for culture-negative samples (P=0.04). Samples with a transport time of <1 hour had 1.81 times the odds (P<0.015) of being culture-positive compared to those with a transport time of >1 hour. Gram smears demonstrated curved Gram-negative bacilli in 60.5% (138/228) of culture-positive samples, but only 4.3% (15/351) of culture-negative samples (P<0.001). Smear-positive samples had 18.8 times the odds (P<0.001) of being culture-positive compared to smear-negative samples. Direct urease testing was positive in 63.2% (144/228) culture-positive samples versus 18.2% (63/347) of culture-negative samples (P<0.001). Urease positive samples had 7.7 times the odds (P<0.001) of being culture-positive compared to urease negative samples. H. pylori isolates were 97.3% susceptible to amoxicillin (216/222), 99.1% to tetracycline (223/225), 50.4% to levofloxacin (68/135), 25.9% to metronidazole (58/224), and 12.9% to clarithromycin (29/224).

Conclusions: H. pylori recovery rates are improved with short transport times, particularly <1 hour. Smear positivity and direct urease test positivity were highly associated with culture success rates. In this population of refractory cases of H. pylori infection, resistance rates to clarithromycin, metronidazole and levofloxacin were high, while amoxicillin and tetracycline remained susceptible. Susceptibility rates were stable over time. These findings could be used to inform treatment regimen selection for patients with a history of prior treatment failure.

#3

ORAL PRESENTATION

Deepak Toor

Supervisior: Dr. Lawrence Lee

Title: Pathological findings (or lack thereof) in benign breast specimens at VGH

AUTHOR(s)

Deepak Toor

ABSTRACT

Background/objectives: Review the rate of pathological findings in benign breast specimens at Vancouver General Hospital.

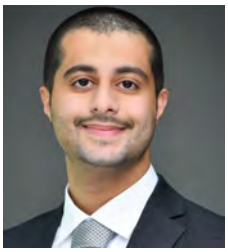
Methods: Sunset search of all benign breast specimens processed in 2017 (in order to have 5 year follow-up) & 2022 (to quantify the increase in caseload).

Results: 1,014 benign breast specimens were reviewed. There were no cases of invasive carcinoma nor DCIS . There were 7 cases total of high-risk lesions (ADH, ALH, LCIS). Clinical management did not significantly change in these 7 cases.

Conclusions: Gross-only evaluation in patients < 35 years old is a safe option at Vancouver General Hospital.

#4

ORAL PRESENTATION



Abdulrahman Almodahka

Supervisior: Dr. Claudine Desruisseaux

Title: Performance of a novel chromogenic agar for primary isolation of pathogenic beta-hemolytic streptococci from throat swabs

AUTHOR(s)

*Claudine Desruisseaux1,2, *Abdulrahman Almodahka2, Rebecca Buckman1, Kim Sy1, Vincent Tang1, Charlene Porter1, Marthe K. Charles1,2

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ABSTRACT

Background/objectives:
Group A Streptococcus (GAS), group C and G streptococci (GCS/GGS) are common causes of bacterial pharyngitis. Chromogenic media are well-established and have been proposed to streamline high-throughput diagnostic workflow. This study aimed to validate the ColorexTM StrepACG (CHROMagarTM, Paris, France) developed to facilitate detection of pathogenic beta-hemolytic streptococci from throat samples.

Methods:
Clinical validation was conducted using 239 throat swabs collected between November 6, 2023 and December 2, 2023. Additionally, 56 spiked EswabsTM (Copan EswabTM) (15 GAS; 7 GCS; 10 GGS; 24 other commensals of pharyngeal flora) were included. Swabs were plated onto ColorexTM and blood agar plates (BAP) and incubated for 18-24 hours at 35-37°C in CO2. ColorexTM plates were examined for colorimetric suspected growth of GAS (orange/red colonies) or GCS/ GGS (light-mauve to purple colonies) by a trained technologist, blinded to results from BAP. Chromogenic agar results were compared with those from BAP. Presumptive growth of beta-hemolytic streptococci was verified by MALDI-TOF. Samples were considered true-positive (TP) if ≥ 1 media yielded growth of beta-hemolytic streptococci, confirmed by MALDI-TOF.

Results:
In total, 100 ColorexTM plates were read as positive, while 195 were negative. Positive and negative-percent agreements with BAP for presumptive growth of beta-hemolytic streptococci were 82.6% (95%CI, 74.4–89.0%) and 97.2% (95%CI, 93.6–99.9%) respectively. As per MALDI-TOF identification, 109 of 295 swabs were considered TP (85 TP-GAS; 24 TP-GCS/GGS. 10.9% (8/73) of ColorexTM with TP GAS yielded brown-purple colonies. After discrepant analysis, sensitivity of ColorexTM and standard BAP were 95.4% (95%CI, 89.7-98.5%) and 97.3% (95%CI, 92.1-99.4%) respectively; whereas specificities were 97.6% (95%CI, 94.6-99.4%) and 94.6% (95%CI, 90.3-97.4%) respectively.

Conclusions:
These findings suggest that ColorexTM StrepACG should be used in combination with BAP for throat samples. Further assessment would be required to establish if this holds true in the context of total laboratory automation paired with artificial intelligence-powered digital plate interpretation modules.

#5

ORAL PRESENTATION

Madeline Lauener

Supervisior: Dr. Kirk Schultz

Title:CD56brightCD16-Perforin- regulatory natural killer cells associated with the suppression of chronic graft-versus-host disease prevent CD4+ T cell proliferation through PD-1 and LAG-3 dependent pathways

AUTHOR(s)

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ABSTRACT

Background/objectives: Chronic graft-versus-host disease (cGvHD) is a major cause of morbidity after Hematopoietic Stem Cell Transplantation (HSCT). Previously, in large cohorts of HSCT patients we identified increased numbers of CD56bright NK cells (NKreg) to be associated with a lack of cGvHD development. Further functional analysis suggested that the NKreg population suppresses CD4+ T cells through a non-cytolytic, and contact-dependent mechanism, though the exact receptor and ligand interaction of which CD4+ T cell populations are impacted is unknown. We hypothesized that the NKreg mechanism may be specific in suppressing CD4+ T cell subpopulations involved in inflammatory responses.

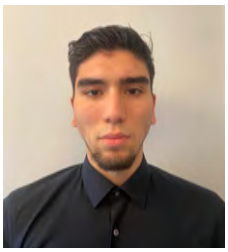
Methods: To investigate the suppressive capacity of NKreg cells, the cells were isolated from healthy donor peripheral blood and co-cultured with CD4+ T cells, CD8+ T cells, Treg cells, B cells, or NK cells for 96hrs. After 96hrs, the proliferation and viability of the responder cells were evaluated via proliferation dye dilution, and 7-AAD dye, respectively. To determine if the CD4+ T cell suppression is specific to a T helper (Th) cell subset we stained the co-cultured cells with a Th1/Th2/Th17 Phenotyping Kit. Further, PD-1 and LAG-3 neutralizing antibodies were added to the NKreg/CD4+ T cell co-culture to determine the receptor dependence. All samples were acquired with the FACSymphony Flow Cytometer and analyzed via Kaluza software.

Results: NKreg cells strongly suppress CD4+ T cell proliferation (approximately 96% suppression of CD4+ T cell proliferation at the 1:1 ratio of NKreg cells to CD4+ T cells). The contact-dependent mechanism of NKreg suppression of CD4+ T cell proliferation was significantly decreased when blocking either the PD-1 (15% decrease in suppression, $p=0.03$), or LAG-3 (29% decrease in suppression, $p=0.04$) receptors, at the 1:2 ratio of NKreg cells to CD4+ T cells. When both receptors are blocked the inhibition of suppressive effect is comparable to that of the LAG-3 blocking antibody being added individually (30% decrease in suppression, $p=0.03$). The suppressive mechanism of NKreg cells was observed to be selective in that they strongly suppress CD4+ T cell proliferation, but do not result in statistically significant suppression of CD8+ T cell, Treg cell, B cell, NK cell, or a specific Th cell subset proliferation ($p>0.05$).

Conclusions: As a result of our studies, we have confirmed the NKreg cell immune suppressive function towards CD4+ T cell proliferation, a main contributor to cGvHD development. Further, we demonstrated a PD-1/LAG-3-dependent direct contact mechanism of NKreg cell suppression, which is selective of total CD4+ T cells, with a lack of suppressive effect towards CD8+ T cells, Treg cells, B cells, and NK cells. The results of these studies contribute to our better understanding of how NKreg cells may induce a cell-specific suppressive function to promote immune tolerance, providing potential cell therapeutic applications for enhancing NKreg cell suppressive function through increasing PD-1/LAG-3 ligand or receptor expression on NKreg cells.

#6

ORAL PRESENTATION



Tali Romero

Supervisior: Cheryl Wellington and Thalia Field

Title: Association of neurological blood-based biomarkers with baseline neuroimaging and cognitive assessments in adults with moderate-severe congenital heart disease

AUTHOR(s)

Tali Romero,1,2 Sophie Stukas,1,2 Vanessa Dizonno, 3 Namali Ratnaweera,3 Bianca Marginean,3 Thalia Field,1,3 Cheryl Wellington1,2

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ABSTRACT

Background: Advances in clinical practice over the past several decades have increased the median lifespan for individuals living with severe congenital heart disease (CHD) by almost 20 years. Consequently, the demographics of this population have evolved such that two-thirds of individuals living with CHD are adults. While the majority of CHD research has focused on cardiac and neurodevelopmental outcomes, recent findings suggest that adults with CHD are at an increased risk of dementia, and cross-sectional studies in younger cohorts indicate an elevated risk of neurocognitive impairment. These observations highlight the need for research investigating the pathological effects of CHD on brain health, as it is still unknown whether these deficits are driven by early-life insults, ongoing accumulating injury, or both. This study measured serum concentrations of neurofilament-light (Nf-L) as a marker of neuronal injury and glial fibrillary acidic protein (GFAP) as a marker of astrocytic activation and vascular damage in a cohort of 93 adults with moderate-severe CHD. The objectives of this study are to (1) assess how individuals with moderate-severe CHD compare to age-matched Canadian normative data and (2) analyze associations between Nf-L/GFAP concentrations with neuroimaging markers and cognitive performance.

Methods: We measured Nf-L/GFAP in an existing cohort of 93 adults with moderate-severe CHD enrolled in a longitudinal brain health study of adults with CHD. Participants undergo cognitive testing, a brain MRI, and a blood draw. Serum specimens were analyzed for Nf-L/GFAP using the Simoa HD-X, an ultra-sensitive semi-automated ELISA platform. Study participants were compared to age-adjusted reference intervals (RI) with the Fisher's Exact test and group-wise comparisons between moderate vs severe CHD were performed using the Mann-Whitney U test. Multivariable regressions, including demographic/clinical variables, will be performed to investigate associations between Nf-L/GFAP and quantitative neuroimaging/cognitive data.

Results: Among study participants, 37 (40%) and 6 (6.4%) were above the 95th percentile RI for GFAP and Nf-L respectively. More individuals above the GFAP 95th percentile RI had white matter hyperintensities (WMH) on MRI (88% vs 45%; Fisher's-exact test, p=0.004, OR=4.3). However, the same was not true for Nf-L (100% vs 52%; Fisher's-exact test p = 0.0618). Additionally, median Nf-L and GFAP concentrations were higher in individuals with WMH vs without (Nf-L: 7.4 pg/mL vs 6.2 pg/mL, p = 0.0045; GFAP 90.85 pg/mL vs 66.4 pg/mL, p = 0.019)

Conclusions: Preliminary analysis demonstrates that there is evidence of elevated GFAP in individuals with CHD who have WMH on MRI. Further analyses examining the relationship between biomarkers and quantitative MRI measures, including WMH and brain volume measurements, and the relationship between biomarkers and cognitive performance, are pending. This work will provide insight into mechanisms of brain injury and the utility of neurological blood biomarkers in the context of adult CHD and may be leveraged to test future preventative and therapeutic strategies in this population.

#7

ORAL PRESENTATION

Ardalan Akbari

Supervisior: Dr. Lynn Hoang

Title: Retrospective Identification of Endometrial Mesonephric-like Adenocarcinomas: Insights Into Morphology, IHC, and Molecular Data

AUTHOR(s)

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AFFILIATION(s)

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2 British Columbia's Gynecological Cancer Research Team (OVCARE), Vancouver, Canada
3 Genetic Pathology Evaluation Center (GPEC) and Molecular and Advanced Pathology Core (MAPCore), University of British Columbia, Vancouver, Canada

ABSTRACT

Background/objectives: Mesonephric-like adenocarcinomas (MLA) of the endometrium, a rare subtype accounting for 0.7-3% of primary endometrial carcinomas, present diagnostic challenges due to their resemblance to other Müllerian neoplasms. This study aimed to retrospectively identify MLA in a Canadian endometrial carcinoma cohort, by using morphology, immunohistochemistry (IHC), and KRAS sequencing.

Methods: Tissue microarrays and IHC were conducted on 1094 endometrial carcinoma cases, identifying 16 potential MLA cases based on GATA3, TTF1, and ER staining patterns, which subsequently underwent targeted KRAS sequencing. A comprehensive histological review by three gynecologic pathologists, integrating additional IHC and molecular information, led to the classification of these cases.

Results: The final analysis yielded 5 MLA cases, 3 excluded cases, and 8 equivocal cases. Our study found that at least 0.5% (5/1094) of the screened endometrial carcinoma cases represented MLA. These cases were all misclassified as endometrioid adenocarcinoma because of similar morphological patterns, predominantly displaying a ductal growth pattern, followed by a tubular growth pattern and low-to-moderate grade cytologic atypia.

Conclusions: The presence of equivocal cases with atypical morphology and abnormal p53 IHC underscores a potential spectrum within MLA, indicating complex diagnostic challenges. This study highlights the importance of a comprehensive diagnostic approach for accurately identifying endometrial MLA. Further exploration into the molecular landscape of MLA is essential for refining diagnostic criteria and developing targeted therapies.

#8

ORAL PRESENTATION

Derek van Pel

Supervisior: Dr. Andrew Churg

Title: Uroplakin-IIIb as a novel immunohistochemical marker for mesothelioma

AUTHOR(s)

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ABSTRACT

Background: Distinguishing mesothelioma from non-small cell lung carcinoma often requires a battery of immunohistochemical stains, as many traditional markers used in mesothelioma lack sufficient specificity to allow them to be used alone. A recent large-scale tissue microarray (TMA) screen identified uroplakin-IIIb (UpIIIb; clone MSVA-736M) as a potentially specific marker for mesothelioma.

Methods: We examined the performance of this antibody using tissue microarrays containing a panel of 48 epithelioid mesotheliomas, 26 sarcomatoid mesotheliomas, and 144 non-small cell lung carcinomas (NSCLCs).

Results: Here we show that UpIIIb has good sensitivity (37/47 evaluable cases positive, 79%) and excellent specificity for distinguishing epithelioid mesothelioma from NSCLC (0/140 evaluable cases positive). UpIIIb sensitivity for epithelioid mesotheliomas was only slightly inferior to the established highly specific mesothelioma marker HEG1 (41/46 evaluable cases positive on the same TMA, 89%). However, UpIIIb did not stain any sarcomatoid mesotheliomas (0/24 evaluable cases positive). We also found that UpIIIb stained a proportion of high-grade serous ovarian carcinomas, a perennial diagnostic confounder in the context of mesotheliomas.

Conclusions: Taken together, our data suggest that UpIIIb can be used as a highly specific and sensitive mesothelial marker when the diagnostic question is epithelioid mesothelioma versus NSCLC; in particular, UpIIIb staining will pick up some number of epithelioid mesotheliomas that are HEG1 negative. Since UpIIIb is known to stain some proportion of urothelial carcinomas as well as gynecologic and a few pancreatic tumors, it should be used with caution in the peritoneal cavity or when the differential diagnosis includes carcinomas from these locations.

#9

ORAL PRESENTATION

Collin Pryma

Supervisior: Luke Y.C. Chen

Title:rosai-dorfman-destombes disease in adults: using a single center experience to navigate diagnostic uncertainty

AUTHOR(s)

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3Division of Hematology, University of British Columbia, Vancouver, BC, Canada

ABSTRACT

Background/objectives:
Rosai-Dorfman-Destombes disease (RDD) is a rare group “R” histiocyte disorder. This condition can be challenging for the pathologist to diagnose, with extranodal disease histologically overlapping with IgG4-related disease. Using a cohort of patients with confirmed RDD, a single-center case series is reviewed to highlight helpful histological, serological, and molecular features that improve diagnosis and lead to optimized treatment strategies for this occasionally morbid disease.

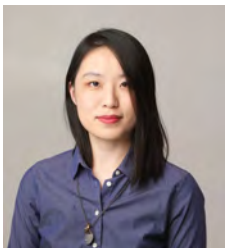
Methods:
This is a retrospective single-center description of 15 adult (age > 17 years) patients with a histopathological diagnosis of RDD from November 2015 to October 2023 at Vancouver General Hospital.

Results:
Fifteen patients with histologically confirmed RDD (five male, ten female, median age 53 years, median follow up 32 months) were included. The mean number of biopsies to achieve diagnosis was 2 (range 1–3), taking a mean of 19.0 months (range 3.0–60.0 months) between symptom onset and diagnosis. Four patients had lymph node involvement, and all 15 patients had extra-nodal disease. Five patients had tissue next-generation sequencing; a KRAS p.K117N mutation was found in one case. Four patients had PET-CT for disease staging. First-line treatments included corticosteroids (nine patients), surgical resection (two patients), rituximab (two patients) and trametinib (for the patient with a KRAS mutation). Six patients with refractory disease received a combination of lenalidomide and dexamethasone; three patients showed complete clinical and radiographic response, three patients had partial response, and none of them developed any significant toxicity.

Conclusions:
A total of 11 of 15 patients presented with extranodal disease only, highlighting the need to be aware of the protean extranodal manifestations of RDD. Many patients had a delayed diagnosis with significant disease refractory to treatment, including cardiac mass with obstructive shock, recurrent pleural effusions, and orbital tumor with vision impairment. In extranodal cases with diagnostic uncertainty, IgG4-related disease was a common differential consideration due to overlapping histologic features. Associated serologic markers such as polyclonal hypergammaglobulinemia, including IgG4, and elevated CRP, as well as identification of MAPK/ERK mutations are useful for achieving definitive diagnosis, which positively influences management decisions.

#10

ORAL PRESENTATION



Yu-Yu Lin

Supervisior: Dr. Kevin Ko

Title:Abnormal p53 Immunohistochemical Patterns Are Associated with Regional Lymph Node Metastasis In Oral Cavity Invasive Squamous Cell Carcinoma At Time Of Surgery

AUTHOR(s)

Tami Yu-Yu Lin1, Rachel Novack1, Kelly Yi-Ping Liu2, Tony L. Ng3, Lynn N. Hoang1, Eitan Prisman2, Catherine F. Poh2, Pushwant Singh Mattu1, Yen Chen Kevin Ko1

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ABSTRACT

Background/objectives: Sixty to eighty percent of oral cavity invasive squamous cell carcinoma (OSCC) demonstrate molecular alterations in TP53. The presence of TP53 mutations in other organ systems has been associated with a more aggressive clinical course. The purpose of this study was to classify OSCC into three subtypes—conventional, Human Papillomavirus (HPV)-associated, and p53 abnormal OSCC—using immunohistochemistry (IHC) to determine if subtype correlates with higher risk of lymph node metastasis at the time of surgery.

Methods: A total of 104 consecutive patients with OSCC resection and associated lymph node dissection were identified from the Vancouver General Hospital database. p53 IHC was performed for all cases and scored into p53 wild-type (conventional: scattered basal, patchy basal/parabasal; HPV-associated: mid-epithelial/basal sparing, markedly reduced [null-like]/basal sparing) and p53 abnormal (overexpression basal/parabasal only, overexpression basal/parabasal to diffuse, null, cytoplasmic) patterns. p16 IHC was performed on 46 cases and scored as 0 (absent staining), 1 (patchy or diffuse staining of less than lower one-third of the epithelium), 2 (diffuse staining of at least lower one-third of epithelium but less than 70% of cells), or 3 (diffuse block-like staining in more than 70% of cells). High-risk HPV RNA in situ hybridization was used to confirmed HPV status. Logistic regression analysis was performed to investigate the association of p53 status, tumor size, depth of invasion (DOI), and pT stage against lymph node status.

Results: We identified 24 cases with p53 wild-type patterns (18 conventional, 6 HPV-associated) and 80 (77%) cases with p53 abnormal patterns (20 overexpression basal/parabasal only, 25 overexpression basal/parabasal to diffuse, 24 null, 11 cytoplasmic). Three of the 24 p53 wild-type cases had positive lymph nodes (2 conventional, 1 HPV-associated; 13%) while 41 of the 80 p53 abnormal cases had positive lymph nodes (51%) (p=0.0008). Nineteen of the p16 cases demonstrated a score of 0, 18 a score of 1, 3 a score of 2, and 6 a score of 3. Among the six p16 score 3 cases, all exhibited wild-type HPV-associated patterns and high-risk HPV RNA ISH was confirmed positive, and all were classified as HPV-associated OSCC. Multivariate analysis showed that p53 abnormal pattern was an independent factor associated with positive node(s) with an odds ratio of 5.12 (95% CI, 1.47-24.1; p=0.02).

Conclusions: p53 abnormal OSCC were significantly more likely to be associated with positive lymph node status compared to p53 wild-type cases at time of surgery. Six cases were previously diagnosed as “conventional” OSCC, but in recognizing the basal-sparing pattern on p53 IHC, we were able to selectively test these cases using HR HPV RNA ISH and establish the HPV-association, thereby reclassifying these cases as HPV-associated OSCC. Further investigation with long-term follow-up is required to determine its clinical application prior to surgery planning.

#11

ORAL PRESENTATION

Fang Fang Li

Supervisior: Dr. Agatha Jassem & Dr. Natalie Prystajecky

Title: Phage immunoprecipitation sequencing in the aetiological diagnosis of neurological disorders

AUTHOR(s)

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ABSTRACT

Background/objectives: Acute flaccid paralysis (AFP) describes a sudden acute onset of muscle weakness that may be preceded by a bacterial or viral infection. Thus, identification of the cause of AFP is often supported by molecular testing. However, viral detection by PCR in cerebrospinal fluid (CSF) is frequently negative, creating a need for antibody testing to complement aetiological diagnosis. Here, we demonstrated the utility of phage immunoprecipitation sequencing (PhIP-Seq) to identify enterovirus D (EV-D)-reactive antibodies in CSF in an EV-D68-related case of AFP.

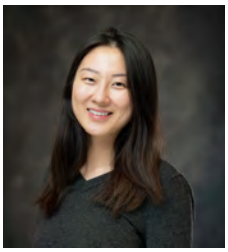
Methods: VirScan, a library of phages displaying viral epitopes of viruses, was used to capture antibodies in specimens. Next-generation sequencing and bioinformatic analysis then identified the epitope-specific reactivities (epitope binding signals, EBS) of bound antibodies. We compared the EBS of EV-D peptides in either CSF or serum of PCR confirmed EV-D cases with (n = 1) and without (n = 6) neurological symptoms against CSF from individuals PCR negative for EV-D with neurological symptoms (n = 10) to demonstrate assay performance of EV-D detection. Enriched peptides, defined as those with EBS ≥ 3.5, were counted and the proportion of enriched peptides were compared between positive and negative specimen. Hierarchical clustering was conducted to assess reactivity patterns against all epitopes in EV-D.

Results: Enriched proportions were observed to be higher in EV-D68 positive serum and CSF specimen (27%) compared to those negative with neurological symptoms (0-8%). EBS for significantly enriched epitopes from EV-D68 positive cases were observed to be significantly different from EV-D68 negative cases ($p<0.001$, Wilcoxon signed-rank test), with epitopes in the capsid protein VP4, non-structural protein 2C, and RNA dependent RNA polymerase being most reactive. When assessing EV-D antibody reactivity patterns by hierarchical clustering, EV-D negative patients clustered separately from those positive. Notably, paired serum and CSF from the clinical case clustered closely, with similar patterns of reactivity observed.

Conclusions: Here, we have demonstrated the utility of PhIP-Seq in the clinical setting to link neurological presentations with infection history to support aetiological diagnosis. Our current work demonstrates PhIP-Seq's ability to detect active/recent EV-D infections in CSF. Future work will aim to validate assay performance on paired specimen to derive cut-offs for clinical use.

#12

ORAL PRESENTATION



Joyce Zhang

Supervisior: Dr. David Huntsman

Title: Single cell transcriptomics identify trajectories of renal sarcomagenesis in a novel transgenic lineage-trackable mouse model of DICER1 syndrome

AUTHOR(s)

Joyce Zhang1, Felix Kommoss2, Shary Chen3, Yana Moscovitz3, Branden Lynch3, Maxwell Douglas3, Janine Senz3,Wild Scott4, Michael Underhill 4, Yemin Wang1, David Huntsman1

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ABSTRACT

Background/objectives:
In 2012 our group discovered recurrent missense mutations in microRNA processing gene DICER1, in Sertoli-Leydig cell tumour. These missense mutations are usually present in trans with a germline DICER1 null mutation, leading to a hemizygous state. “DICER1 syndrome” refers to tumours associated with germline DICER1 mutations, which can have pulmonary or extra-pulmonary manifestations.

Given no cell line models exist, development of animal models is crucial. Many of DICER1 cancers are sarcomas; we therefore postulate the cell of origin is mesenchymal. To this end, we developed a tamoxifen inducible, tdTomato-trackable, Hypermethylated in Cancer 1 (HIC1)-creERT2 driven transgenic mouse strain. Hic1 marks mesenchymal progenitors. With this strain, we successfully created a model that histologically recapitulate the 3 renal tumours seen in DICER1 syndrome: cystic nephromas, Wilms tumours, and anaplastic sarcomas. We propose to construct the oncogenesis continuum to gain insights into early tumorigenesis events. Our objective is to identify oncogenic events underlying Dicer1 mutation-driven murine kidney tumour development with single cell RNA-sequencing (scRNA-seq) technologies.

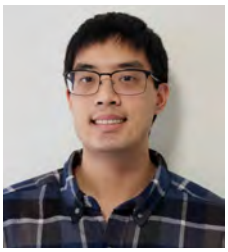
Methods: Prior to mapping temporal trajectory, we conducted scRNA-seq with 5 murine renal tumours. To build the tumorigenesis continuum, we will harvest kidneys (wild type and hemizygous genotype) post tamoxifen injection at 1, 3, 6, months and perform scRNA-seq. Cell differentiation trajectory analysis will be performed. We will identify key cell populations that emerge and expand, as well as genes/oncogenic pathways at the pre-malignant stage and determine how they evolve along the course of tumour development.

Results: Integration of 5 tumours revealed diverse cell populations: epithelial, mesenchymal, endothelial cells, lymphocytes, and macrophages. We further clustered the mesenchymal populations and identified sub-populations such as highly proliferative cells, muscle satellite cells, and terminally differentiated muscle cells. These mesenchymal cells also show high expression of markers for blastema (Ncam1, Sox11) - a histological component in Wilms tumours. Subsequently we will establish a temporal trajectory of tumorigenesis with time point samples.

Conclusions: With scRNA-seq, we begin to unravel the heterogeneity of these murine tumours. Our study will verify the utility of the first DICER1 syndrome-associated cancer model, enabling researchers to utilize it to improve patient management.

#13

ORAL PRESENTATION



Jamie Lee

Supervisior: Dr. Maziar Riazy

Title:Simultaneous Lupus Nephritis and Disseminated Tuberculosis with Renal Involvement: To Immunosupress or Not?

AUTHOR(s) Jamie Lee 1, Maziar Riazy 1

AFFILIATION(s) 1Department of Pathology and Laboratory Medicine, University of British Columbia

ABSTRACT

Background/objectives:
Lupus nephritis is a common and serious manifestation of systemic lupus erythematosus. In addition to a high rate of progression to end-stage kidney disease, patients with lupus nephritis are at high risk of death due to infection. We present a renal biopsy case of active lupus nephritis with concurrent tuberculosis, with organisms detectable in the renal parenchyma. We discuss the histopathological findings and the clinical conundrum of balancing immunosuppresion with antimicrobial therapy.

#14

ORAL PRESENTATION

Sirim Kim

Supervisior: Dr. Lawrence Lee

Title: Assessing the diagnostic accuracy of p16 immunohistochemistry stain on head and neck fine needle aspirate cytology specimens

AUTHOR(s)

Sirim Kim1, Lawrence Lee1

AFFILIATION(s)

Department of Pathology and Laboratory Medicine, University of British Columbia, Vancouver, British
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ABSTRACT

Background/objectives: Head and neck cancers often present as a painless neck mass in middle aged patients. The top differential diagnosis is a metastatic Human Papilloma Virus (HPV) associated oropharyngeal squamous cell carcinoma. Fine needle aspirate of the neck mass is often the first tissue available to make the diagnosis. Herein, assessing whether the carcinoma is HPV related or not is critical in determining the prognosis and possible treatment pathways.

Pathologists attempt to determine the HPV status of the carcinoma tissue obtained in the fine needle aspirate using the p16 immunohistochemical stain, which is a surrogate marker of the HPV virus infection.

Determining the p16 status on the fine needle aspirate specimen, however, has a high rate of false negativity due to the nature of tissue processing. One of the ways of improving the diagnostic accuracy is to modify the p16 antibody clone.

The pathology department at the Vancouver General Hospital has recently switched the p16 immunohistochemical antibody clone from CINTEC (the old clone) to Cell-Marque (the new clone). The consensus among the pathologists is that the new clone has higher diagnostic accuracy compared to the old clone.

A systematic study is performed to quantify the diagnostic accuracy of the new clone compared to the old clone.

Methods: Sixty cytology cases with CINTEC (the old clone) p16 immunohistochemical stain, and thirty-two cases with Cell-Marque (the new clone) p16 immunohistochemical stain were analyzed. The resulting cytology p16 staining pattern was compared to the permanent resection's p16 staining pattern and the patient's clinical HPV status. Sensitivity, specificity, and the overall diagnostic accuracy were calculated for each p16 clone.

Results: The overall diagnostic accuracy was improved from 53% to 88%, with a significant improvement in the sensitivity from 35% to 80% while both clones maintaining 100% specificity.

Conclusions: This systematic study has provided quantitative evidence for the improved diagnostic accuracy of the Cell-Marque p16 clone compared to the CINTEC p16 clone in the setting of assessing the HPV status of the fine needle aspirate specimens.

#15

ORAL PRESENTATION

Genevieve Amaral

Supervisior: Dr. Aleksandra Stefanovic

Title: Evaluation of the NG-Test® CTX-M MULTI lateral flow immunoassay

AUTHOR(s)

Geneviève Amaral1, Calvin K.F. Lo1, Jennifer Bilawka2, Gordon Ritchie1,2, Marc G. Romney1,2, Willson Jang2, Christopher Lowe1,2, Nancy Matic1,2, Michael Payne1,2, Aleksandra Stefanovic1,2

AFFILIATION(s)

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2 Department of Pathology and Laboratory Medicine, Division of Medical Microbiology and Virology, St. Paul's Hospital, Vancouver, Canada

ABSTRACT

Background/objectives:
With increasing incidence of extended-spectrum beta-lactamase (ESBL) producing Enterobacterales, rapid ESBL identification can facilitate early antimicrobial optimization for these infections. NG-Test® CTX-M MULTI (NG-BIOTECH, Guipry-Messac, France) is a rapid, qualitative multiplex immunochromatographic assay detecting cefotaximase-Munich (CTX-M)-type ESBL (groups 1, 2, 8, 9, 25) with a previously reported sensitivity 90-100% and specificity 96-100%. We evaluated NG-Test® CTX-M MULTI performance compared to two polymerase chain reaction (PCR)-based CTX-M detection methods.

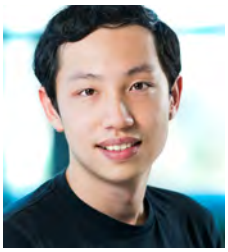
Methods:
Seventy-eight frozen Gram-negative isolates (2013-2023) recovered from previous submitted multidrug-resistant and non-ESBL clinical samples, along with carbapenemase-screening specimens, were sub-cultured to blood agar plates. After 48 hours incubation, 3 colonies were suspended in the NG-Test® CTX-M MULTI commercial buffer and applied to the test cassette as per the manufacturer's instructions. Results were compared to a reference standard: either commercial BioFire® FilmArray® Blood Culture Identification 2 (BCID2) Panel (bioMérieux, Marcy-l'Étoile, France) (n=32), or an internally validated, laboratory-developed PCR (n=46). Discrepant results were resolved with repeat NG-Test® CTX-M MULTI, phenotypic combination discs, and an alternative PCR platform.

Results:
NG-Test® CTX-M MULTI detected CTX-M in 26/78 isolates (33.3%), compared to 30/78 (38.5%) by PCR methods. Post-discordance analysis revealed: positive percent agreement (PPA) 89.7% (95%CI 71.5-97.2%), negative percent agreement (NPA) 100% (95%CI 90.9-100%), and overall agreement 96.2% (95%CI 88.4-99.0%). No false positives and only three false negatives were identified, possibly due to freezing-related plasmid loss or low-level enzyme expression: Raoultella ornithinolytica (n=2), Klebsiella oxytoca (n=1). One Escherichia coli was phenotypically negative for CTX-M but PCR positive; whole-genome sequencing confirmed the presence of a non-functional blaCTX-M gene.

Conclusions:
NG-Test® CTX-M MULTI performed well, consistent with previous studies, with overall categorical 96.2% agreement for CTX-M detection compared to composite reference standard. Although limited to CTX-M, rapid turnaround time and minimal technical expertise could support earlier escalation to carbapenem therapy for ESBL infections. Further studies are needed to support clinical implementation through diverse testing methodology (e.g., directly from blood culture) and correlation with patient outcomes.

#16

ORAL PRESENTATION



Allen Zhang

Supervisior: Dr. Julia Naso

Title: Tracing tissue of origin in squamous cell carcinomas of unknown primary by methylation profiling

AUTHOR(s)

Allen W. Zhang1, Andrew Galbraith2, Vahid Akbari2,3, Wan Lam1,4, David Huntsman1,5, Steven Jones2,3, Steven Yip1, Julia Naso1

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ABSTRACT

Background/objectives: Squamous cell carcinomas are one of the most common cancer types worldwide, and can arise from multiple different anatomic sites. In patients with multiple squamous cell carcinomas, identifying the primary site can be crucial for clinical management. Whereas multiple independent primaries can potentially be cured by resection, metastases may require systemic treatment, the nature of which may be dependent on the site-of-origin. Confusion can arise when determining tissue of origin in squamous cell carcinomas due to a lack of site-specific histologic, immunohistochemical, and mutational features. Epigenetic signatures, including DNA methylation, differ between cells of different tissue lineages and sites, and therefore we aimed to determine whether DNA methylation profiling can accurately distinguish squamous cell carcinomas from different sites.

Methods: We analyzed array-based DNA methylation data for n=1851 primary and metastatic squamous cell and squamous-like (urothelial) carcinomas from 5 different primary sites (head and neck, lungs, cervix, esophagus, and bladder). We then optimized a neural network to predict primary site. Classifier robustness to batch effects and unknown sample class was tested by iteratively excluding selected datasets and primary sites from training. We additionally validated the network on matched normal tissue (n=118) from all 5 sites and on Oxford Nanopore sequencing data for squamous cell carcinomas (n=6) from the Personalized OncoGenomics (POG) program.

Results: Our neural network accurately classified 467 out of 474 (99%) squamous cell carcinomas in the test set, including 30 out of 31 metastases (97%), and was robust to batch effects. When trained on only a subset of cancer types, our model was able to distinguish known from unknown types (AUC 0.90). In addition to accurately classifying cancer samples, the network trained on cancer samples correctly classified 113 out of 118 (96%) matched normal samples from each corresponding site, showing recognition of methylation patterns retained from the cells of origin of these carcinomas. Feature analysis showed that the most important genes used by the classifier were those involved in anatomical patterning and tissue lineage specification. Our classifier was directly applicable to low-depth Nanopore sequencing data generated in a clinical setting, showing concordance with consensus-determined primary site in 5 out of 6 metastatic squamous cell carcinomas (AUROC 0.92).

Conclusions: Leveraging site-intrinsic methylation patterns, our classifier accurately infers tissue of origin in squamous cell carcinomas from array-based methylation data. Our preliminary results show robust transferability to low-depth Nanopore sequencing data, which could enable rapid, cost-effective primary site determination and inform optimal management of patients with multiple squamous cell carcinomas.

#17

ORAL PRESENTATION

Erin Tanaka

Supervisior: Dr. Ramon Klein Geltink

Title:Mitochondrial Complex II Modulates CD8+ T cell Function

AUTHOR(s)

Erin Tanaka1,2, Juhee Oh1,2, Liam Johnston2,3, Anne-Sophie Archambault2, Rachel Cederberg3,4, Meredith Clark3,4, Annette Patterson2, Kevin Bennewith1,3,4, Will Bailis5, Ramon Klein Geltink1,2,3

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ABSTRACT

Background/objectives: CD8+ T cells are important immune cells that have shown success in cellular therapy for the treatment of cancer. However, many challenges still prevent efficient clearance of cancer by CD8+ T cells. To improve treatment outcomes and reduce cancer relapse, it is critical to generate CD8+ T cells with higher cytotoxic function during the expansion process in vitro, which can be achieved through modulation of T cell metabolism. Preliminary studies revealed that inhibition of mitochondrial Complex II (CII) is able to produce T cells with substantially improved tumour control and persistence when transplanted to tumour-bearing mice. CII is a protein complex that plays important roles in cellular metabolism, cell proliferation and epigenetics. However, recent research diverges in the effects of CII for either improving or hampering function of T cells. Therefore, the complete picture of how CII affects T cell function is still insufficiently elucidated. We aim to uncover how CII regulates T cell function and the differences in effects of CII during T cell differentiation and expansion.

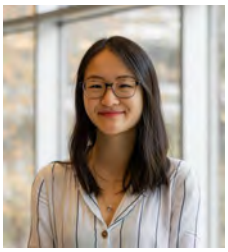
Methods: To investigate the effects of timing on CII inhibition, we isolated and activated CD8+ T cells from transgenic antigen-specific mice, and treated the CD8+ T cells with CII inhibitors overnight either early or late during activation. Function of the CD8+ T cells was determined by the production of pro-inflammatory cytokines post re-stimulation and by co-culturing T cells with a tumor cell line expressing the T cell specific antigen.

Results: We found that T cells treated with CII inhibitors during early activation reduced production of anti-tumor cytokines, while CII inhibition after full activation increased cytokine production amounts. Timing of CII inhibition also affected mitochondrial energy generation potential. When late CII-inhibited CD8+ T cells are transplanted to tumour-bearing mice, the T cells showed superior tumour control and T cell persistence. Both early and late CII inhibition reduced proliferative speed of the CD8+ T cells, and both cases induced altered mitochondrial mass and membrane polarization.

Conclusions: These results highlight how CII activity differentially affects CD8+ T cells dependent on the T cell activation state and timing. To improve cellular therapy for cancer, we can utilize these specific requirements on the metabolic regulation of function for CD8+ T cells, in order to produce more functional CD8+ T cells with superior tumour control and ensure effective treatment of the cancer.

#18

ORAL PRESENTATION



Renying (Loulou) Cai

Supervisior: Dr. Helene Cote

Title: The association of hiv, and seven other chronic/latent viral infections with markers of immune aging

AUTHOR(s)

Renying (Loulou) Cai1,2,7, Nancy Yang1,2, Anthony Hsieh1, Mel Krajden1,6, Melanie Murray3,4,5,7, Helene Cote1,2,3,7, for the CIHR Team on Cellular Aging and HIV Comorbidities in Women and Children (CARMA

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6 BC Centre for Disease Control, Vancouver, British Columbia, Canada
7 Edwin S.H. Leong Healthy Aging Program, University of British Columbia, Vancouver, British Columbia

ABSTRACT

Background/objectives: As effective antiretroviral therapies have drastically increased the lifespans of people living with HIV (PLWH), research priorities have shifted towards investigating the accelerated biological aging in PLWH. PLWH experience accelerated cellular and immunological aging, evident by the higher incidence of age-related diseases such as hypertension, diabetes, and cancer that occur earlier in life than HIV- individuals. This may be influenced by co-infection with other chronic/latent viruses (including CMV, EBV, HHV-8, HSV-1, HSV-2, HCV, and HBV) that are known to be individually associated with markers of aging or age-associated diseases. Our aim was to determine associations between sex, HIV status, number of chronic/latent viral infections, and markers of immune aging, in a cohort of PLWH versus controls.

Methods: CARMA cohort participants, balanced for age[1-76y], sex (n=48 female, n=49 male) and HIV status (n=49 HIV+, n=48 HIV-), with a broad range of viral coinfections were selected for this analysis. Infection status for CMV, EBV, HHV-8, HSV-1, and HSV-2 was determined serologically; HIV, HCV, and HBV were self-reported. Stored peripheral blood mononuclear cells were used to assess the CD4:CD8, proliferative:senescent CD8 T-cell (CD8+,CD28+:CD28-), and CD8+naïve:effector T-cell ratios via flow cytometry. Participants were dichotomized by sex, HIV status, and above and below median number of non-HIV chronic/latent viral infections. Associations between number of viruses, HIV status, and age were assessed using Mann-Whitney and Spearman's correlation.

Results: PLWH exhibited a lower CD4:CD8 (0.9[0.5-1.3] vs 1.8[1.5-3.2], p<0.0001) and CD8+,CD28+:CD28- (1.0[0.5-1.7] vs 1.7[1.1-4.3], p=0.0002) but no change in CD8+naïve:effector T-cell ratios compared to controls. Individuals with above-median chronic/latent viral infections (excluding HIV) exhibited lower CD4:CD8 (1.0[0.5-1.4] vs 1.7[1.3-3.2], p<0.001), CD8+,CD28+:CD28- (1.3[0.6-2.2] vs 2.1[1.2-4.8], p=0.0065) and CD8+naïve:effector (0.2[0.1-0.4],0.3[0.2-0.6], p=0.02) T-cell ratios compare to those with below-median chronic/latent viral infections. Age was found to be associated with 1) and increased number of non-HIV chronic/latent viral infections (rho=0.21, p=0.04), 2) decreased CD8+,CD28+:CD28- ratio (rho=-0.26, p=0.009) and 3) decreased CD8+naïve:effector T-cell ratio (rho=-0.52, p<0.001). No significant associations were found in terms of sex, however female sex appeared to have slightly higher CD4:CD8 ratio (p=0.06).

Conclusions: All three immune aging markers were associated with an increase in non-HIV chronic/latent viral infections, while HIV was only associated with CD4:CD8 and CD8+,CD28+:CD28- ratio, potentially implicating that a high burden of other viral infections contributes highly to immunological aging. Although no significant sex-specific associations were observed, a larger sample size may elucidate if they are present. We plan to increase the sample size to 350 CARMA participants, with well-balanced age, sex and HIV status to allow for multivariable analysis and identification of independent associations, giving a better insight into immune aging.

#19

POSTER PRESENTATION - UNDERGRADUATE

Boaz Li

Supervisior: Dr. Ying Wang

Title: Quality controls for spatial gene expression analysis in formalin-fixed paraffin embedded tissues

AUTHOR(s)

Boaz Li1,2, Samuel Leung1,2, Melody Cheng1,2, Maria Elishaev1,2, Yuancheng Mao1,2, Coco Ng2, Tiffany Chang2, Gurpreet Singhera2, Bobby Grewal3, Basak Sahin2, Chi Lai1,3, Amrit Singh2,4, Ying Wang1,2.

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4 Department of Anesthesiology, Pharmacology and Therapeutics, University of British Columbia, Vancouver, British Columbia, Canada

ABSTRACT

Background/objectives: Formalin-Fixed Paraffin-Embedded (FFPE) tissue samples are the most widely used format for biobanking and now tools, such as Visium, are available to obtain high-throughput spatial gene expression data from FFPE samples. There is, however, a lack of benchmarking of how biobanking procedures will affect the analysis results. Especially for most cardiovascular biobanks that collects heart tissues from autopsies, the post-mortem cell lysis may lead to RNA degradation. We hypothesize that the use of autopsy tissues will introduce bias in spatial gene expression analysis.

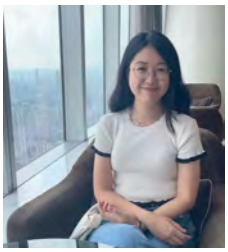
Methods: FFPE tissue sections of coronary arteries from autopsy and freshly collected hearts were obtained from the Bruce McManus Cardiovascular Biobank. RNA amount and RNA fragmentation (quantified via DV200) were assessed as a pre-sequencing quality control for Visium to select samples of high RNA quality. Autopsy and freshly collected coronary arteries that all have passed the current RNA quality standard (DV200>30%, n=3 in each group) were proceeded to Visium sequencing. Mitochondrial counts were measured to assess sample quality after sequencing, as an indicator of cell death or autolysis. Furthermore, cell deconvolution and differentially expressed gene analysis assessed how the two sample types varied in their transcriptome.

Results: Coronary arteries from autopsy samples indicated a 50 percent decrease in RNA amount and a higher mitochondrial count compared to those from freshly isolated hearts, which indicated a total RNA degradation. However, this degradation was not reflected by a lower DV200 value, Visium's recommended RNA quality test. It suggests that total RNA fragmentation alone does not accurately predict RNA quality for Visium spatial gene expression. Cell deconvolution and differentially expressed gene analysis showed that in regions with enriched smooth muscle cells, autopsy samples have decreased expression of contractile proteins and missing phenotype of smooth muscle cells. Instead, autopsy samples have increased expression of mitochondrial gene caused by post-mortem cell lysis, which led to the misdiagnosis of mitochondrial myopathy by gene ontology analysis.

Conclusions: Cell lysis in autopsy samples will introduce bias in spatial gene expression analysis if DV200 is the sole standard for quality control. Post-sequencing results indicate smooth muscle cell lysis for autopsy tissues that is not reflected in Visium's pre-sequencing RNA quality test. Using autopsy samples for spatial biology may lead to an inaccurate interpretation of cell phenotype and pathological diagnosis.

#20

POSTER PRESENTATION - UNDERGRADUATE



Carly Lin

Supervisior: Dr. Honglin Luo and Dr. Yasir Mohamud

Title: Viral proteins 3A and 3D synergistically mediate mitochondrial dysfunction in coxsackievirus B3-induced myocarditis

AUTHOR(s)

Carly Lin1,2, Wendy Hwang1,2, Yasir Mohamud1,2, Honglin Luo1,2

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1 Department of Pathology and Laboratory Medicine, The University of British Columbia
2 Centre for Heart Lung Innovation

ABSTRACT

Background/objectives: Myocarditis, characterized by the inflammation infiltration of the myocardium, stands as a leading cause of sudden death in young people, with approximately 1.5 million cases worldwide annually. Among all infectious causes, viral infection is the most prevalent etiology of myocarditis. Our preliminary data revealed mitochondrial damage in the myocardium in viral myocarditis induced by coxsackievirus B3 (CVB3), a pathogen commonly employed in preclinical models to study this disease. Mitochondria play a crucial role in various biological functions, and impaired mitochondria lead to decreased energy production, elevated reactive oxygen species (ROS) production, cell death, and inflammation. However, the precise mechanism by which the virus damages mitochondria remains elusive. In this study, we aim to elucidate the role of viral proteins, particularly membrane-associated viral proteins 3A and 3D, in mitochondrial dysfunction and to explore the potential of antioxidants to minimize mitochondrial damage by reducing ROS.

Methods: HeLa and HEK293 cells were transfected with constructs to overexpress CVB3 viral proteins 3A and 3D. Western blotting was conducted to quantify protein levels. Moreover, confocal microscopy was employed to visualize the localization of viral proteins. Additionally, mitochondrial function assays were conducted to assess ROS production, mitochondrial membrane potential, and permeability. Quantitative PCR was utilized to detect the release of mitochondrial DNA into the cytosol upon overexpression of these viral proteins.

Results: Our investigation confirms the localization of membrane-associated viral proteins (3A and 3D) to the mitochondrial membrane during CVB3 infection and its consequential impact on mitochondrial function. These findings reveal a synergistic impairment of mitochondrial functions by the overexpression of 3A and 3D, leading not only to diminished mitochondrial potential but also to the mislocalization of mitochondrial DNA. Furthermore, the release of phosphatidylserine decarboxylase (PISD), an inner mitochondrial membrane protein, from the mitochondria to the cell periphery, where it co-localizes with LC3, unveils intricate interaction between viral proteins and mitochondrial dynamics. We will continue to quantify the change in the LC3-II/I ratio to examine the role of PISD in inducing autophagy, ultimately resulting in apoptosis.

Conclusions: When CVB3 viral proteins 3A and 3D localize to the mitochondrial membrane, the mitochondria become damaged. Direct viral damage causes mitochondrial dysfunction in viral myocarditis. Elucidating the role of viral proteins in mitochondrial dysfunction in viral myocarditis is crucial for developing targeted treatment strategies.

#21

POSTER PRESENTATION - UNDERGRADUATE

Edward Sobczak

Supervisior: Dr. Helene Cote

Title: Placental mitochondrial dna heteroplasmies in women living with hiv and their association with preterm birth

AUTHOR(s)

Edward Sobczak1, 2, Zeshuo Li1, 2, Hélène C.F. Côté1, 2, 3

AFFILIATION(s)

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2 Centre for Blood Research, University of British Columbia, Vancouver, BC; 3Women's Health Research Institute

ABSTRACT

Background/objectives: There are approximately 20.2 million women and girls living with HIV worldwide. Despite effective antiretroviral treatment, women living with HIV (WLWH) experience a significantly higher prevalence of preterm birth (PTB) than their HIV-negative counterparts. The reasons for this are still unclear, but there is accumulating evidence that impaired placenta mitochondrial function, HIV infection, and antiretroviral medication may be associated with PTB. Our first objective was to examine the relationship between mtDNA heteroplasmy, HIV status, and incidence of PTB in a cohort of women living with and without HIV. Our second objective was to examine the presence of confirmed pathogenic heteroplasmies across tissues and between mother and child.

Methods: The fetal placental mtDNA of 61 participants balanced for age and HIV status was sequenced using Illumina Next-Generation Sequencing, and mtDNA heteroplasmy was characterized. The frequency of pathogenic and non-synonymous heteroplasmy was calculated. Mouth swab, cord tissue, and/or blood sample DNA from both the mother and child were also sequenced for participants who were previously identified as having pathogenic heteroplasmy.

Results: Our preliminary data corroborates the presence of a previously identified pathogenic heteroplasmy in fetal placental tissue. Interestingly, this heteroplasmy is also present in the fetal cord tissue, but does not appear in the maternal whole blood, indicating a de-novo mutation. Further analysis will determine if this mutation is present in fetal whole blood and placenta tissue facing the maternal side.

We have also observed a higher proportion of non-synonymous mutations in the preterm birth group, but no difference in synonymous/non-synonymous mutation ratio between WLWH and HIV-negative women. Further analysis will focus on the rate of heteroplasmy with respect to HIV status and preterm birth, as well as haplogroup analysis and coding vs noncoding mutations.

Conclusions: Higher proportion of non-synonymous mtDNA mutations in fetal placental tissue of preterm babies suggests that preterm birth may be associated with placental mitochondrial DNA mutations. As the next part of this project, we will increase the sample size and perform further analysis to investigate if mtDNA mutations are associated with HIV status, and if identified pathogenic mtDNA mutations have developed de novo or been inherited.

#22

POSTER PRESENTATION - UNDERGRADUATE

Florence Sanjaya

Supervisior: Dr. Helene Cote

Title: Effect of Switching to an Integrase Inhibitor on Mitochondrial DNA content in Women with HIV.

AUTHOR(s)

Renying Cai1,2,3, Florence Sanjaya1, Marcela Ardengue Prates Da Silva4,5, Shelly Tognazzini6, Angela Kaida4,6, Melanie CM Murray3,4,5,7,8, Helene CF Cote1,2,3,4,7, on behalf of the Children and Women: Antiretrovirals and Markers of Aging (CARMA; CTN 277) and the British Columbia CARMA-CHIWOS Collaboration (BCC3; CIHR CTN 335)

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ABSTRACT

Background: Antiretroviral Therapy (ART) has greatly increased the lifespan of people living with HIV. However, these drugs are lifelong, and clinical trials do not investigate potential long-term side effects and consequences. Despite being one of the newer classes of ART, integrase inhibitor (INSTI)-containing regimens are currently the most recommended around the world for their tolerability and efficacy. Older ART classes have had various side effects, including hepatic and neural toxicities, and mitochondria depletion. Recently, clinical outcomes such as weight gain have been observed when individuals initiate INSTIs, potentially implicating metabolic off-target effects. Mitochondria play an important role in metabolism, and mitochondrial DNA (mtDNA) content is a biomarker for mitochondrial dysfunction. Here, we aimed to measure mtDNA content changes in women with HIV who have switched to an INSTI-based regimen, compared to women who remained on their protease inhibitor (PI)- or non-nucleoside reverse transcriptase inhibitor (NNRTI)-containing regimens.

Methods: This was a retrospective cohort study of women enrolled in the CARMA and/or BCC3 study who had blood specimens available before and after switching to INSTIs. Each woman was matched 1:1 for age, ethnicity, PI/NNRTI pre-switch, and smoking status with women who remained on their PI/NNRTI-containing regimens. Blood mtDNA was determined using monochrome multiplex qPCR, then statistical differences between groups were compared for each category: age, ethnicity, smoking status, time between visit, undetectable viral load at both visits, CD4 count, and mtDNA % change per year.

Results: Fifty-six women (40 PI-, 16 NNRTI-regimen) were matched with 56 controls. While older age was associated with lower mtDNA content ($p=0.0045$) and non-switchers had shorter time between visits compared to their INSTI-switcher counterparts ($p<0.014$), no significant differences in the % change in mtDNA/year were detected between women who switched to INSTI and those who continued their regimens ($p=0.77$). There were no differences for age, ethnicity, smoking status, undetectable viral load at both visits, and CD4 count ($p=0.22$ for visit 1, $p=0.42$ for visit 2). Moreover, mtDNA content changes were not associated with ethnicity, smoking status, viral load or CD4 count.

Conclusions: Switching to INSTI showed no association with mtDNA content in women, a reassuring finding and a step further in understanding how ART affects mitochondrial health. However, given the large variance observed, associations between changes in mtDNA and other mitochondrial health markers should be further investigated. In addition, detecting the difference in effects between individual INSTI drugs was beyond the scope of this study, and can warrant further investigation.

#23

POSTER PRESENTATION - UNDERGRADUATE

Ghazal Sokhanran

Supervisior: Dr. Andre Mattman

Title: Macrocytosis in Mitochondrial DNA Deletion Syndromes

AUTHOR(s)

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ABSTRACT

Background: Single large mitochondrial DNA (mtDNA) deletions (SLD, 5 kb) syndrome is a rare inborn error of metabolism. SLDs may lead to highly variable phenotypes ranging from more common presentations, such as myopathy and chronic progressive external ophthalmoplegia (CPEO), to more severe rare ones such as Kearns–Sayre (KSS) and Pearson marrow-pancreas syndrome (PMPS). While hematological disorders are associated with SLD, macrocytosis has not been linked in the medical literature. Based on an index patient, we conducted a review of a potential association within our clinic's adult patient cohort.

Methods: Review of Adult Metabolic Diseases Clinic (AMDC) patients between 2016-2022 with SLD and a heteroplasmy level of > 10% in muscle. Comparison group of patients with a single pathologic mtDNA variant other than a deletion. For both cohorts, we collected hemoglobin, MCV and additional tests relevant to macrocytosis.

Results: We identified 26 SLD patients and 16 comparison patients. The most common diagnosis in the SLD cohort was CPEO, while the most common diagnosis in the comparison group was MELAS or MIDD. The SLD cohort was significantly older than the comparison group. 10/ 26 (38%) of mtDNA deletion patients had macrocytosis with elevated mean corpuscular volume (MCV), median of 108 fL (102–114 fL). Seven of the patients with macrocytosis had none of the recognized medical conditions that predispose to macrocytosis. None of the comparison group patients had macrocytosis while one had microcytosis. There was a significant difference ($p = 0.000$) between the MCV and MCH in the SLD cohort as compared to the comparison group. There was no other significant difference detected for these two groups in hemoglobin ($p = 0.351$), RDW ($p = 0.851$), vitamin B12 ($p = 0.845$), or homocysteine levels ($p = 0.077$).

Conclusions: The findings indicate a relatively high rate of macrocytosis in adult SLD that is not shared by adult patients with other mtDNA associated clinical syndromes. If substantiated in follow up studies, this SLD specific finding may relate to two possibilities: i) a unique pathophysiology associated with SLD or ii) the older age of the SLD variant cohort as compared to the comparison group. The etiology remains unclear. One possibility is that a relative deficiency of vitB12, or folate, within the normoblast is the causative mechanism. To explore this possibility, a prospective vitamin supplement trial is planned to determine if the macrocytosis corrects with pharmacologic vitamin supplementation (Vit B12 and folinic acid NCT06186154).

#24

POSTER PRESENTATION - UNDERGRADUATE

Ghazal Sokhanran

Supervisior: Dr. Andre Mattman

Title: Steps to Improving Glomerular Filtration Rate estimation in British Columbia

AUTHOR(s)

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ABSTRACT

Background: Glomerular filtration rate (GFR) is the primary indicator of kidney function, and it is widely used in the diagnosis, treatment and monitoring of chronic kidney disease (CKD). Therefore, quantifying this factor is a critical aspect of kidney care. GFR quantification is achieved using serum creatinine levels and patient demographics as variables in the GFR estimation equation, CKD-EPI 2009, which has been used in BC since 2014. Estimating GFR (eGFR) through different newly available equations, using creatinine, cystatin C, or both, will be compared to the iohexol clearance rate within a cohort of patients representing different ethnic subpopulations living within BC. However, before any guidelines for eGFR determination in BC can be formalized, the proper methodologies for measuring and evaluating eGFR and measured GFR must be made available for local validation. Therefore, this project aims to develop proper methodologies for measuring cystatin C and iohexol in BC.

Methods: A series of different processes were performed to develop a methodology for accurately measuring iohexol using an internal standard (iohexol-d5), and blank pooled serum spiked with in-house quality control (QC) stock solution. The overall accuracy and precision of the methodology for 46 samples of iohexol by liquid chromatography tandem mass spectrometry (LC-MS/MS) obtained from Queen's University were compared to the methodology developed at Saint Paul's Hospital. On the other hand, 42 cystatin C samples were obtained from the University of Alberta. Following the Cobas Pro manufacturing guidelines, the methods and measurements from Alberta were compared with those of Saint Paul's Hospital. Comparisons were performed using the Passing Bablok method for both markers.

Results: The method comparison regression line for the iohexol methodology has a slope of 1.06 [0.0.998,1.132], intercept of -0.418 [-4.33,3.178], R2 of 0.9666 and a median difference of 5.271%. As for cystatin C, the method comparison regression line has a slope of 1.031 [1.000 1.067], intercept of 0.0178 [-0.034 0.055], R2 of 0.9960 and the overall difference between the two methods is 6-12%.

Conclusion: This project shows that proper and accurate methodologies have been developed for measuring cystatin C and iohexol in BC. Hence, the first step toward developing the fundamental methodologies has now been completed, and the study can move forward with the next, which is recruiting participants and determining whether the eGFR equation used in BC is the best possible option for this community.

#25

POSTER PRESENTATION - UNDERGRADUATE

Jennifer Wu

Supervisior: Dr. Vilte Barakauskas

Title: Clinical validation of the atellica chemistry analyzer for hemoglobin A1C testing

AUTHOR(s)

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ABSTRACT

Background/objectives:
Hemoglobin A1C is a glycated form of hemoglobin that is formed via the non-enzymatic addition of a sugar residue to the N-terminal valine of the B hemoglobin chain. Its formation reflects blood glucose levels, and it is used for diabetes screening, diagnosis and monitoring. The BC Children's and Women's Hospital (C&W) laboratory currently uses a benchtop point-of-care style, immunoassay cartridge-based system for A1C determination. However, test volumes and staffing constraints calls for a new testing method that can facilitate the clinical needs of C&W. The Siemens Atellica chemistry analyzer is an automated, random access core laboratory analyzer that offers improved workflow and turnaround time for A1C testing. The aim of the study is to validate the analytical performance of the assay according to standards set by the National Glycohemoglobin Standardization Program (NGSP), and characterize assay performance in patients with hemoglobin variants commonly encountered in BC to ensure methods are suitable for use in a local setting and support appropriate result interpretation.

Methods:
The Atellica assay was evaluated for precision, accuracy, and Hb variant interference according to NGSP guidelines. The samples used in this study were leftover clinical samples from C&W and rocked for 10 minutes to ensure thorough mixing. The impact of introducing whole blood samples to the Atellica analyzer was assessed through sample carry-over, mixing, and stability studies. Method accuracy was evaluated through method comparison with three different methods across three different clinical laboratories. HbF interference was assessed by spiking samples with neonatal samples containing high levels of HbF to determine the threshold for interference. All results were analyzed using EP Evaluator software.

Results:
%CV for simple and complex precision met design claims of <1.5% and <2.0%, respectively. No sample carry-over was observed, and results are not expected to be impacted for a standing time of up to 1 hour after mixing. Contrary to design claims, sample stability when stored at 5-7°C was reduced from 7 to 4 days, while stability when stored at room temperature was extended from 2 to 4 days. Comparison of the Atellica with three other methods demonstrated a consistent negative bias of varying extent. HbF results were interpreted within the context of the pre-existing negative bias of the Atellica. Results were inconclusive, with no discernable bias associated with increasing HbF.

Conclusions:
The negative bias associated with the Atellica analyzer was unexpected, given both the current and proposed assays were validated by the same vendor. Nevertheless, the bias observed falls within the 5% total allowable error limit set by the NGSP. A1C testing on the Atellica chemistry analyzer has been validated according to NGSP guidelines. Technicians and clinicians should ensure samples are thoroughly mixed prior to testing, and exercise caution when interpreting the results of samples with known Hb variants.

#26

POSTER PRESENTATION - UNDERGRADUATE

Jennifer Yi

Supervisior: Dr. Lien Hoang

Title: Molecular Stratification of Vulvar Squamous Cell Carcinoma

AUTHOR(s)

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ABSTRACT

Background/objectives: Currently, vulvar squamous cell carcinomas (VSCC) are treated as a homogeneous entity, but recent literature has shown that VSCC can be separated by human papillomavirus (HPV) into HPV-associated (HPVA) and HPV-independent (HPVI) types. There is emerging evidence that abnormal p53 portends a worse prognosis within the HPVI group, but few large-scale studies have examined this. Our objective was to compare the clinicopathologic differences between VSCC stratified by both HPV and p53 status using a large institutional cohort.

Methods: This was a retrospective cohort study of patients diagnosed with VSCC via surgical resection between 2008 and 2021 at Vancouver General Hospital (VGH). Cases were stratified using p16 (a surrogate marker for high-risk HPV infection) and p53 immunohistochemistry, into three groups: (1) HPVA, (2) HPVI p53 abnormal (p53abn) and (3) HPV-I p53 wild-type (p53wt). Age, pathologic features (size of tumor, depth of invasion, perineural invasion, lymphovascular invasion, lymph node involvement, invasion into nearby anatomical structures, precursor lesions), progression free survival and overall survival were compared between the three groups.

Results: A total of 379 patients were identified: 129 (34%) HPVA, 188 (50%) HPVI p53abn and 62 (16%) HPVI p53wt tumors.

In comparison to HPVA, patients with HPVI VSCC were older (mean 72.3 vs 66.1 years, $p < 0.0001$), had larger tumor size (mean 3.33 vs 2.75 cm, $p < 0.04$), were more likely to have a background of lichen sclerosus ($p<0.01$), invade into adjacent organs ($p<0.03$), and have positive margins ($p=0.006$). When considering p53, HPVI p53abn tumors exhibited larger depth of invasion ($p<0.008$) and were more likely to have lymph node metastases ($p=0.004$) compared to HPVI p53wt tumors. Patients with HPVA were less likely to suffer from disease recurrences compared to HPVI p53wt and HPVI p53abn (20%, 37% and 59% respectively, < 0.00001) and less likely to die from the disease (13%, 25% and 32% respectively, < 0.00001). There were no differences between groups for perineural or lymphovascular invasion.

Conclusions: This study reaffirms that HPV status in VSCC has prognostic implications, and the addition of p53 further prognosticates the HPVI group. HPVI p53abn VSCC was associated with the worst clinicopathologic features and outcomes, compared to both HPVI p53wt and HPVA VSCC. VSCC should be stratified into not two, but three different diseases based on HPV and p53 status.

#27

POSTER PRESENTATION - UNDERGRADUATE

Kate Fitzsimmons

Supervisior: Dr. Julia Naso

Title: Utilization and Impact of Ultra-Stat Idylla EGFR Testing for Non-Small Cell Lung Carcinomas

AUTHOR(s)

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ABSTRACT

Background/objectives: The initiation of EGFR-targeted therapy for non-small cell lung cancer (NSCLC) depends on the results of molecular testing. Ultra-stat Idylla EGFR testing at the BC Cancer Genetics and Genomics Laboratory (CGL) recently became available upon clinical request for NSCLC. This test has the potential for faster turnaround time, but is limited to a single gene, such that subsequent Focus Panel next-generation sequencing is needed if results are negative. We aimed to (i) assess how patients with a high likelihood of EGFR mutation and an urgent need for treatment are being selected for ultra-stat testing, and (ii) assess the impact of ultra-stat testing on clinical workflows and patient outcomes.

Methods: Ultra-stat Idylla EGFR test requests between February 2, 2022 and November 2, 2023 were identified from CGL records, and clinical information on the ultra-stat tested patients was retrospectively compiled through chart and database review. Associations were assessed using Fishers exact tests, and survival analysis was performed using cox proportional hazards models.

Results: Fifty-six NSCLC patients with ultra-stat Idylla EGFR test results were identified. Ultra-stat testing was most frequently requested by medical oncologists (38/56 cases, 68%) and respirologists (8/56 cases, 14%). The median laboratory turnaround time for ultra-stat testing was 1 day (range 0 to 7 days). Five samples failed ultra-stat testing, and 35 of the remaining 51 (69%) were positive for EGFR mutations, much greater than the 19.7% EGFR positivity rate for Focus Panel NSCLC CGL testing between May 2021 and February 2024 (n=3,287). The presence of an EGFR mutation was significantly associated with Asian race (36/51tested patients; P=0.002) and never smoking (42/51 tested patients; P=0.020). Of the EGFR mutation-positive patients, 29 (82.9%) received EGFR-targeted therapy, delivered a median of 6 days (range 0 to 28 days) after the ultra-stat result was reported. Over a median of 223.5 days of follow-up after tissue was received for testing (range 0-723 days), significantly longer overall survival was seen for patients who received targeted therapy after a positive Idylla result compared to other ultra-stat tested patients (P=0.042; HR 0.35, 95% CI 0.13-0.96). Other targetable alterations were identified through Focus Panel testing in 9 out of 20 patients (45%) who had a negative or failed ultra-stat test. The median Focus Panel turnaround time was 10 days (range 7-29 days). Five of the 21 (24%) ultra-stat tested patients with failed/negative ultra-stat results died less than 14 days after the specimen was received for ultra-stat testing, underscoring the clinical urgency of test results.

Conclusions: Ultra-stat Idylla testing allows rapid identification of actionable EGFR mutations and prompt initiation of targeted treatment. Clinicians effectively identified patients with a high probability of EGFR mutation, with race and smoking status predictive of a positive result within the ultra-stat tested cohort. Results from this study will be used to develop decision aids for identifying patients likely to benefit from ultra-stat testing.

#28

POSTER PRESENTATION - UNDERGRADUATE

Kaya Frese

Supervisior: Dr. Jacqueline Quandt

Title: Antiphospholipid IgM distinguish the clinical phenotypes of multiple sclerosis

AUTHOR(s)

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ABSTRACT

Background/objectives: Multiple sclerosis (MS) is a chronic inflammatory, neurodegenerative disorder of the central nervous system. Lipids make up more than 80% of myelin, the protective sheath around axons and potential target of MS pathology. Demyelination can release lipids (cholesterol, phospholipids (PL), and glycolipids) from membrane scaffolds. Elevated antiphospholipid antibodies (aPL) have been reported in MS but are poorly described across clinical phenotypes. We aim to characterize prevalence patterns of a broad panel of aPL in MS.

Methods: We conducted an exploratory cross-sectional study of serum IgM levels to cardiolipin (CL), phosphatidyl-choline (PC), -ethanolamine (PE), -inositol (PI), -serine (PS), and sphingomyelin (SM) measured by ELISA in healthy controls (HC, n=35), clinically isolated syndrome (CIS, n=20), relapsing remitting (RRMS, n=33), secondary progressive (SPMS, n=30), and primary progressive (PPMS, n=20) participants.

Results: Independent of age and sex, elevated levels of IgM to all PL examined were associated with a higher likelihood of being CIS relative to HC ($p<0.005$), whereas only PI and PS aPL were increased in clinically definite MS relative to HC ($p<0.05$). Within MS subgroups, increased levels of IgM to CL, PI, PS, and SM were associated with a greater likelihood of being SPMS over RRMS ($p<0.05$) independent of age, sex, disease duration (DD), and EDSS. There was no association of aPL levels and sex. Increased PI, PS, or CL aPL in the CIS cohort ($p<0.05$) was associated with being older, but age associations did not exist in other subgroups. Several positive associations with DD were observed in RRMS and/or SPMS cohorts (but not CIS or PPMS): PE aPL in RRMS ($p=0.032$); PE, PI, and SM aPL in SPMS ($p<0.04$), and PC, PE, and SM aPL in pooled RRMS and SPMS ($p<0.02$), with PI and PS aPL trending similarly ($p=0.056$, 0.083). Notably, elevated PE, PI, PS, and SM aPL in the PPMS group were associated with higher EDSS scores ($p\leq0.05$) whereas SM ($p=0.039$) or PI, PS, and CL aPL ($p<0.1$) were instead inversely associated with EDSS in SPMS participants. Positive responses (aPL level > HC level+1.5SD) to single PL were detected in 3-11% of HC, 25-40% of CIS, 3-18% of RRMS, 13-33% of SPMS, and 0-20% of PPMS participants. More than responses to any one PL, the breadth of IgM responses was highly associated with one MS subtype over another. Broad positivity (positivity to three or more PL) was greater in the CIS group (40%) than HC (2.9%) or MS (19.3%) ($padj<0.0125$), and greater in the SPMS group (33.3%) than RRMS (12.1%) or PPMS (10%). Increasing numbers of positive responses in a participant predicted a higher likelihood of being CIS or SPMS over other groups: CIS or MS over HC (beta-estimate (b)=0.876 (CI 0.328-1.424), $p=0.002$; $b=0.442$ (CI 0.031-0.854), $p=0.035$), and SPMS over RRMS or PPMS ($b=0.434$ and 0.474, $p<0.05$).

Conclusions: Findings highlight the prevalence of broad aPL responses in CIS and SPMS over HC and other MS subtypes. Longitudinal studies matched to imaging/clinical correlates are needed to describe the relevance of aPL to MS pathophysiology and utility as a biomarker of disease course or severity. Conclusions: Ultra-stat Idylla testing allows rapid identification of actionable EGFR mutations and prompt initiation of targeted treatment. Clinicians effectively identified patients with a high probability of EGFR mutation, with race and smoking status predictive of a positive result within the ultra-stat tested cohort. Results from this study will be used to develop decision aids for identifying patients likely to benefit from ultra-stat testing.

#29

POSTER PRESENTATION - UNDERGRADUATE

Kristen Danielle Go

Supervisior: Dr. Cheryl Wellington

Title: Comparison of Multiplex Immunoassays for the Measurement of Neurological Blood Biomarkers

AUTHOR(s)

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ABSTRACT

Background/objectives: Blood-based neurological biomarkers are a more accessible, less invasive alternative to traditional methods for diagnosing neurological conditions such as Alzheimer's disease, Multiple Sclerosis, and traumatic brain injury. Many biomarkers have demonstrated strength in their ability to diagnose and prognose these conditions, including neurofilament light chain (NfL) and glial fibrillary acidic protein (GFAP). NfL is an axonal intermediate filament protein that increases in plasma with brain injury or neurodegeneration. GFAP is an astrocytic intermediate filament protein, and its elevations are thought to indicate excessive GFAP expression due to astrocyte activation and neuroinflammation. Several methods for quantifying plasma brain biomarker levels are commercially available as multiplexed immunoassay kits. However, little work has been done to assess the comparability of results produced by different assay formulations. In this study, we performed a cross assay analysis to determine how comparable results are from three different assay kits measuring NfL and GFAP in blood. The assays analyzed were the Quanterix Neurology 2-Plex B Advantage (N2PB;103520), Neurology 4-Plex B Advantage (N4PB; 103345), and Neurology 4-Plex E (N4PE;103670) assays. In addition to NfL and GFAP, the 4-plex assays measure other biomarkers. N4PB also measures biomarkers Tau protein and ubiquitin carboxy-terminal hydrolase L1, and N4PE also measures beta-amyloid 42/40.

Methods: To cross assay formulations, 4 separate experiments were performed, using a total N=160 samples. For each cross, N=40 blood samples from previously analyzed de-identified cohorts were selected based on the dynamic range of results of the original analysis. Blood biomarker concentrations were measured using Quanterix Simoa HD-X Analyzer. The following crosses were performed (original:crossed to): N4PE:N2PB, N4PE:N4PB, N2PB:N4PE, and N4PB:N4PE. Samples originally measured using N4PE and N4PB were plasma, while samples originally measured using N2PB were serum. Assays were performed as per manufacturer's specifications. Biomarker concentration comparisons were done using Spearman correlation and Bland-Altman analysis to assess the bias between assays.

Results: For Nfl, the following correlations between assay formulations were found – N4PE:N2PB ρ=0.9442 ($p<0.0001$), N4PE:N4PB ρ=0.9550 ($p<0.0001$), N2PB:N4PE ρ=0.8577 ($p<0.0001$), and N4PB:N4PE ρ=0.9208 ($p<0.0001$). For GFAP, we found the following correlations – N4PE:N2PB ρ=0.9831 ($p<0.0001$), N4PE:N4PB ρ=0.9644 ($p<0.0001$), N2PB:N4PE ρ=0.8848 ($p<0.0001$), and N4PB:N4PE ρ=0.8155 (<0.0001). Bland-Altman analysis (% bias (SD)) for NfL revealed for N4PE:N2PB 28.82% (15.17), N4PE:N4PB 46.96% (14.24), N2PB:N24PE -6.88% (21.95), and N4PB:N4PE -23.04% (14.86) bias. For GFAP measurements, the biases found were for N4PE:N2PB -27.28% (21.13), N4PE:N4PB -29.80% (18.43), N2PB:N4PE 22.30% (26.29), and N4PB:N4PE 25.13% (36.21).

Conclusions: For both NfL and GFAP, concentrations tightly correlate between assay formulations. However, there is significant bias between some assays. This indicates the need to correct results between assay formulations.

#30

POSTER PRESENTATION - UNDERGRADUATE

Longyijie Wei

Supervisior: Dr.David Huntsman

Title: Dual targeting of cystathionine gamma-lyase and mTOR pathway in clear cell ovarian cancer

AUTHOR(s)

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ABSTRACT

Background/objectives: Clear cell carcinoma of the ovary (CCC) is the 2nd most common ovarian cancer and is histologically and clinically distinct from other subtypes. Deep endometriosis of the ovary is the most common precursor for CCC. Recently, we identified cystathionine gamma-lyase (CTH), a key enzyme in the transsulfuration pathway, as a marker of Mullerian tract derived ciliated cells and CCC of both the ovary and uterus regardless of which mutations are present. Also, CTH is highly expressed in CCC and in endometriosis adjacent to CCC, but not other subtypes of ovarian cancers. In a preliminary study employing a CTH knockout (KO) CCC cell line in a mouse model, a notable decrease in CCC's metastatic capacity was observed, alongside an increase in both proliferative potential and apoptosis. Facing a scarcity of clinically viable CTH inhibitors, our team conducted a computer-aided screening using the ZINC22 database, which houses over 4 billion commercial compounds, ultimately identifying 17 compounds with the potential to inhibit CTH by binding to its active site. Further, we found that CTH confers CCC with metastatic fitness via post-transcriptional regulation of hypoxia-inducible factor 1 alpha (HIF1α). In CCC, HIF1α expression is driven mainly by the highly active PI3K/AKT/mTOR pathway, which enhances HIF1α translation. While mTOR inhibitors such as Everolimus successfully inhibit HIF1α in CCC, it has no impact on CTH expression. So, we hypothesized that dual targeting CCC with CTH and mTOR inhibitors might represent a novel treatment strategy for patients with CCC.

Methods: We used well-established CCC cell lines, including OVISE, OVMANA, and RMG1. Cells were treated with CTH inhibitor Aviglycine, mTOR inhibitor Everolimus, or combined treatment, and effects on cell proliferation and mTOR pathway activity were assessed using IncuCyte, western blotting, and MTT assay. Growth rate differences will be measured using the Hill slope coefficient in GraphPad Prism, with significance assessed by a Student's t-test against the Hill slope of Aviglycine hydrochloride, a commercial CTH inhibitor.

Result: Our in vitro data demonstrate that targeting CTH alone using Aviglycine increases cell proliferation, which, surprisingly, confers a therapeutic advantage when combined with an mTOR inhibitor. Intriguingly, the combination of Aviglycine and 10nM Everolimus was more potent than treatment with 20nM Everolimus alone. Among the 17 identified CTH inhibitors, 4 exhibited superior synergy with mTOR inhibitors compared to Aviglycine, the control CTH inhibitor from prior research. This suggests a novel therapeutic strategy for CCC through CTH and mTOR inhibitor combination.

Conclusions: Targeting CTH in CCC and potentially other cancers might represent a novel and impactful therapeutic approach. Particularly, the combined treatment of CTH and mTOR inhibitors may present novel therapeutic strategies for individuals with CCC. The current research underway is optimizing better dose usage and CTH inhibitors (among the 17) for applying combined treatment in both in vitro and in vivo studies.

#31

POSTER PRESENTATION - UNDERGRADUATE

Lucy Ogoke

Supervisior: Dr. Wei Xiong

Title: Comprehensive Comparative Analysis of MOLLI (Magnetic Occult Lesion Localization and Imaging) Seed-Guided Excision Versus Fine-Wire-Guided Excision in Breast Cancer Surgery: a pathologic study

AUTHOR(s)

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ABSTRACT

MOLLI seeds in identifying targeted lesions, comparing them with traditional fine-wire-guided excision techniques.

A total of 17 consecutive fine-wire-guided excisions and 58 consecutive Molli-Seed-guided excisions were meticulously examined. Key parameters, including tumor size, specimen size, pathologic diagnosis, margin status for invasive carcinoma and in-situ carcinoma, and the distance to the closest margin by DCIS, were systematically recorded. Biomarker status was also documented.

Results revealed the comparative efficacy of the two methodologies. In terms of the overall positive margins involved by invasive carcinoma or carcinoma in-situ, our findings indicated a marginally lower rate in Molli-seed-guided excision cases (8 of 58 cases, 13.80%) compared to fine-wire-guided cases (4 of 17 cases, 17.65%), with no statistically significant difference observed ($p>0.05$). In cases where only invasive carcinoma was present, there was no significant difference in the positive margin ratio between the two groups. Similar findings were also observed in the cases with only in-situ carcinoma. In addition, the ratio of negative margins < 2mm from in-situ carcinoma did not show a significant difference between the two groups. Further analysis encompassed the volume of the specimen and biomarker status, revealing no significant differences between Molli-seed-guided and fine-wire-guided excision cases.

In **conclusion**, the study demonstrated that the Molli-seed method was not inferior to the fine-wire-guided method in terms of margin positivity for both invasive carcinoma and in-situ carcinoma. The key advantage of Molli seeds, their wire-free nature, was underscored by the absence of injury risk for staff handling specimens, differentiating them from traditional fine wires. Furthermore, the seeds' ability to remain within the tumor during grossing emerged as a crucial factor contributing to more precise tumor localization during the pathological examination. These results not only affirm the effectiveness of MOLLI technology but also highlight its potential to enhance patient safety and streamline surgical procedures.

#32

POSTER PRESENTATION - UNDERGRADUATE

Mckenna Stuart

Supervisior: Cheryl Wellington & Mehwish Anwer

Title: Investigating axonal damage after traumatic brain injury (TBI) using whole-brain machine learning image analysis tools

AUTHOR(s)

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ABSTRACT

Background/objectives: Traumatic Brain Injury (TBI), a major global health concern, induces a myriad of effects on neurological function through diffuse axonal injury (DAI), inflammation, vascular damage, and neuronal dysfunction. Axonal integrity and function is compromised due to shearing or stretching of axons during primary injury or through inflammatory secondary injury processes following TBI. Human studies show that DAI is associated with axonal swellings, known as axonal varicosities, as well as axonal vacuoles and terminal bulbs. The Closed Head Model of Engineered Rotational Acceleration (CHIMERA) model is a non-surgical murine model that produces concussion-like TBI and unrestricted head motion similar to human TBI. Here we aim to develop a workflow for 3D mapping of TBI-induced axonal damage using light sheet microscopy (LSM) and machine (deep) learning tools. As an exemplar dataset, we leveraged samples from an ongoing study investigating whether CHIMERA TBI results in concurrent damage to spinal tracts and if CHIMERA can be used to model polytrauma i.e. TBI and Spinal cord injury (SCI).

Methods: Thy-1-YFP-16 reporter mice, which express yellow fluorescent proteins in cortical and subcortical neurons and their projections to spinal cord, were used. Mice were randomized to sham or TBI groups with a neutral, rostral, or caudal interface position to induce CHIMERA with or without cervical hyperextension. Sham mice received all procedures except for impact. At 7 days post-TBI, paraformaldehyde fixed whole brains with the cervical spinal cord intact were collected for 3D histological analysis. Tissue was SHIELD-fixed, passively cleared to render them optically translucent, and refractive index matched to achieve optical transparency prior to imaging on a Zeiss Z.1 LSM. The volumetric imaging dataset was stitched in arivis Pro software for 3D rendering and analysis.

Results: Large 3D regions of interest (ROI), including brainstem and cervical spinal cord, were extracted from the whole brain imaging dataset for deep learning workflow optimization using arivis Cloud, an AI-powered web-based machine learning platform. First, to train a segmentation model, image datasets were annotated in arivis Cloud to distinguish between varicosities and background. The trained model was then tested on a subset of data within the cloud. To validate the training of the deep learning model, segmentation accuracy was also manually inspected. Next, the trained segmentation models were imported into arivis Pro software to run analysis pipelines for ROIs from all datasets. Our trained models successfully distinguished axonal varicosities from background in all spinal cord ROI's and the bead-on-string morphology of damaged axons was confirmed with 3D renderings.

Conclusions: We successfully developed a workflow to train a model to identify axonal damage in cleared mouse brains using machine learning based image analysis tools. This work provides essential analytical tools for subsequent analysis of murine brain injury and its relationship with spinal cord damage.

#33

POSTER PRESENTATION - UNDERGRADUATE

Navtej Kang

Supervisior: Cheryl Wellington & Mehwish Anwer

Title: Comparison and characterization of opal fluorescent dyes

AUTHOR(s)

Navtej Kaur Kang

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ABSTRACT

Background/objectives: Multiplex immunofluorescence staining allows for the simultaneous detection of different biomarkers in a single tissue section. This study aimed to compare and characterize eight commonly used Opal™ fluorescent dyes (Akoya Biosciences) in automated multiplex immunofluorescence assays.

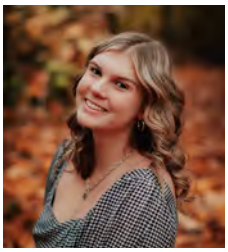
Methods: Monoplex staining of Pan Cytokeratin (PanCK+), a tumor epithelial marker, was performed on 4 um serial sections of a tissue microarray (TMA) containing ovarian carcinoma specimens (Gynecologic Cancer Tissue Bank). Sections were stained with the following Opal fluorescent dyes: Opal 480, 520, 540, 570, 620, 650, 690, and 780 at the same concentration, and counterstained with 4',6-diamidino-2-phenylindole (DAPI). The dyes were characterized by the signal intensity within their designated channel and the signal bleed-through in unintended channels. Signal intensity and bleed-through were quantified using HALO v3.6.4134 (Indica Labs). Signal strength was normalized against DAPI to account for slight variations in section thickness. The same 40 TMA cores were analyzed across each section to ensure consistency and reliability during the analysis.

Results: Significant differences were observed in signal intensity among the Opal fluorescent dyes (Kruskal-Wallis test, $p < 0.05$). Opal 650 exhibited the highest mean signal intensity, while Opal 690 and Opal 780 exhibited the lowest. Varying levels of signal bleed-through were observed, with Opal 480, Opal 520, and Opal 540 showing higher proportions of bleed-through events than other Opal dyes.

Conclusions: The observed differences emphasize the importance of selecting the appropriate combination of fluorescent dyes for accurate and reliable multiplex biomarker detection. Understanding the variation between different reagents that serve the same function is crucial to efficient research and development, ensuring the optimization of experimental protocols in multiplex immunofluorescence staining.

#34

POSTER PRESENTATION - UNDERGRADUATE



Nicola Wray

Supervisior: Dr. Jacqueline Quandt

Title: The biomarker potential of neurofilament light and heavy chains in preclinical models of multiple sclerosis

AUTHOR(s)

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ABSTRACT

Background/objectives: Multiple sclerosis (MS) is an inflammatory, neurodegenerative disease characterized by the demyelination of neurons, leading to axonal injury and loss in the central nervous system. Progressive disease is thought to be characterized by neurodegeneration with a relative decline in inflammatory responses. Neuroaxonal damage releases cytoskeletal proteins into the blood, including neurofilament light (NfL) and heavy (NfH). While neurofilaments are under evaluation for their promise as biomarkers for monitoring disease activity, severity, and onset in MS, their utility in preclinical models and translatability in target identification and therapeutic development is unclear. We aimed to evaluate NfL and NfH levels at various stages of an autoimmune, demyelinating model of MS, experimental autoimmune encephalomyelitis (EAE), and determine their relevance to pathophysiology including time of symptom onset, disease severity, recovery, and their relevance to each other.

Methods: Mice were immunized with the myelin oligodendrocyte glycoprotein (MOG)35-55 peptide that drives an acute clinical attack with minimal recovery followed by relapse and chronic disability. Daily clinical assessments recorded symptom onset and disease severity with serum samples collected from EAE mice at acute (day 17-20) and chronic (day 62) stages postimmunization to compare to healthy non-EAE or sham-immunized mice. Meso Scale assays were used to measure serum Nf levels.

Results: NfL and NfH levels in non-EAE mice were low and negligible (278±57, 0.62±0.02pg/mL), while levels were consistently elevated in mice at the time of acute EAE (17516±1153, 63±5.9pg/mL). After symptom onset, NfL and NfH levels tended to increase, peak at 6 days post onset (18290±13275; 77±78pg/ml), and subsequently decrease. For mice at acute EAE, cumulative disease severity scores moderately correlated with NfL and NfH levels ($r=0.51$, $p=0.0008$; $r=0.51$, $p=0.0007$) as NfL and NfH levels strongly correlated with each other ($r=0.74$, $p<0.0001$). Later in the chronic phase of EAE when disability was still evident, serum NfH levels (1.5±0.76pg/mL) were comparable to healthy, non-EAE mice. In contrast, NfL levels (1384±334 pg/mL) were higher than healthy, non-EAE mice yet lower than mice at acute EAE.

Conclusions: The tendency for NfL and NfH to peak at comparable times during acute EAE development shows comparable pathology and neuroaxonal damage at this disease phase. The positive association between NfL and NfH with each other and disease severity in acute EAE mice supports their reliability as biomarkers of early neurodegeneration. In contrast, elevated NfL and negligible NfH levels in chronic EAE mice reflect differences in neurodegenerative processes at later disease stages. Just as these models are not considered progressive and lack neurodegeneration, they model findings where the progressive stages of MS are no longer considered to be as neurodegenerative as early events in MS. These findings may inform future preclinical investigations on the monitoring efficacy of novel or repurposed MS therapeutics and improve their translatability to early and late stages of MS.

#35

POSTER PRESENTATION - UNDERGRADUATE

Puneet Kaur Arora

Supervisior: Dr. Calum MacAulay

Title: Immune Cell-Tumor Cell Neighbor Pair Analysis to Guide Personalized Lung Cancer Treatment in Canada

AUTHOR(s)

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ABSTRACT

Background/objectives: In Canada, non-small cell lung cancer (NSCLC) is the most common cause of cancer-related mortality. Patients with early-stage NSCLC are amenable to surgery and have a better prognosis compared to patients with later stages of the tumor. However, many patients experience recurrence without long-term survival often caused by mutations in the tumor genes and the patient's immune cells. Therefore, there is an unmet need to decrease tumor recurrence and increase survival outcomes for Canadians with early-stage NSCLC. Immune cell-tumor cell interaction in lung cancer tissue can predict a) recurrence after surgery, and b) response to immune therapy.

Methods: Multiplex immunohistochemistry (IHC) was performed on primary tumors of 40 resected early-stage NSCLC treated with immune therapy to identify immune cell-tumor cell interactions using 12 cell markers. Imaging was performed using the hyperspectral cell sociology (HCS) platform which also allows to quantify cell subsets based on their staining intensities. Cell-cell interactions were analyzed via nuclei segmentation and phenotyping was performed to group cells based on their antibody staining intensity. Multiple markers across multiple sections were identified by IHC stain thresholding using the VisioPHARM software.

Results: Although not all 40 cases have been analyzed yet, we expect to see a difference in cell distributions between the recurrent and non-recurrent cohorts. Preliminary analysis of a subset of cases (n=13) indicates the potential to differentiate between recurrent (n=5) and non-recurrent (n=8) based on the percentages (%) of CD-8, PD-1, and PDL-1 cell surface markers and their combinations. For these cases, statistical significance ($p < 0.05$) was observed for the % T-regulatory (Treg) cells that comprise of CD8-PD1+ cell surface makers. However, no statistical significance ($p > 0.05$) was noted for other T-cell populations [(CD8-PD1-); (CD8+PD1+); (CD8+PD1-)]. Furthermore, neighborhood analysis revealed that there is a higher likelihood of a favorable outcome when there is a greater presence of CD-8 cells surrounding PDL-1 cells. This observation held true with an 86% accuracy for the analyzed cases. We aim to perform similar analysis for other cell populations for each patient biopsy.

Conclusions: The results of this research are expected to build a framework for cell sociology analysis for immune cell-tumor cell interactions. This framework, in turn, is anticipated to carry significant clinical implications for patients who stand to benefit from targeted immune therapy.

#36

POSTER PRESENTATION - UNDERGRADUATE

Vincent Labador

Supervisior: Dr. Wei Xiong

Title: Cytosponge pathology test has high specificity in detecting barrett's esophagus-related neoplasia in patients undergoing surveillance

AUTHOR(s)

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AFFILIATION(s)

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ABSTRACT

Background: Barrett's esophagus (BE) is a precancerous lesion defined by goblet cells in the mucosa >1 cm from the gastroesophageal junction. BE increases the risk of esophageal adenocarcinoma. Endoscopic surveillance is recommended for patients with BE because endoscopic intervention is highly effective for high-grade dysplasia and cancer. However, repeated endoscopy has associated harms and access has been limited during the COVID-19 pandemic. We aimed to evaluate the role of a non-endoscopic device (Cytosponge) coupled with laboratory-histochemical studies in patients with concurrent biopsies. The Cytosponge is a non-invasive method that allows for the entirety of the gastrointestinal region to be sampled. This study aims to determine if a Cytosponge can be used as an alternative to a biopsy (Bx).

Methods: 46 patients with a diagnosis of BE have been enrolled in the study since 2021 and is currently ongoing. Each patient underwent both a Bx and cytosponge at the time of their appointment. A Cytosponge sample was collected and processed to generate H&E and Alcian Blue 2.5 Periodic Acid Schiff (AB2.5/PAS) stained slides. The adequacy of Cytosponge samples was determined by the presence of glandular cells. The presence or absence of goblet cells and dysplasia were recorded for all adequate cases. Indefinite for dysplasia was recorded if a definitive dysplasia could not be determined. The concurrent Bx tissue was processed in the histology lab to generate H&E slides. The diagnosis of BE, dysplasia (indefinite, low grade or high grade) was recorded. Statistical analysis to determine sensitivity (SE), specificity (SP), positive predictive values (PPV), and negative predictive values (NPV) were calculated.
Results: The median age of these patients was 70 with a range of 53 - 85 years old. Most of the patients were male (92%). Of the 46 samples, 2 were excluded because concurrent biopsies were not performed. 33 of the remaining 44 cytosponge samples were adequate (75.00%) for pathology assessment. Goblet cells were detected in 21 of 33 adequate cases (65.63%) while goblet cells were identified in 34 of 44 Bx cases (77.00%). Indefinite for dysplasia and dysplasia were detected in 11 adequate cases (33.33%) versus 15 of 44 biopsies (34.09%). The SE and SP of Cytosponge to detect dysplasia was 72.73% and 100.00%, respectively. The PPV and NPV for dysplasia were 100% and 86.96%, respectively. The SE and SP of cytosponge to detect goblet cells were 70.37% and 33.33%, respectively. The inadequate cases were mostly found in the age group >=71 yo (56.673%) compared to <= 70 yo (17.65%) ($p = 0.000354$).

Conclusions: Cytosponge examination had a high specificity and moderate sensitivity to detect dysplasia in BE patients. There were no clinical complications in the enrolled patients. Our study suggested that cytosponge could be used as a surveillance method to identify BE patients with an increased risk of developing cancer. These patients could benefit from early endoscopic intervention. Our results also suggested that Cytosponge may not be the most suitable surveillance method in patients >=71 yo because of the high inadequacy rate.

#37

POSTER PRESENTATION - UNDERGRADUATE

Zheng Fang Yang

Supervisior: Dr. Eric McGinnis

Title: When classifications of myeloid neoplasms clash

AUTHOR(s)

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ABSTRACT

Background/objectives: Acute myeloid leukemia (AML) and myelodysplastic neoplasms (MDS) are heterogenous malignancies that have relied on the World Health Organization (WHO) Classification of Tumors of Haematopoietic and Lymphoid Tissues criteria for classification. In 2022, two independent systems were proposed to update the widely used WHO fourth edition (WHO4): the WHO fifth edition (WHO5) and the International Consensus Classification (ICC) systems. Both use recurring genetic changes, disease biology and clinical features to categorize AML and MDS. Differences between the classification systems impacts diagnosis, prognosis, and management of these diseases. It is unknown which of the classification systems best reflects behaviours of AML or MDS seen in clinical practice. We hypothesize that applying the WHO5 and ICC criteria to historical diagnostic bone marrow specimens from patients with AML or MDS will reveal changes and discrepancies between classification systems.

Methods: To compare WHO5 and ICC, we reviewed cases of AML and MDS with reported cytogenetic, sequencing, and clinical data which were diagnosed at Vancouver General Hospital between 2016 and 2022. Potential cases were identified from the BC Cancer Agency genetic laboratory database query for MDS or AML. The participant list was refined by further review of electronic medical record databases for pathology records. Additional data was collected on patient demographics, clinical features, laboratory findings, bone marrow findings, and cytogenetic results. Diagnoses according to WHO4, WHO5, and ICC were assigned to each case based on the clinical history and laboratory results.

Results: We collected 446 myeloid neoplasm cases of AML and MDS, and 90 cases showed discrepancies between WHO5 and ICC. ICC includes the new entity AML with mutated TP53, applying to 28 cases, while the corresponding category is absent in WHO5. Under ICC, the new MDS/AML entity is subdivided based on AML characteristics, while these 39 cases are defined as either MDS with biallelic TP53 mutations, MDS with increased blasts, or MDS with fibrosis under WHO5. In the patient cohort, 4 cases of AML with mutated RUNX1 and 4 cases of AML with trisomy-8 are genetically defined under ICC, but these specific mutations are not defined under WHO5. WHO5 upgrades 2 cases of MDS to AML with mutated NPM1, but these cases do not meet the AML blast cut-off for ICC. Of the remaining, 10 cases of MDS differ in terminology, and 3 cases of MDS or AML are at the criteria margins.

Conclusions: While most reclassifications from the WHO4 system are concordant between WHO5 and ICC, there are minor differences affecting a subset of patients. Both WHO5 and ICC have softened the border between MDS and AML, but they take different approaches such as having different blast thresholds for AML with mutated NPM1 and using the MDS/AML category that follows AML subclassifications. The biggest discrepancy is seen in the new category of AML with mutated TP53. Patient outcomes in each system should be further explored to elucidate clinical behaviours of different myeloid neoplasms.

#38

POSTER PRESENTATION - GRADUATE

Alexandra Witt

Supervisior: Dr. Ed Pryzdial

Title: Development of double mutant clotting factor X as a novel thrombolytic agent

AUTHOR(s)

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ABSTRACT

Background/objectives: The favoured clot-dissolving drug (i.e. thrombolytic agent) is a recombinant (r) version of tissue plasminogen activator (tPA), which activates plasminogen to plasmin, the clot-busting enzyme. However, the administration of rtPA has risk, because the high dose of rtPA required to dissolve clots causes life-threatening hemorrhage in up to 7% of patients, resulting in large part from systemic, rather than clot-localized, enzyme activity. Our lab has previously demonstrated a non-enzymatic thrombolytic function for the plasma protein clotting factor X (FX) in accelerating the generation of plasmin by tPA, and has generated a recombinant mutant to act as a replacement for rtPA. Several key characteristics are desirable: 1) a blocked active site to prevent clotting; 2) stability in plasma; and 3) accessibility of sites integral to clot-localization and thrombolysis. The hypothesis addressed here is that the gamma-carboxyglutamic acid (Gla)-domain of FX, which is known to enable binding of FX to anionic phospholipid membranes and fibrin, is key to localizing the thrombolytic effects of FX, facilitating enhanced safety, and that a recombinant variant of FX with thrombolytic function is superior in safety compared to rtPA.

Methods: Wild type rFX (rFXwt) and double mutant rFX with inhibited clotting function (i) and plasmin cleavage-resistant (c) mutations (rFXic) were produced in HEK 293 cells and purified. Their plasmin-cleavage profile and prothrombin clotting times were evaluated. Calcium-dependent binding to anionic phospholipid was tested to further confirm post-translational modification and clot-localizing function of the Gla-domain. Acceleration of thrombolysis was evaluated using a plasmin-selective chromogenic substrate. Purified Gla-domainless plasma-derived FX was generated proteolytically by chymotrypsin treatment and tested in the same assays to further understand the mechanism of clot-localization.

Results: Compared to rFXwt, which was cleaved into the expected rFX-beta and FX-gamma species by plasmin, proteolysis of rFXic was limited to production of rFX-beta. This is predicted to stabilize thrombolytic activity in plasma. In contrast to rFXwt, rFXic had undetectable clotting activity in reconstituted FX-deficient plasma. Neither mutation impacted intrinsic ability to bind anionic phospholipids in a calcium-dependent manner, while removing the Gla-domain inhibited this localization ability. In preliminary efficacy tests, rFXic generated 10-fold more plasmin than rFXwt, indicative of thrombolytic acceleration. Calcium enhanced the solution-phase acceleration of rtPA by rFX and protected cleavage by chymotrypsin, implicating an involvement of the calcium-binding sites of FX.

Conclusions: These data support the hypothesis that rFXic has thrombolytic activity and uses the calcium-binding Gla-domain to localize clot lysis. Next, we will assess thrombolytic efficacy in vivo and therapeutic safety ex vivo, and anticipate highlighting rFXic as both an effective and safer alternative to rtPA.

#39

POSTER PRESENTATION - GRADUATE

Amir Parham Pirhadi Rad

Supervisior: Dr. Babak Shadgan

Title: Quantifying consistency in tissue oxygenation across anatomical landmarks in healthy individuals through near-infrared spectroscopy

AUTHOR(s)

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ABSTRACT

Background/objectives: Near-infrared spectroscopy (NIRS) is a non-invasive method that leverages light penetration in tissues to capture tissue oxygenation. NIRS sensors provide a monitoring method of oxygen delivery and consumption in critical care patients by estimating oxygen saturation within the tissue; this is a parameter known as regional tissue oxygenation (STO2). STO2 measured from the forehead has been commonly used in clinical settings to monitor cerebral tissue oxygenation. However, there is a clinical need to determine additional body measurement sites that may be used as a reliable STO2 reference in tissue oximetry applications.
The objective of this study is to determine the reliability of STO2 across common body measurement sites and to determine a normal general STO2 range (establish baseline values for general tissue oxygenation level) from healthy adult individuals at rest. STO2 will be measured from 10 bilateral regions + Sternum and tongue of the participant's body. Data collection sites include bilateral Tibia, bilateral Quadriceps, bilateral Thenar, Sternum, bilateral Ramus, bilateral Orbital rim, Bilateral Temporal, bilateral TMJ, bilateral Forehead and tongue in quiescent (relaxed posture) participants.

Methods: NIRS sensors (Root with O3 Regional Oximetry) were affixed to the participant measurement zones using double-sided tape. Data were collected continuously at a sampling rate of 0.5 Hz for 30 seconds per participant. Prior to the placement of each NIRS sensor, the subcutaneous tissue thickness, consisting of the thickness of the skin and underlying subcutaneous fat layer, was measured using a skinfold calliper (Skyndex LLC, Albuquerque, USA). The colour sensor (Nix Mini 3 Color Sensor, Nix Sensor Ltd, Canada) was used to capture tissue colour pigmentation for all the data collection sites except the tongue by placing the sensor on the data collection site. A cohort of 13 healthy adults were recruited for participation in the study (10 Male and 3 Female, Mean age 32.8). Participants laid down supine for 5 minutes before data collection. NIRS sensors were placed on the data collection sites to record the STO2 of the region of interest. For the tongue data collection, the sensor was fixed on a tongue depressor covered by a disposable transparent film.

Results: 13 participants, including 10 male and 3 female healthy volunteers between the ages of 25 to 45 years old (Mean age 32.8) participated in the study. The sternum had the highest STO2, with an average of 75 ± 4. The lowest STO2 standard deviation was recorded on Sternum at 2.7 and the highest reported was 10.6 for the tongue. The standard deviation of STO2 on the face was 4.6 ± 1.5 with the lowest on the rim of Orbit at 3.1 and the highest temporal at 6.1.

Conclusions: We demonstrated that regional STO2 measurements from the medial sternum demonstrated less variability. Due to less subcutaneous fat, the forehead and sternum can provide more accurate STO2 measures; therefore, they can be considered more reliable reference sites for monitoring STO2 levels in healthy individuals

#40

POSTER PRESENTATION - GRADUATE

Ali Khajegili Mirabadi

Supervisior: Dr. Ali Bashasashti

Title:Beyond words: evaluating pathology large vision models with large language models

AUTHOR(s)

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ABSTRACT

Background/objectives: Large Vision Models (LVMs) have emerged as central tools in digital pathology, particularly for analyzing Whole Slide Images (WSIs). However, there remains uncertainty regarding the semantic representation capabilities of these models. Currently, Large Language Models (LLMs), exemplified by ChatGPT, have demonstrated proficiency in representing textual data. This study endeavors to bridge this gap by employing LLMs in conjunction with pathology reports to develop a novel semantic measure for evaluating medical image representations provided by LVMs.

Methods: This study adopts an innovative methodology, amalgamating LLMs and pathology reports to assess the semantic quality of LVMs. Utilizing the Cancer Genome Atlas dataset, which includes pathology images and corresponding reports, provides a robust foundation. The LLM model employed is Command-R-V1, boasting 35 billion parameters and trained on extensive text data, facilitating the representation of pathology reports. Four LVMs, namely Phikon, UNI, CTransPath, and Lunit-Dino, are subjected to evaluation. The semantic metric is crafted based on the topological similarity of retrieval outcomes from the LLM model and the LVM model, thereby enabling a comparison between visual and semantic representations.

Results: Preliminary findings reveal the inadequacy of conventional metrics like accuracy, precision, and F1 Score in fully assessing Visual Language Models (VLMs), as they may overlook crucial semantic nuances. The developed metric highlights discrepancies in semantic information captured by different VLMs, suggesting a misalignment with semantic representations from pathology reports. Additionally, VLMs performance vary across cancer types, indicating the significant influence of training data on semantic proficiency. These observations underscore the necessity for nuanced evaluation frameworks to better understand and harness the potential of VLMs in medical image analysis, transcending traditional metrics to encompass semantic intricacies essential for accurate interpretation in pathology practice.

Conclusions: This study exposes the limitations of traditional metrics in gauging the semantic capabilities of Large Vision Models (LVMs) in digital pathology. By integrating Large Language Models (LLMs) with pathology reports, we unveil discrepancies in semantic representations among different VLMs, further influenced by varying performance across cancer types. These findings underscore the necessity for nuanced evaluation frameworks to accurately assess VLMs and optimize their utility in pathology practice, thereby enhancing diagnostic precision and patient care outcomes.

#41

POSTER PRESENTATION - GRADUATE

Bob Lin

Supervisior: Dr. Honglin Luo

Title:Investigating the Phosphatidylinositol 4-phosphate Pathway of Coxsackievirus B3-Induced Non-Canonical Autophagy

AUTHOR(s)

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ABSTRACT

Background/objectives: Worldwide, Coxsackievirus B3 (CVB3) causes a quarter of all cases of dilated cardiomyopathy and myocarditis in children. CVB3 is a positive-sense single-stranded RNA enterovirus that manipulates host cells' autophagy system, a mechanism crucial for cellular component recycling and virus destruction. CVB3 exploits autophagy through an alternate pathway, bypassing steps typically required for autophagy and subsequently facilitating viral replication. While much of this alternate pathway remains unknown, research has shown that a knockdown of phosphatidylinositol 4-kinase beta (PI4KIIIβ) significantly inhibits CVB3-induced microtubule-associated protein 1A/1B-light chain 3 (LC3) lipidation, a marker for autophagosome formation. This observation strongly suggests that phosphatidylinositol 4-phosphate (PI4P), synthesized by PI4KIIIβ, plays a role in CVB3-induced autophagy. PI4P is also known to be involved in recruiting CVB3 viral polymerase 3D to initiate viral RNA synthesis. A preliminary proximity-dependent biotin identification (BioID) experiment was recently conducted to identify cellular proteins proximal to CVB3 viral polymerase 3D and found a list of protein candidates. Sorting nexin 6 (SNX6) stood out among the candidates by additionally showing interaction with PI4KIIIβ, a PI4P kinase. We hypothesize that SNX6 is a downstream effector of PI4P that is integral to the activation of CVB3-induced autophagy. The primary aims of this project are to determine the role of SNX6 in CVB3-induced autophagy and viral propagation.

Methods: To validate SNX6 as a PI4P interactor, we cultured HeLa cells to over 90% confluency in growth media before lysis. We then performed a pulldown assay employing PI4P lipid-coated beads using the lysate, followed by Western blot analyses utilizing anti-SNX6 rabbit antibody. To further investigate the role of SNX6 in CVB3-induced autophagy, we will be silencing and deleting SNX6 expression in CVB3-infected cells using RNA interference and CRISPR-Cas9 gene editing approaches. Indicators of CVB3 infection and autophagosome formation, such as viral protein (VP1) expression, LC3 lipidation, and LC-puncta, will be monitored using Western blotting, immunostaining, and fluorescence microscopy assays. We will also examine the role of SNX6 in viral propagation through SNX6 silencing and deletion, with viral propagation to be assessed by infectivity and plaque assays.

Results: Preliminary findings from Western blot analyses have yielded inconclusive data on the interactivity of PI4P and SNX6.

Conclusions: While our preliminary data is inconclusive, literature analysis supports our assessment of PI4P and SNX6 interactivity. Currently, we have only scratched the surface of this project. Subsequent investigations will continue toward elucidating the precise role of SNX6 in CVB3-induced autophagy and viral propagation. Continued exploration of the CVB3-induced autophagic pathway may generate further insights into how CVB3 manipulates cellular autophagy mechanisms, potentially providing avenues to explore therapeutic solutions to CVB3 infection.

#42

POSTER PRESENTATION - GRADUATE

Cyril Helbling

Supervisior: Dr. DeMarco

Title: Navigating Challenges in the Development of Seed Amplification Assay for Synucleinopathies

AUTHOR(s)

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ABSTRACT

Background: A biofluid biomarker is needed to enable early differentiation of synucleinopathies like Parkinson's disease (PD), from phenotypically related disorders. Fortunately, with the continued development of seed amplification assays (SAA) we now have ultrasensitive methods that leverage prion-like behaviour of pathologically misfolded alpha-synuclein (a-syn) found in synucleinopathies. Challenges with current SAA include variable performance, methodological complexity, multi-day analytical run-times, and high cost-per-test. Focusing on the first challenge—variable performance—we developed a baseline a-syn cerebrospinal fluid (CSF) SAA workflow and explored various methodological modifications toward improved performance.

Methods: For the testing matrix, we used pools of CSF from non-neurological ("normal") controls spiked with neuropathologically confirmed brain homogenate that is either positive or negative for a-syn pathology. These spiked CSF samples (n=5 per group) were used for method optimization experiments to make efficient use of limited CSF samples with clinical and neuropathological neurodegenerative diagnoses. The impact of different sample preparation steps on seeding kinetics was examined, including no equilibration between sample mixing steps versus lengthy equilibration. The ability to distinguish seeded from spontaneous aggregation was examined using different assay substrates: wild-type recombinant a-syn and a K23Q sequence variant with an N-terminal his-tag. To perform a preliminary assessment of diagnostic performance, CSF (without spiking) from individuals with a clinical neurodegenerative diagnosis (PD n=3; non-neurological controls n=3) were tested.

Results: Shorter average lag time to seeded aggregation (11.1 ± 1.6 h versus 55.9 ± 8.3 h) was observed when frozen samples were equilibrated at room temperature for 30 minutes prior to the usual sample preparation before analysis. Improved ability to distinguish seeded from spontaneous aggregation was observed with the K23Q sequence variant, due to reduced spontaneous aggregation kinetics. The average lag time to spontaneous aggregation of the assay using K23Q substrate was 50.1 ± 14.7 h, compared to that of wild-type of 42.3 ± 16.3 h, while the average lag time to seeded aggregation using K23Q substrate was 28.9 ± 13.8 h, compared to that of wild-type: 50.1 ± 14.7 h. Using the optimized SAA protocol and testing the individual (non-spiked) CSF samples, the assay demonstrated 100% sensitivity (3/3) and 100% specificity (3/3) for the detection of PD.ConclusionBy modifying the spiked CSF sample preparation and selecting different assay substrates, we obtained faster seeded aggregation and slower spontaneous aggregation kinetics, enhancing assay reproducibility and accuracy. Published SAA are lengthy multi-day tests, generally run in quadruplicate to overcome reproducibility issues. Our optimizations may lead to a decreased assay duration and required replicates, reducing the cost-per-test. Subsequent research will continue to evaluate further optimization, followed by a formal analytical validation including a large case control study to formally assess diagnostic accuracy.

#43

POSTER PRESENTATION - GRADUATE

Fabiola Wu Wu

Supervisior: Dr. Kirk Schultz

Title: Different metabolomic profiles in adult compared to pediatric chronic graft-versus-host Disease

AUTHOR(s)

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ABSTRACT

Background/objectives: Chronic graft-versus-host disease (cGvHD) is the leading cause of morbidity following allogenic hematopoietic stem cell transplant (HSCT). Previously, we (Subburaj et al., 2022) found biological differences associated with cGvHD in a large pediatric study, ABLE1.0 (PBMTC1202) and validated these patterns using archival samples from a Children's Oncology Group trial, ASCT0031. In 2 pediatric cGvHD, we demonstrated significant elevation of alpha-ketoglutarate, kynurenine, glutamic acid and decreased C8. Therefore, in this study we aim to compare metabolomic cGvHD profiles in a separate adult cohort compared to previous metabolomic changes seen in children.

Methods: One hundred thirty-three patients were enrolled in an adult cGvHD biomarker study. This study included 18 patients with cGvHD onset between 100 – 365 days compared to 115 patients with no cGvHD with samples obtained at 3, 6, and 12 months post-HSCT. In the pediatric study, 63 patients were enrolled, including 38 with cGvHD onsets, and 25 with no cGvHD. Plasma was separated from whole blood and examined using direct injection mass spectrometry with reverse-phase LC-MS/MS for ~142 metabolites. Differences in metabolite levels between cGvHD and non-cGvHD patients were compared using multiple regression and considered significant if a metabolite met all 3 of the following criteria: (1) p-value < 0.05; (2) effect ratio of ≥1.3 or ≤0.75; and (3) receiver operator characteristic AUC ≥0.60.

Results: A mixed analysis revealed significant decreases in trimethylamine N-oxide and Hippuric acid levels at cGvHD onset compared to non-cGvHD. Trimethylamine N-oxide showed early significance (3 and 6 months), while hippuric acid was significant only at 3 months. Interestingly, indole acetic acid was associated with late onset cGvHD (8–12 months). Comparing plasma metabolomic profiles in adults and pediatric patients at cGvHD onset to controls without cGvHD, adult profiles exhibited decreased trimethylamine N-oxide and Hippuric acid levels, while pediatric profiles showed elevated α-ketoglutarate, succinic acid, and glutamic acid, and decreased aspartic acid and glutamine that validate primary metabolome changes observed in the ABLE1.0 pediatric cohort.

Conclusions: We were unable to see significant increase of metabolites associated with the onset of pediatric cGvHD. The adult cohort instead was characterized by alteration in intestinal microbiome associated metabolites including: trimethylamine N-oxide and hippuric acid in early onset cGvHD and indole acetic acid in late onset cGvHD. This suggest there may be different age-related metabolite changes seen in adult versus pediatric cGvHD and requires validation separate cohorts. The metabolomic profile at the onset of cGcHD in the ASCT0031 pediatric cohort validates a lot of the primary metabolome changes seen previously in the ABLE1.0 pediatric cohort.

#44

POSTER PRESENTATION - GRADUATE

Forouh Kalantari

Supervisior: Dr. David Huntsman

Title: Human endometrial organoid model implicates G6PD-dependant metabolism as a potential targetable pathway in ARID1A mutant gynecological cancers including clear cell ovarian cance

AUTHOR(s)

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ABSTRACT

Background/objectives: AT-rich interactive domain-containing protein 1A (ARID1A) serves as a structural core subunit of the Switch/Sucrose Non-Fermentable (SWI/SNF) chromatin remodeling complex. ARID1A is frequently mutated in gynecological cancers including clear cell ovarian cancer (CCOC). Our lab discovered inactivating mutations of ARID1A in about 50% of CCOC, often co-occurring with a PIK3CA activating mutations. In this project, we hypothesized that ARID1A deficiency in endometrial-associated gynecological cancers results in temporal epigenetic and transcriptomic alterations along the transformation continuum which may provide insights on the biology of these cancers and suggest potential therapeutic options.

Methods: We characterized transcriptomic and epigenomic features of the dissociated organoids by single cell RNA and ATAC sequencing using the 10X Genomics platform. The transcriptomes and chromatin accessibility were compared between non-transduced, single or double mutant conditions to elucidate the mechanisms underlying oncogenic transformation.

Results: : Double ARID1AKO PIK3CAH1047mutant organoids were larger than non-transduced organoids, and at later passage, the organoids manifested CCOC histopathology, including hobnail cells. Single cell gene expression profiles from passage 1 (LogFC 0.8 / FDR 1) and passage 6 (logFC 2.2 /FDR 7.29E-33) experiments showed upregulation of S100A4. S100A4 is a calcium binding protein which interacts with structural proteins to promote metastasis of cancer cells. However, the exact mechanism is still unknown. These data correlated with the increased accessibility of the chromatin of the S100A4 gene in passage 6 of the mutant organoids observed in scATAC-seq. We further validated these data in wildtype and ARID1A mutant CCOC and endometrial cancer cell lines. Additionally, S100A4 immunohistochemistry on CCOC (p-value=0.022) and endometrial cancer (p-value= 1.8e-06) from tissue microarrays showed significant association between S100A4 expression and ARID1A loss independent of PIK3CA mutation. The functional experiments from endometrial primary epithelial cells overexpressed with S100A4 showed significant proliferation and fast wound healing (p-value<0.05) compared to control transduced cells. In order to understand the mechanism of S100A4 in our model we performed immunoprecipitation of S100A4 followed by mass spectrometry analysis (IPMS) in 4 different ARID1A mutant cancer cells to analyse the interactome partners of S100A4. The results demonstrated glucose -6-phosphate dehydrogenase (G6PD) to interact directly with S100A4 (BFDR=0, saint score=1) in all 4 cell lines. G6PD is a rate limiting enzyme in pentose phosphate pathway that supplies the energy to the cells in oxidative damage response by maintaining the level of NADPH.

Conclusions: Understanding the biological function of S100A4 and G6PD interaction may lay the groundwork for the development of new therapeutics for ARID1A mutant gynecological cancer patients

#45

POSTER PRESENTATION - GRADUATE

Fumi Inaba

Supervisior: Dr. Martial Guillaud and Dr. Calum MacAulay

Title: Large-scale DNA Organization Identifies Aggressive Prostate Cancer in Low and Intermediate-Risk Patients Treated with Radical Prostatectomy

AUTHOR(s)

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ABSTRACT

Background/objectives: Prostate cancer (PCa) is the most prevalent cancer among men in Canada where its pathological heterogeneity and limited efficacy of current prognostic methods make precise treatment a challenge. The current standard prognostic method is the D'Amico classification, stratifying patients into low, intermediate, and high-risk categories. However, 15% of low-risk and 30-40% of intermediate risk patients experience biochemical recurrence (BCR) following radical prostatectomy (RP); a non-trivial proportion of patients experiencing adverse clinical outcomes following a treatment of curative intent. This highlights the potential benefit of a refined prognostic tool to inform initial treatment planning. For this, we propose large-scale DNA organization (LDO), a biomarker derived from an image analysis pathomics approach that encompasses nuclear morphology, DNA quantity, and chromatin organization of individual nuclei.

Methods: For a given biopsy scan, LDO of individual nuclei are analyzed by leveraging a DNA-stoichiometric Feulgen-thionin stain and nuclei instance-segmentation. Segmentation identifies the pixels in the biopsy scan belonging to a single nucleus, from which 168 LDO features are calculated. Unsupervised clustering analysis of LDO measurements defines ten subpopulations of nuclei. Within each nuclei subpopulation, a binary classifier is trained to predict if a nucleus originates from a patient with BCR. Predictions are summarized as the proportion of nuclei in each subpopulation predicted to originate from a BCR patient. These proportions were used to train a linear discriminant analysis model to predict if the biopsy is from a BCR patient. Patients with more than half of their biopsies predicted to originate from a BCR patient are predicted to experience BCR. The analysis pipeline was trained and assessed on a cohort of 115 RP-treated PCa patients from Slovenia, 27 of which experienced BCR and 88 without. The 295 available biopsy scans were downsampled to 160 to balance the number of biopsy scans between the two patient cohorts, then used to train the linear discriminant model.

Results: The biopsy-wise classifier had a balanced accuracy of 82.5% on the test set of 40 biopsy scans. On a patient level, the balanced accuracy was 90.89%, correctly classifying 23 of the 27 BCR patients, and 85 of the BCR-free patients. Confusion matrices show higher specificity for both biopsy and patient classification, correctly classifying more BCR-free biopsies and patients than those with BCR.

Conclusions: The proposed image analysis framework and LDO as a prognostic marker to predict BCR following RP treatment shows promising results. It may be able to refine initial treatment plans for patients by predicting the likelihood of BCR following RP prior to the procedure. However, this framework needs to be validated on an independent cohort to ensure generalizability and validity. For this framework to be a robust tool for treatment plan refinement, it must also be applicable to other PCa treatments. Training the framework for other treatments will be completed upon validation of the current proposed framework.

#46

POSTER PRESENTATION - GRADUATE

Jennifer Cooper

Supervisior: Dr. Cheryl Wellington

Title: Validating the utility of plasma biomarkers to diagnose autopsy confirmed Alzheimer's disease

AUTHOR(s)

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ABSTRACT

Background: Plasma biomarkers have been proposed as a low cost, accessible tool to aid in the diagnosis of Alzheimer's disease (AD). They can detect the underlying pathology of AD, with the top candidate biomarkers being: amyloid beta 42/40 (Aβ42/40), reflecting amyloid plaques; phosphorylated tau-181 (p-tau-181), reflecting neurofibrillary tangles; neurofilament light (NfL), reflecting axonal damage observed in neurodegeneration; and glial fibrillary acidic protein (GFAP) reflecting neuroinflammation through astrocyte activation. Although these biomarkers can differentiate those with AD from non-demented individuals, the ability to differentiate AD from other forms of dementia is more complex. One major confounder is the presence of co-pathologies from other dementias. Often biomarker studies are conducted to look at concordance with other measures of AD specific neuropathological hallmarks, which can miss co-pathologies only detectable upon autopsy. This study investigates the ability of plasma biomarkers to detect autopsy confirmed AD pathology and understand the influence of co-pathologies.

Methods: Plasma samples were obtained from the UBC Hospital Clinic for Alzheimer Disease and Related Disorders from patients diagnosed with dementia who had subsequent brain autopsy to determine what neuro-pathologies are present. Biomarkers were analysed on the Quanterix Simoa HD-X analyzer using commercial Neurology 4-plex E and p-tau-181 assays. Group comparisons were performed using Mann-Whitney test and Fisher's exact test. Area under receiver operating curve (AUROC) analysis was done to determine diagnostic potential of a combination of biomarkers.

Results: Of the N=96 participants, N=45 (47%) we're female, and the median age of death was 75 (65-82) years. N=60 (63%) of participants had the presence of AD pathology. Those with AD were significantly older than those without (77 vs 64 y, p<0.0001), and there was no significant difference in sex distribution between groups (p=0.2916). In those with AD, Aβ42/40 (0.049 vs 0.061 pg/ml, p<0.0001) was significantly lower, while p-tau-181 (4.7 vs 2.3 pg/ml, p<0.0001) and GFAP (277 vs 131 pg/ml, p<0.0001) were significantly higher than those without AD. NfL was not significantly different between the groups (p=0.1136). When combining Aβ42/40, p-tau-181, and GFAP, while accounting for age and sex the AUROC was 0.884. Of those with AD, N=25 (42%) participants had purely AD, while N=35 (58%) had additional co-pathology. Compared to non-AD, Aβ42/40 was significantly lower in both pure AD (p<0.0001) and co-pathology groups (p<0.0001), ptau-181 and GFAP were significantly higher in both pure AD (p-tau-181 p<0.0001, GFAP p<0.0001) and co-pathology groups (p-tau-181 p<0.0001, GFAP p=0.0007), and NfL had no significant differences. None of the biomarkers significantly differed between those with pure AD and co-pathology.

Conclusion: Plasma biomarkers can distinguish patients with dementia due to autopsy-confirmed AD versus non-AD dementia with good diagnostic accuracy, and were not impacted by co-pathology. However, these biomarkers cannot differentiate those with pure AD pathology and those with co-pathologies.

#47

POSTER PRESENTATION - GRADUATE

Guadalein Tanunliong

Supervisior: Dr. Inna Sekirov, Dr. Mel Krajden

Title: Anti-SARS-CoV-2 IgA and IgG antibodies in the classification of recent COVID-19 among pregnant individuals

AUTHOR(s)

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ABSTRACT

Background/objectives: Coronavirus Disease 2019 (COVID-19) infection and/or vaccination elicits host immunoglobulin A and G (IgA and IgG) antibody responses that can fight against the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection. While laboratory testing for the presence of IgA and IgG antibodies has yielded powerful applications in diagnostics and surveillance, its utility in determining the timing of SARS-CoV-2 infection remains unknown. We hypothesize that IgA and IgG antibody levels against COVID-19 differ over time following infection in individuals who were unvaccinated or vaccinated prior. Our objective was to evaluate the performance of anti-SARS-CoV-2 IgA and/or IgG antibodies as biomarkers in classifying recent COVID-19 among vaccinated and unvaccinated pregnant individuals.

Methods: We retrospectively selected residual sera from pregnant individuals across British Columbia with PCR-confirmed COVID-19 who were either two-dose vaccinated (N=137) or unvaccinated (N=171) prior to infection, with their confirmed infections occurring 0.5 to 3 months before serum collection. All serum specimens were tested for IgA and IgG antibodies against SARS-CoV-2 spike (S), receptor binding domain (RBD), and nucleocapsid (N) using the Meso Scale Diagnostics multiplexed immunoassay. Kruskal-Wallis tests, Pearson and Spearman correlation tests, and sensitivity and specificity calculations were carried out.

Results: Median IgA levels against SARS-CoV-2 N significantly differ over time following infection for both unvaccinated-infected ($P<0.0001$) and vaccinated-infected ($P=0.0002$) individuals, decreasing over time to below positivity cutoff at one month for vaccinated-infected individuals ($R=-0.34$) and two months for unvaccinated-infected individuals ($R=-0.43$). These differences were not observed for median IgG levels. Combined IgA and IgG dual markers yielded suboptimal sensitivities and specificities in classifying recent infections within two months of serum collection among both unvaccinated-infected and vaccinated-infected individuals and cannot be used to stratify individuals by the timing of their COVID-19 infection. Interestingly, combined IgA and IgG markers can be used to distinguish between unvaccinated-infected from vaccinated-infected individuals.

Conclusions: This work demonstrates that anti-N IgA levels, but not IgG, differ over time following infection regardless of vaccination status in a pregnant population. However, combined serum IgA and IgG levels cannot be used to inform timing of COVID-19. Our findings provide profound insights into both the utility and limitations of COVID-19 antibody testing in laboratory and public health applications.

#48

POSTER PRESENTATION - GRADUATE

John Perrier

Supervisior: Dr. Ed Pryzdial

Title: Coagulation initiated by tissue factor on a coronavirus

AUTHOR(s)

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ABSTRACT

Background/objectives: Abnormal blood clotting is a leading cause of death worldwide. Furthering our understanding of how viral infection and blood clotting are connected, our lab discovered that the cell membrane initiator of coagulation, tissue factor (TF), is integrated into the outer envelope of oral herpes virus and is required for its infection in vivo. Here we study the ubiquity of this finding by addressing the question, “Can human coronavirus 229E (HCoV-229E) infection and hyper-coagulation be simultaneously targeted to reduce associated pathologies?” In the current project, the effects of coagulation inhibitors on clot formation and cell infection will be assessed to demonstrate that envelope TF is pivotal in pathology and viral infection. These data will further support our hypothesis that any enveloped virus can acquire TF if replicated in a TF-bearing cell, providing a novel broad-spectrum antiviral therapeutic target.

Methods: HCoV-229E was propagated in human hepatoma-derived Huh7 cells and purified via sucrose gradient ultracentrifugation. Immunogold electron microscopy and/or western blot were used to characterize TF antigen associated with the virus and host cells. The enzymatic function of TF on purified virus surface was evaluated by chromogenic and plasma clotting assays. Virus-mediated factor X activation was measured on a kinetic microplate reader (VMax, Molecular Devices), using chromogenic reagent S-2765 and TF-specific inhibitors. A prothrombin time test was used to measure virus-initiated clotting time in pooled citrated human plasma.

Results: We demonstrated that both purified HCoV-229E and the cell from which it emerges express TF antigen. Immunogold electron microscopy confirmed that TF is associated with the purified virus. Purified HCoV-229E exhibits TF-dependant acceleration of clotting factor VIIa-dependent factor X activation, comparable to the physiological clotting cascade. Plasma clotting times are shortened by purified HCoV-229E in a concentration-dependant manner. These functional effects of virus were inhibited by a TF-specific monoclonal antibody.

Conclusions: To understand how viral infection may affect the blood clotting system, we have identified TF on the surface of HCoV-229E. Cofactor function and procoagulant activity observed in TF on HCoV-229E, combined with the observation of TF on several members of Herpesviridae, supports the notion that all types of enveloped viruses have surface TF and provides a mechanism for the thromboinflammatory complications observed in patients infected with other Coronaviridae members, such as SARS-CoV-2. Further studies will define the effect of TF-axis clotting proteases, FXa, FVIIa, and thrombin, on HCoV-229E infection of cells, serving as the framework for broad-spectrum antiviral therapeutic development.

#49

POSTER PRESENTATION - GRADUATE

Julliet Zama

Supervisior: Dr. Hélène Côte

Title: The Impact of hepatitis C virus (HCV) clearance on markers of immune aging and inflammation among women living with and without human immunodeficiency virus (HIV) over time.

AUTHOR(s)

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ABSTRACT

Background: Despite the availability of effective antiretroviral therapy (ART), people living with HIV (PLWH) experience signs of premature aging. Women living with HIV (WLWH), who constitute more than half of PLWH globally and 23% of PLWH in Canada, experience shorter lifespans and health spans than both men living with HIV and HIV-negative women. Premature aging is a condition when PLWH develop age-related conditions such as arteriosclerosis, diabetes, neurological disorders, and renal damage earlier in life compared to the general population. It has been linked to inflammation and immune dysfunction, which can accelerate the shortening of leukocyte telomere length (LTL), a marker of immune aging. Chronic HCV infection, associated with inflammation and T-cell (CD4+ and CD8+ T cells) exhaustion is also associated with shorter LTL. Furthermore, oxidative stress seen in HIV/HCV viral infections is associated with mitochondrial DNA (mtDNA) alterations which can affect mitochondrial function and contribute to immune senescence and aging. Unlike HIV, HCV can be eliminated through effective antiviral therapy using direct-acting antiretrovirals which many women have received over the past 5-10 years, with many achieving HCV sustained viral response. However, the effect of clearing HCV on immune aging has not been described. This research aims to understand the effect of HIV and HCV clearance on several markers of immune aging and inflammation in WLWH.

Hypothesis: Successful clearance of HCV will be associated with 1) slower loss of LTL, 2) increased mtDNA content, 3) increased CD4:CD8 ratio, and/or 4) a decrease in CD8+ T cell CD28+:CD28- ratios, and 5) decreased markers of inflammation.

Methods: In this case-control retrospective study, WLWH and HIV-negative women (controls) ≥ 16 years with a specimen collected before and after HCV clearance (study group, n≥60) will be selected from two cohorts; the Children and Women AntiRetrovirals and Markers of Aging (CARMA) or the British Columbia CARMA-CHIWOS collaboration (BCC3) cohort study. Study group participants will be matched with controls (no HCV, or no HCV clearance) by balancing socio-demographic characteristics (age, ethnicity) and time elapsed between visits, as best as possible. Using plasma, HCV RNA status (pos/neg) will be confirmed by RT-qPCR. Markers of immune aging investigated will include LTL and mtDNA content, both measured by monochrome multiplex qPCR on whole blood DNA. We will use live peripheral blood mononuclear cells to quantify CD4:CD8 T cell and CD8+ T cell CD28-:CD28+ ratios, two markers of immune aging, both measured by flow cytometry. In plasma, we will quantify a panel of selected markers of inflammation (IL-6, TNF-α, IL-10, INF-α, CRP, and INF-γ) using the mesoscale kits, as well as ALT, AST.

Significance: Treatment for HCV is costly and access to it is not always equitable, determining its potential effect on aging markers will help inform the care of all women, including often marginalized and vulnerable WLWH.

#50

POSTER PRESENTATION - GRADUATE

Katlyn Richardson

Supervisior: Dr. David Granville

Title: Granzyme k induces keratinocyte proliferation and inflammation: implications for psoriasis pathogenesis

AUTHOR(s)

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ABSTRACT

Psoriasis is a chronic inflammatory disease that currently affects over one million Canadians. It is characterized by increased keratinocyte proliferation and inflammation forming skin plaques. Activation of the transcription factor, signal transducer and activator of transcription 3 (STAT3), is a hallmark of keratinocyte proliferation and inflammation in psoriasis. Still, knowledge regarding the mechanisms underlying keratinocyte activation remains incomplete, averting potential for efficacious therapeutics. Previously, we demonstrated that the serine protease, Granzyme K (GzmK), is elevated in human psoriasis lesions. Further, in a murine model of psoriasis, GzmK knockout mice exhibited reduced disease severity compared to wild-type mice. Notably, GzmK has been shown to induce cellular proliferation and the release of pro-inflammatory cytokines in other cell types through the activation of protease-activated receptor 1 (PAR-1). Thus, we hypothesized that GzmK mediates STAT3-driven keratinocyte proliferation and inflammation in psoriasis through PAR-1 activation.

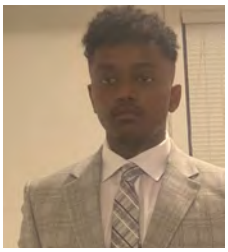
Human keratinocytes were initially transfected with siRNA targeting F2R (PAR-1) or a negative control for 120 h, followed by evaluation of siRNA knockdown efficiency by Western blot. Following transfection, keratinocytes were incubated with or without GzmK for 48 h, and cellular proliferation was assessed using cell counts and Ki-67 immunocytochemistry. To further elucidate the mechanisms underlying GzmK-mediated keratinocyte proliferation in vitro, phosphorylation levels of both MAPKs and STAT3 were evaluated by Western blot. To investigate whether GzmK-mediated activation of STAT3 depends on MAPK signaling, keratinocytes were pre-treated with a p44/42 MAPK inhibitor, and STAT3 activation was assessed in the presence or absence of GzmK. Findings were validated in vitro through the examination of pro-proliferative and pro-inflammatory markers in mouse skin sections from a mouse model of psoriasis, comparing GzmK knockout to wild-type mice.

In vitro, human keratinocytes stimulated with GzmK exhibited a significant increase in cellular proliferation, accompanied by a robust phosphorylation of both p38 and p44/42 MAPK as well as STAT3. These effects were markedly decreased in cells transfected with F2R (PAR-1) siRNA. Additionally, pre-treatment of keratinocytes with a p44/42 MAPK inhibitor abrogated the phosphorylation of STAT3 by GzmK. In vivo, skin tissues from GzmK knockout mice displayed significantly reduced levels of cellular proliferation and inflammation compared to wild-type mice. Specifically, histological examination revealed that GzmK deficiency resulted in decreased epidermal hyperplasia (thickening), staining for the proliferation marker Ki-67 within the epidermis, inflammatory cell infiltrate, and pro-inflammatory cytokine levels.

GzmK is elevated in human and murine psoriasis and contributes to disease severity by promoting keratinocyte proliferation and inflammation. In vitro, GzmK induced keratinocyte proliferation and inflammation occurs through a PAR-1/MAPK/STAT3 mechanism. Inhibition of GzmK may represent a novel therapeutic approach for psoriasis management.

#51

POSTER PRESENTATION - GRADUATE



Kidus Achalu

Supervisior: Dr. Cheryl Wellington

Title: Comparison of Plasma Proteomic Profiles of Adolescent Athletes Pre- and Post-Concussion

AUTHOR(s)

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ABSTRACT

Background: Sport-related concussion (SRC) is a major health concern with an estimated 200,000 cases reported annually in Canada; the majority of which are in children and adolescents. However, SRCs are underreported suggesting an underestimation of reported concussion rates. Symptoms following an SRC are often subtle, which can affect the reliability of clinical diagnostic methods that rely on subjective patient-reported measures and recall, resulting in reporting bias. As undiagnosed concussions can lead to long-term health issues and have unfavourable neurological implications, developing objective diagnostic tools could improve early detection and diagnosis, inform prognosis, and refine treatments. Recent research has demonstrated the potential utility of blood-based biomarkers for SRC diagnosis, offering a quantitative, minimally-invasive, and cost-effective method to assess changes in the brain. Some biomarkers have shown group-level differences between athletes with an SRC and controls, however, large overlap between these groups reduces clinical relevance at the individual level. To identify novel biomarkers with greater diagnostic potential in adolescent athletes, a targeted proteomic discovery approach may be useful. This study will analyze the proteomic profile of adolescent athletes pre- and post-SRC to find putative diagnostic biomarkers with better sensitivity and specificity than biomarkers studied to date.

Methods: Blood samples are collected through the SHRed Concussions study, a Canadian multisite study in which male/female athletes (13-16 years old) are recruited who actively partake in sports with high-risk for concussion including rugby, soccer, and football. Following enrollment, participants undergo preseason testing. Those who sustain an SRC will undergo SRC assessment and blood draws at 72 hours, 1 week, and every 2 weeks until cleared to play. Paired preseason and post-SRC plasma samples will be analyzed by Alamar's NULISA platform, a proximity-extension assay capable of detecting 120 proteins in their central nervous system panel with attomolar sensitivity. Machine learning techniques will be used to reduce data dimensionality and data will be analyzed using paired t-test, repeated measures ANOVA, and logistic regression.

Results: To date, over 1200 participants have been enrolled with 2,144 blood samples collected; 35% of these samples being post-SRC. A sub-cohort of 76 participants (n = 45 males) who have a median age of 16 and have both a preseason and post-SRC blood sample(s) will be used in this . We hypothesize that we will identify novel blood-based biomarkers that may offer improved clinical diagnostic utility and inform on an athlete's SRC status.

Conclusion: Blood-based biomarkers have the potential to improve SRC diagnosis, prognosis, and treatment by adding objective measures that complement subjective patient-reported clinical measures. Furthermore, the use of paired samples reduces the impact of confounding variables often observed in studies that have a distinct control group. This project has the potential to advance accuracy of SRC diagnosis enabling more precise and timely interventions.

#52

POSTER PRESENTATION - GRADUATE

Lauren Deneault

Supervisior: Dr. Cheryl Wellington

Title: The role of cysteine residues in the binding of novel therapeutics for prostate cancer

AUTHOR(s)

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AFFILIATION(s)

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ABSTRACT

Background/objectives: Current therapies for prostate cancer (PC) involve pharmaceutical castration by targeting the androgen receptor (AR) ligand-binding domain (LBD). These therapies are not curative with the malignancy progressing to castration-resistant PC (CRPC). Most CRPC remains driven by the AR through multiple resistance mechanisms, e.g., constitutively active AR splice variants that lack LBD (AR-Vs), or gain-of-function mutations in LBD. This has led to the clinical development of EPI analogs ("EPI") which target the AR N-terminal domain (NTD) to block the transcriptional activities of AR-Vs and mutated ARs. The EPI-binding pocket of the AR-NTD is enriched in cysteine residues. We hypothesize that free cysteine residues, with the ability to interact and form disulfide bonds, may alter AR transcriptional activity, and impact the binding mechanism of EPI analogs. Here we begin to elucidate the role of cysteines in the inhibitory mechanism of EPI on AR activity.

Methods: The role of cysteines in the covalent binding mechanism of EPI to AR used a fluorescein-labelled EPI with fragments of recombinant AR-NTD (rNTD) with or without the addition of iodoacetamide. Iodoacetamide is an alkylating agent that caps reduced cysteines thereby eliminating potential covalent binding of EPI to cysteines. Site-directed mutagenesis was used to create AR expression vectors with a point mutation at C264 and C509. AR plasmids and androgen-response luciferase reporter gene constructs were transfected into CV-1 or PC-3 cells allowing the comparison of wildtype and mutant AR transcriptional activities. Half-maximal inhibitory concentration (IC50) was calculated to assess the impact of cysteine mutations on inhibition of prostate specific antigen (PSA) luciferase activity.

Results: Capping cysteine residues with iodoacetamide reduced the amount of covalent binding of EPI. Additionally, EPI bound preferentially to the oxidized form of rNTD suggesting the importance of disulfide bridges. Cysteine mutations led to reporter-specific and cell-specific increases in AR transcriptional activity in response to androgen. Cysteine mutations did not significantly impact the ability of EPI-7170 to inhibit androgen-induced PSA-luciferase activity.

Conclusions: Cysteine residues in the AR-NTD are essential for covalent binding of EPI. Cysteine mutations at the AR-NTD play an important role in the AR transcriptional activity. Future work will assess additional EPI analogs and look to evaluate the impact of the cysteine mutations in AR splice variants. Ultimately, this work provides valuable insight into the unique mechanisms of EPI analogs and the continual development of more specific and potent therapies for prostate cancer.

#53

POSTER PRESENTATION - GRADUATE

Liam Byrne

Supervisior: Dr. Natalie Prystajecky

Title: extracting antibiotic resistance genes from wastewater

AUTHOR(s)

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2 Pathology and Laboratory Medicine, University of British Columbia, Vancouver, BC

ABSTRACT

Background/objectives: The World Health Organization has called antimicrobial resistance (AMR) “one of the biggest threats to global health, food security, and development”. In 2018, an estimated 14,000 Canadian deaths were associated with an antimicrobial resistant bacterial infection. Moreover, the cost of treating antimicrobial resistant infections in Canada is expected to grow from $1.4 billion in 2018 to $6 billion in 2050. Despite the ever-increasing burden of AMR, the true prevalence of antimicrobial resistant organisms is unknown due to insufficient testing at the population level. Problematically, current clinical AMR testing is biased towards nosocomial infections and the techniques used such as culturing or deep read sequencing are time consuming and not easily scalable. New techniques are needed to establish a better understanding of the communal prevalence of AMR. The utility of wastewater testing as a less biased, population wide surveillance method has been demonstrated recently for viral pathogens like SARS-CoV-2, but current wastewater processing methods result in poor recovery of bacterial targets. We sought to develop a new wastewater processing method that would improve the sensitivity of AMR surveillance in wastewater.

Methods: A total of 20 wastewater samples were collected from five wastewater treatment plants across four dates. Wastewater was processed in parallel via 3 different processing methods (vacuum filtration, differential centrifugation, ultrafiltration) and DNA was extracted. Resulting DNA was tested with a tetraplex qPCR targeting 4 clinically important antibiotic resistance genes: OXA48, KPC, NDM, and MCR1. Standard curves and volumetric analyses were used to quantify each antibiotic resistance gene. Mean AMR gene concentrations from each wastewater processing method were compared.

Results: The vacuum filtration processed samples had a 97.5% antimicrobial resistance gene detection rate contrasted with 83.8% and 52.5% for differential centrifugation and ultrafiltration respectively. Moreover, vacuum filtration yielded antimicrobial resistance genes at 7-fold higher concentrations on average than the second-best method, differential centrifugation.

Conclusions: We demonstrated that vacuum filtration results in greater concentration of AMR genes from wastewater and a higher detection rate than other methods examined. Future wastewater surveillance of AMR will employ vacuum filtration.

#54

POSTER PRESENTATION - GRADUATE

Mayur Mallya

Supervisior: Dr. Ali Bashashati

Title: deep learning-based foundation models predict ovarian bevacizumab response using histopathology images

AUTHOR(s) Mayur Mallya, Ali Mirabadi, Hossein Farahani, Ali Bashashati

AFFILIATION(s) University of British Columbia

ABSTRACT

Background/objectives:
Epithelial ovarian cancer (EOC) is a lethal gynecological cancer with high mortality and recurrence rates to traditional treatment of surgery and chemotherapy. Bevacizumab is a targeted therapeutic agent which in conjunction with traditional therapy has shown to inhibit tumor angiogenesis in clinical trials leading to its FDA approval for the treatment of advanced EOC. However, due to the lack of effective biomarkers for the prediction of therapeutic outcomes, the use of bevacizumab for personalized treatment remains challenging to this day. Additionally, given the high cost and potential toxicity, it is imperative to identify predictive methods for EOC treatment using bevacizumab. In this work, we leverage the routinely acquired histopathology images for predicting patient-specific treatment response to bevacizumab using data-driven AI methods.

Methods:
Given the success of deep learning (DL) methods in the automated analysis of histopathology images in the last decade and the ubiquity of digitized whole slide images (WSI) in comparison to molecular analysis, in this work we predict the treatment response of bevacizumab from the WSI of the patients. To train the DL models, we use the ATEC23 dataset that was curated as part of the EOC bevacizumab response clinical trial at the Tri-Service General Hospital in Taiwan and made publicly available in 2023. In our experiments, we use the Foundation Models, which in the recent months have shown tremendous success in a variety of downstream WSI analysis tasks, along with the traditionally used multiple instance learning (MIL) techniques for treatment response prediction to bevacizumab therapy.

Results:
We analyze the prediction performance across a combination of 5 different MIL models and 12 different feature encoders of the WSI. Among the feature encoders, we use 6 traditionally used image encoders pre-trained on large volume of natural images and 6 FM encoders trained on large-scale WSI datasets. Our rigorous 3-fold cross-validation experiments across multiple combination of MIL models and feature encoders show that our models can predict the patient-specific binary bevacizumab response upto 70% accuracy on the internal test set. We also observe that the FM encoders provide higher prediction performance relative to the traditionally used encoders for this task.

Conclusions:
While we achieve a promising performance of over 70% in the patient-specific bevacizumab response prediction, it is only the first step in the identification of histological biomarkers. To this end, we further propose to validate our models on external datasets such as the bevacizumab clinical trials conducted in Europe (OVAR) and also investigate the performance of AI models on more robust problem settings such as the patient-specific survival analysis from WSI in addition to the currently employed binary classification setting. Finally, we aim to discuss our findings with pathologists to identify clinical biomarkers for bevacizumab treatment.

#55

POSTER PRESENTATION - GRADUATE

Mehdi Nouri Zadeh

Supervisior: Dr. Babak Shadgan

Title: Potential application of near-infrared spectroscopy in monitoring muscle spasticity; a feasibility study in healthy individuals

AUTHOR(s)

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AFFILIATION(s)

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ABSTRACT

Background/objectives:
People with incomplete spinal cord injury (SCI) commonly experience upper extremity muscle atrophy and dysfunction, which reduce their ability to handle their activities of daily living and independence. High-intensity resistance exercise programs (60-80% of one's one-repetition maximum -1RM),) are considered the most effective intervention to improve muscle strength and hypertrophy. However, most people with SCI cannot safely tolerate high-intensity exercise protocols and cannot afford upper limb muscle overuse injuries.

Methods:
In this observational study, 22 healthy male and female volunteers performed a series of forearm muscle contractions at varying intensities. These contractions were applied and measured by a hand dynamometer. To record the changes in tissue oxygenation during muscle contractions, a continuous wavelength NIRS sensor was used. Additionally, muscle activations were monitored using an EMG sensor.

Results:
Preliminary results indicated a strong correlation between muscle contraction levels and changes in muscle tissue oxygenation index (TOI).

Conclusions:
The study demonstrates the feasibility and function of muscle NIRS to determine various levels of skeletal muscle contraction in healthy individuals. This research may assist in developing a new objective technique for the non-invasive monitoring of muscle spasticity. Such a technique

#56

POSTER PRESENTATION - GRADUATE

Michael Lane

Supervisior: Dr. David Granville

Title: Granzyme b: a novel mechanism for dermal-epidermal junction degradation in stevens-johnson syndrome/toxic epidermal necroylsis

AUTHOR(s)

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ABSTRACT

Background/objectives: Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN) are life-threatening, immune-mediated, cutaneous adverse drug reactions with a paucity of effective treatments. SJS/TEN is characterized by the separation of the epidermis from the dermal layer of the skin, resulting in severe blistering and peeling. Through degradomic and other approaches, we have recently identified that the extracellular proteolytic activity of serine protease Granzyme B (GzmB) contributes to the onset and progression of bullous pemphigoid, the most common autoimmune blistering condition in the elderly. Dermal-epidermal junction proteins that anchor the epidermis to the dermis – alpha6/beta4 integrin, collagen VII, and collagen XVII – were previously identified as substrates of GzmB cleavage, and their levels decreased in autoimmune blistering conditions. In the present study, we hypothesize that GzmB accumulates extracellularly, is proteolytically active, and cleaves key dermal-epidermal junction proteins in SJS/TEN.

Methods: Skin biopsies collected from SJS/TEN patients (n=8) and healthy participants (n=8) were analyzed using immunohistochemistry to assess protein levels of GzmB and its substrates, alpha6/beta4 integrin, and collagen XVII. ELISA was used to quantify GzmB levels in blister fluid from patients with SJS/TEN (n=6) and was compared to those of bullous pemphigoid patients (n=2). Western blotting was used to identify fragments of collagen XVII in SJS/TEN (n=6) and bullous pemphigoid (n=2) blister fluid samples.

Results: Epidermal and dermal GzmB levels appeared elevated in SJS/TEN compared to healthy skin. SJS/TEN sections exhibited reduced alpha6/beta4 integrin and collagen XVII at the dermal-epidermal junction compared to healthy skin. Increased levels of GzmB as well as fragments of collagen XVII (around 120 kDa and 97 kDa, as previously observed in GzmB cleavage assays in vitro) were detected in all SJS/TEN blister fluid samples, suggesting that the extracellular proteolytic activity of GzmB is sustained in SJS/TEN.

Conclusions: The present study provides a potential novel pathological mechanism of action in SJS/TEN whereby elevated levels of extracellular GzmB mediate degradation of dermal-epidermal junction proteins, leading to separation of the epidermis from the dermis.

#57

POSTER PRESENTATION - GRADUATE

Mohammad Ghodsi

Supervisior: Dr. Cheryl Wellington

Title: Preliminary overview of neurological blood-based biomarkers in survivors of acute intimate partner violence

AUTHOR(s)

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ABSTRACT

Background/objectives: It is estimated that over 200,000 Canadian women experience Intimate Partner Violence (IPV)-caused Brain Injury (BI) annually. BI caused by IPV (IPV-BI) can occur from non-fatal strangulation (resulting in anoxic BI), head impact (resulting in traumatic BI), or a combination of both. The prevalence of IPV-BI is likely underestimated due to a lack of BI awareness in this population and the hesitancy of survivors to seek medical treatment. This underdiagnosis not only impedes survivors' access to vital support systems but also increases the risk of the development of chronic symptoms. This highlights the urgent need for objective, feasible, and IPV-BI sensitive diagnostic tools to identify and characterize acute BI among survivors. While blood-based neurological biomarkers have emerged as promising tools for objective examination of BI in many neurological indications, including sport-related concussions, hypoxic-ischemic BIs, and neurodegenerative disorders, their diagnostic utility in IPV-BI has not yet been explored. The objectives of this study are to describe (1) biomarkers in survivors of IPV with and without suspected IPV-BI compared to Canadian normative Reference Intervals (RI), and (2) the relationship between biomarkers and acute BI symptoms experienced by IPV survivors.

Methods: 29 females who experienced IPV within the last 30 days were enrolled (median=10 days, SD=6.8 days). Participants were divided into two groups based on whether or not they experienced non-fatal strangulation and/or head impact during the incident (suspected IPV-BI [n=22] vs. without IPV-BI [n=7]). Plasma was analysed for Neurofilament-Light (NfL: a marker of axonal damage) and Glial Fibrillary Acidic Protein (GFAP: a marker of astrocyte activation and inflammation). Biomarker concentrations were compared to age-adjusted Canadian normative RI by expressing them as within or above the 95th percentile. Mann-Whitney tests compared biomarkers between those with suspected IPV-BI grouped based on whether or not they experienced headache (n=13 with, n=9 without) and dizziness (n=13 with, n=9 without) at the time of the incident.

Results: Of the 22 participants with suspected IPV-BI, n=4 (16%) were above the RI for NfL, and n=2 (8%) were above the RI for GFAP. Of the 7 participants without IPV-BI, n=1 (12.5%) was above the RI for NfL and no participants were above the RI for GFAP. NfL and GFAP were not significantly different in participants reporting headache (NfL: p=0.56, r=0.14; GFAP: p=0.74, r=0.08) or dizziness (NfL: p=0.096, r=0.36; GFAP: p=0.79, r=0.06) at the time of the incident.

Conclusions: Low proportions of participants with suspected IPV-BI had biomarkers outside of population norms, leading to the need for future investigation of these individuals. Biomarkers were not significantly associated with reports of headaches or dizziness at the time of the incident. Dizziness showed a medium effect size for NfL, indicating the need for a larger sample size. Moving forward, we will continue the recruitment (recruitment target of 500 participants), and assess a wider profile of central nervous system-specific biomarkers (~120 analytes).

#58

POSTER PRESENTATION - GRADUATE

Parisa Golesorkhi

Supervisior: Dr. Jayachandran Kizhakkedathu

Title: Development of an anti-adhesive and antibacterial coating for platelet storage bags to improve quality of platelets during storage

AUTHOR(s)

Parisa Golesorkhi

ABSTRACT

Background/objectives:
Platelet transfusion is a lifesaving therapy to prevent bleeding in trauma, surgery and other hematological conditions. Despite their importance, platelets have a very short shelf-life during storage. It is hypothesized that the reason might be the non-ideal material (Polyvinyl chloride) that platelet bags are made up of. This material has a hydrophobic surface that triggers the adhesion and activation of platelets that leads to deterioration of the whole bag by time. Moreover, Storage of platelet products at room temperature (22 to 24C) provides ideal conditions for bacterial proliferation. Beside that, we hypothesize that residual red blood cells (RBCs) in platelet units may provide bioavailable iron that promotes bacterial growth. Thus we hypothesize that applying a hydrophilic based coating that contains iron chelators and antimicrobial peptides on the interior of the storage bags will extend platelets shelf-life by inhibiting platelet adhesion and bacterial proliferation. inclusion of iron chelators will bind and remove iron, thereby inhibiting bacterial growth in both culture medium and platelet concentrates. Also, antimicrobial peptides have shown promising result in killing the bacteria in contact.

Methods:
To test these hypotheses, we first developed DFO conjugated polydopamine based poly(N,N-dimethyl acrylamide) coating and we did optimization and characterization tests through iron quantification assays and bacterial growth inhibition investigations. Then we are going to use antimicrobial peptide to conjugate to our optimized iron-chelator containing coating and antibacterial activity tests will be conducted in both culture media and platelet rich plasma. At the end, platelet storage bags will be coated with the final coating and we will utilize flow cytometry, LDH assay, ... for the cell viability, biocompatibility and platelet quality measurements (platelet adhesion and activation). Also we will measure glucose level, pO2, pCO2 of the platelets by time to investigate if platelets are breathing happily in the new developed device.

Results:
Development and characterization assessment of the coating demonstrated a successful conjugation of deferoxamine as an iron-chelator to the coating which is able to bind to available iron and remove that from the media. After applying the coating to bags, we expect our results to demonstrate a significant decrease in platelet adhesion to the surface of the coated platelet bag and, in turn, preserving the cells' quality during storage. Also, we expect to see a significant inhibition of bacterial contamination and growth in stored platelet.

Conclusions:
The integration of iron chelators alongside antimicrobial peptides presents a promising strategy for mitigating transfusion-transmitted bacterial infections and enhancing platelet safety. By incorporating these advancements into platelet storage devices, there is potential to elevate the quality of stored platelets while diminishing the likelihood of bacterial contamination. Consequently, such measures could extend storage durations, lower expenses, and enhance the safety of transfusions for patients.

#59

POSTER PRESENTATION - GRADUATE

Nikolay Alabi

Supervisior: Dr. Ali Bashashati

Title: Novel deep learning-based diagnosis of micropapillary carcinoma in bladder cancer whole-slide pathology images

AUTHOR(s)

Graham Archibald1* , Maryam Asadi1* , Nikolay Alabi1* , Ali Khajegili Mirabadi1, Alberto Contreras-Sanz2, Walid Eshumani2 , Hossein Farahani1 , Peter Black2** , Gang Wang3** , and Ali Bashashati1,3**

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* These authors contributed equally to this work.
** These authors jointly supervised the study.

ABSTRACT

Background/objectives: There are many distinct variant histological subtypes of urothelial bladder cancer that are biologically more aggressive than conventional urothelial carcinoma (UC). Micropapillary carcinoma of the bladder (MPC) stands out as a highly aggressive form of UC with a poor prognosis. It is frequently encountered in advanced stages and is consistently correlated with an aggressive clinical course and unfavorable prognosis. This histological subtype variant exhibits a pronounced inclination to invade the lymphovascular system, leading to early metastasis in lymph nodes and various organs during the disease's progression. However, the identification of MPC often encounters difficulties leading to interobserver disagreement in the diagnostic process. Due to the complex and heterogeneous histological characteristics, there is a poor concordance between pathologists, with 61% of centers reaching no agreement on the diagnosis of this variant histology. The absence of a reliable and robust method for the identification of MPC has significant ramifications in the realm of bladder cancer diagnosis, treatment, and patient outcomes. This deficiency often translates into delayed or missed recognition of MPC, leading to inappropriate treatment.
Our primary goal in this study was to elucidate a generalizable (i.e. applicable to data from different institutions) deep learning-based strategy for improving histological subtype variant diagnosis between conventional UC and the rare MPC variant.

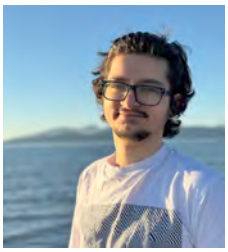
Methods: Our data consisted of two separate cohorts of H&E-stained whole slide images reviewed by two expert genitourinary pathologists to reach a consensus UC or MPC classification. We trained models based on deep convolutional neural networks to classify images. Performance was assessed through cross-validation on the training set (262 WSIs belonging to 86 patients), and on an independent test set (160 WSIs belonging to 72 patients). We applied a color normalization strategy with multiple normalization methods to produce patches that strike a balance between similarity and variability to improve the robustness of the model and overcome the color variation of H&E images. To demonstrate our model's utility, we simulated the clinical diagnostic process by computing the patient-level accuracy for all these experiments by classifying a case as MPC if at least one of its slides is classified as such.

Results: Our results demonstrate the promise of our proposed models with a balanced accuracy reaching 95.8% in our external test set. Misclassified cases were reviewed and interpreted by a pathologist to provide a rationale for the error (i.e. artifact).

Conclusions: We demonstrate the promise of a deep learning-based digital pathology workflow for bladder cancer micropapillary carcinoma classification based solely on H&E stained images that is robust to the color variation present in data from differing pathology departments. The model performance is sufficient to be implemented into the clinical setting as an adjunct for histological subtype variant diagnosis and supporting bladder cancer diagnosis and treatment after further validation on larger cohorts.

#60

POSTER PRESENTATION - GRADUATE



Peyman Malek Mohammadi Nouri

Supervisior: Dr. Jayachandran Kizhakkedathu

Title: Immune protection of chimeric antigen receptor T cells via direct enzymatic polysialylation to improve persistence for cell therapy

AUTHOR(s)

Peyman Malek Mohammadi Nouri1,2, Haiming Luo2,3, Vivian Fung4,5, Lyann Sim3, Majid Mojibian4,5, Megan K. Levings4,5,6, Stephen G. Withers3, Jayachandran N. Kizhakkedathu1,2,3,6

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ABSTRACT

Background/objectives:Chimeric Antigen Receptor (CAR) T cell therapy has gained much attention in recent years for its promising results against certain B cell malignancies. This approach involves engineering T cells to express CARs targeted at specific antigens, which are then infused into patients to eliminate cells carrying the designated antigen. However, a drawback is the premature clearance of a portion of CAR T cells, partly due to the host's immune response against CAR. This premature clearance diminishes the efficacy of CAR T cell therapy, necessitating a higher quantity of CAR T cells to achieve the desired effect and contributing to the substantial costs associated with the treatment. To enhance the persistence of CAR T cells, I will exploit the anti-adhesive and immunosuppressive properties of polysialic acid (PSA), a homopolymer, comprised of sialic acid units, involved in cell signaling and immunomodulation in various immune cells. I hypothesize that polysialylation of CAR T cells could shield them from the host immune system transiently and enhance persistence of CAR T cells. To investigate this hypothesis, the following objectives will be addressed: 1. optimization of polysialylation conditions, 2. investigation of the immunosuppressive and shielding effect of PSA on CAR T cells.

Methods: To polysialylate T cells, PSA is grafted from the surface of the cells by addition of sialic acids from cytidine monophosphate-Sia (CMP-Sia) donor molecules to the non-reducing end of sialic acids on the surface of the cells, mediated by polysialyltransferase-109 (PST-109) enzyme. Firstly, PSA growth and shedding kinetics on T cells were optimized by measuring PSA content at various conditions via flow cytometry. Furthermore, the effect of PSA on activation and proliferation of human leukocyte antigen (HLA)-A2-reactive CAR T cells was evaluated in vitro via flow cytometry. Finally, a peripheral blood mononuclear cell (PBMC)-CAR T cell co-culture model was utilized to study how polysialylation influences CAR T cell viability and cytotoxicity, when interacting with its target.

Results: Our preliminary findings showed that polysialylation using optimal conditions can decrease the expression of CD25 and CD69 activation markers in CAR T cells. Moreover, proliferation assay on PSA-CAR T cells suggested PSA-mediated hindrance of cell proliferation by blocking the interaction of cells with activation signals. Additionally, our co-culture model revealed that polysialylation could increase CAR T cell viability and decrease their cytotoxicity when cultured with PBMCs in a transient manner.

Conclusions: Low persistence of CAR T cells hinders both the effectiveness and cost-efficiency of CAR T cell therapy, requiring higher doses for desired outcomes. Our data suggests that polysialylation could lead to a milder activation of CAR T cells, thus improving their viability and lowering their cytotoxicity against their target cells in a transient manner. Therefore, this study provides preliminary data for development of a polysialic acid-based surface engineering method to improve CAR-T cells persistence and efficacy.

#61

POSTER PRESENTATION - GRADUATE

Razieh Sadat Banijamali

Supervisior: Dr. Honglin Luo

Title: Mesenchymal stem cell-derived extracellular vesicles: a new strategy for delivering oncolytic coxsackievirus B3 in triple-negative breast cancer treatment

AUTHOR(s)

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ABSTRACT

Background/objectives: Breast cancer is among the most prevalent cancers and the leading causes of cancer-related death in women. Triple-negative breast cancer (TNBC) is a subtype of breast cancer known for its high aggressiveness and treatment challenges, highlighting the imperative for the development of innovative therapies. Coxsackievirus B3 (CVB3) is a naturally occurring oncolytic virus recognized for its ability to selectively target and kill various tumor cells. Our laboratory has previously demonstrated that CVB3 serves as a potent and safe oncolytic virus against TNBC both in vitro and in vivo. By genetically modifying CVB3 via a miRNA-detargeting strategy, we significantly reduce its cardiac and pancreatic toxicity while retaining its oncolytic potency. However, a significant challenge in oncolytic virotherapy lies in delivering naked viruses to the target site, due to the presence of neutralizing antibodies. To overcome this obstacle, utilizing carriers, such as extracellular vesicles (EVs), offers a promising approach to conceal the therapeutic viruses from the host's immune system and enhance treatment efficacy. In this study, we aim to investigate the efficacy and safety of miRNA-modified CVB3 encapsulated into mesenchymal stem cells-derived EVs (MSC-EVs) against TNBC in cultured cells and in mice.

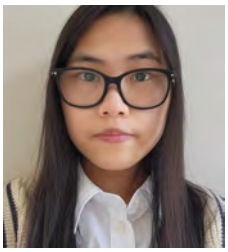
Methods: MSCs were isolated from the adipose tissue of BALB/c mice through collagenase enzymatic digestion, followed by infection with miRNA-CVB3 at an MOI of 1 overnight. EVs were subsequently extracted from the supernatant using a commercial kit. Following the treatment of 4T1 mouse TNBC cells with miRNA-CVB3-loaded MSC-EVs, we evaluated the efficacy of this delivery system and its impact on cancer cell death by measuring the viral load, cell viability and cleaved caspase-3, respectively.

Results: We demonstrated that MSC-EVs carrying miRNA-CVB3 successfully delivered the viruses to mouse 4T1 TNBC cells. Subsequent assessment of cell viability using the MTS assay revealed a significant decrease in cell viability post-treatment with miRNA-CVB3-loaded MSC-EVs. Additionally, analysis of apoptosis demonstrated a notable increase in the level of cleaved caspase-3 in 4T1 cells treated with miRNA-CVB3-loaded MSC-EVs compared to the sham group, suggesting the induction of apoptotic cell death.

Conclusions: Our preliminary data indicate that using MSC-EVs as a vehicle for miRNA-CVB3 delivery provides a novel and promising therapeutic approach for the treatment of TNBC. Further assessment is underway to thoroughly evaluate the efficacy and safety of MSC-EV-mediated delivery of miRNA-CVB3 in a mouse model of breast cancer.

#62

POSTER PRESENTATION - GRADUATE



Rebecca Ho

Supervisior: Dr. David Huntsman

Title: Targeting metabolic reprogramming in ARID1A/B dual-deficient dedifferentiated endometrial carcinoma

AUTHOR(s)

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ABSTRACT

Background/objectives: Endometrial cancers are common gynecologic neoplasms consisting of many histologic subtypes with vastly different outcomes. For example, endometrial adenocarcinoma is the most common type of cancer consisting of well-differentiated endometrioid glands. People with this cancer have high survival rates. But occasionally, these tumours can dedifferentiate, creating a cancer called dedifferentiated endometrial carcinoma (DDEC) with differentiated and undifferentiated components. Compared to endometrial adenocarcinoma, DDEC is rare and aggressive, with limited treatment options. Likewise, some DDECs have co-loss of ARID1A and ARID1B, two switch/sucrose non-fermentable (SWI/SNF) complex subunits. This complex regulates gene expression, and ARID1A/B mutations are implicated in driving DDEC progression. As such, we hypothesize that ARID1A/B dual-deficient DDEC have unique genetic dependencies that create targetable vulnerabilities that allow for the development of new treatment options.

Methods: Therapeutic targets were identified by mining CRISPR/Cas9 knockout data on the Cancer Dependency Map. First, we analyzed ARID1A and ARID1B mutation data to establish ARID1A/B dual-deficient and proficient cell lines. Next, gene effect scores, a measure of cell response to gene knockout, were compared between the two groups. Genes essential to dual-deficient cell lines have a negative score; thus, we conducted a pathway enrichment analysis to process negatively scoring genes to determine their biological function. The putative essential pathways were validated by treating ARID1A/B dual-deficient and proficient cells with drugs and comparing the difference in drug sensitivities between the two groups. To account for genomic differences between the different cell lines we either 1. Re-expressed ARID1A or ARID1B in a dual-deficient cell line or 2. Knocked out ARID1B in proficient cell lines to understand the role of ARID1A and ARID1B in mediating drug response.

Results: Gene Ontology enrichment analysis identified mitochondrial dependencies in ARID1A/B dual-deficient cancers. As the core function of the mitochondria is to generate energy through oxidative phosphorylation (OXPHOS), we treated endometrial cancer cell lines with OXPHOS inhibitor IACS-010759. ARID1A/B dual-deficient cell lines were more sensitive to the drug, and re-expression of either ARID1A or ARID1B in a dual-deficient cell line can partially rescue dual-deficient cells from drug toxicity. Furthermore, ARID1B knockout in a proficient cell line can sensitize the cells to the drug.

Conclusions: Our data suggests that ARID1A and ARID1B dual-deficient DDEC may have increased reliance on the mitochondria for survival compared to their proficient counterparts. This highlights the possibility of targeting the mitochondria as a potential treatment option for those living with this cancer. Furthermore, SWI/SNF defects in subunits other than ARID1A/B are common in many other cancers. Thus, these findings could provide preliminary data on the generalizability of targeting the mitochondria in cancers with other SWI/SNF defects.

#63

POSTER PRESENTATION - GRADUATE

Ryan Chan

Supervisior: Dr. Brian Kwon

Title: Histopathology of posttraumatic syringomyelia in a porcine model of spinal cord injury

AUTHOR(s)

Ryan Chan1 , Jing Wang1, Shenani Basnayake1, Sigrun Jarlsdottir1, Femke Streijger1, Juliana Mitchell1, Ali Zaidi1, Brian K. Kwon1,2

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ABSTRACT

Background/objectives: Spinal cord injury (SCI) affects over 85000 Canadians, with 1800 cases reported annually. Along with characteristic motor and sensory deficits, there are many debilitating chronic effects of SCI. Posttraumatic syringomyelia (PTS) is one such chronic condition characterized by the development of cerebrospinal fluid (CSF)-filled cysts, also known as syrinxes, in the spinal cord, months or years after the initial injury. Despite the prevalence of this condition, the causal mechanisms of initial cyst formation and progressive growth remain poorly understood. As such, this study evaluates the potential usefulness of a porcine model of SCI as a useful intermediary in the pre-clinical testing of novel PTS pharmacological treatments. We hypothesize that the porcine model is an accurate histopathological model for posttraumatic syringomyelia.

Methods: A T10 contusion-compression injury was induced in female Yucatan miniature pigs (n = 40) by dropping a 50 g impactor onto the exposed spinal cord. In-vivo high-resolution ultrasound imaging was performed to visualize the cord at baseline, 30 minutes, 2 weeks, and 14 weeks post-injury. Cords were harvested between 12 to 14 weeks later and stained with eriochrome cyanine for histological quantification. Cavitations were manually traced in Zeiss ZEN 3.4 to calculate volume in histological sections.

Results: Multilocular cavities were observed up to 46 mm from the epicenter of impact. These cavities were detected in several ultrasound scans and histological sections and were found to contain low-density tissue within the syrinx space. Cord regions caudal to the impact epicenter displayed relatively higher cavitation volume and length compared to rostral regions, with a cavity length of 4.9 ± 2.8 mm. Syrinxes were located in greater volume within the white matter. Additionally, while the contusion was applied to the dorsal side of the cord, syrinxes presented in greater volume in the lateral and ventral regions.

Conclusions: Porcine cavitations exhibit a volume and distribution that can be compared to that of human PTS. The aforementioned similarities may have significant implications in providing valuable insights into the mechanisms underlying early syrinx formation and in providing a platform for the preclinical testing of potential therapeutic interventions. Further studies will explore immunohistochemical markers of PTS, such as GFAP upregulation at the syrinx border.

#64

POSTER PRESENTATION - GRADUATE

Taylor Da Silva

Supervisior: Dr. Hugh Kim & Dr. Dana Devine

Title: Investigating the role of platelet factor 4 in asthma

AUTHOR(s)

Taylor Da Silva1,2, Lynn Huang1, Ahmed Kabil1,3,4, Michael Hughes1,3,4, Kelly McNagny1,3,4, Tillie Hackett5, Dana Devine1,2, Hugh Kim1,6,7

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ABSTRACT

Background/objectives:
Asthma is a chronic inflammatory lung disease that affects over 3 million Canadians. Interleukin-33 (IL-33), interleukin-6 (IL-6), interleukin-8 (IL-8), and thymic stromal lymphopoietin (TSLP) are cytokines produced by lung fibroblasts and epithelial cells that mediate inflammation in asthma. Platelets contribute to asthma and other inflammatory diseases. Platelet factor 4 (PF4) is a pro-inflammatory chemokine released during platelet activation and is elevated in patients with asthma. The immune response in PF4 knockout and wild type mice was assessed in a papain asthma model. PF4 knockout mice had less eosinophil recruitment than wild type mice, indicating that PF4 contributes to eosinophil recruitment. However, the way that PF4 mediates the pathogenesis of asthma isn't fully understood. We hypothesize that treatment with PF4 increases production of IL-33, IL-6, IL-8, and TSLP by human lung fibroblasts (HFLs) in a dose-dependent manner.

Methods:
HFLs were cultured for 24 hours, serum-starved for 24 hours, then cultured with different concentrations of PF4 for 72 hours. Human IL-33, IL-6, IL-8, and TSLP were measured in the supernatants by enzyme-linked immunosorbent assay (ELISA).

Results:
Experimental conditions are in the process of being optimized for IL-33, IL-6, IL-8, and TSLP ELISAs.

Conclusions:
Experiments will be replicated using the optimized ELISA conditions. mRNA expression of each cytokine will be measured at different PF4 concentrations and immunofluorescence staining of PF4 and IL-33 will be compared between normal and asthmatic lung tissues. Understanding how PF4 affects cytokine production by lung cells will help to further elucidate the inflammatory role of platelets in asthma.

#65

POSTER PRESENTATION - GRADUATE

Tetiana Povshedna

Supervisior: Dr. Helene Cote

Title: Self-reported hiv viral load is reliable and not affected by adverse lived experiences of women living with hiv in british columbia

AUTHOR(s)

Tetiana Povshedna1,2,3, Shayda A Swann4,5,6, Marcela A.P. Silva4,5, Shelly Tognazzini7, Melanie Lee7, Angela Kaida4,7, Melanie CM Murray1,3,4,5,6, Helene CF Cote1,2,3,4, on behalf of the British Columbia CARMA-CHIWOS Collaboration (BCC3; CIHR CTN 335)

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ABSTRACT

Background/objectives: HIV viral load (VL) is a key predictor of long-term health for women living with HIV (WLWH). Here, we investigate how self-reported HIV VL in a cohort of WLWH in the British Columbia CARMA-CHIWOS Collaboration (BCC3) Study relates to women's knowledge of their own key HIV-related health parameters, and whether it is associated with selected socio-demographic characteristics.

Methods: For women enrolled between March 2021-August 2023 (n=219), self-reported HIV VL (undetectable≤40 copies/ml or detectable>40 copies/ml) was compared to VL obtained from chart review closest to but before the date of self-reported VL. Sensitivity, specificity, predictive values and likelihood ratios were calculated overall and for socio-demographically defined subgroups.

Results: Ninety-five percent of WLWH (208/219) in the study estimated their most recent HIV VL via self-report, and 189/219 (86%) estimated it correctly. Among women who self-reported HIV VL, 200/208 (96%) were on antiretroviral therapy, half reported history of homelessness and 30% reported current substance use. Knowledge about "Undetectable=Untransmittable" was lower (4/10; 40%) among women who didn't report their most recent VL than for those who did, 153/208 (74%). Importantly, self-reported undetectable HIV VL performed similarly across socio-demographic subgroups and showed high sensitivity (Table 1). Enacted, internalized, and disclosure concerns-related HIV stigma scores did not differ between women who estimated their HIV VL correctly or incorrectly.

Conclusions: Our findings replicate previous reports of high awareness about HIV VL by women in BC, despite high prevalence of adverse socio-demographic experiences in our cohort. Our data further suggest that despite highly stigmatized life experiences, WLWH in BC self-report their VL detectability reliably.

#66

POSTER PRESENTATION - GRADUATE

Wan Hei Cheng

Supervisior: Dr Ying Wang

Title: Characterizing inflammation in coronary artery disease

AUTHOR(s)

Wan Hei Cheng, Yuancheng Mao, Samuel Leung, Coco Ng, Gurpreet Singhera, Basak Sahin, Amrit Singh, Chi Lai, Ying Wang

ABSTRACT

Background/objectives:
Coronary artery disease (CAD) is the build-up of atherosclerotic lesions on coronary arteries, causing occlusion of blood flow to the heart. Despite traditional risk factors such as high cholesterol levels, inflammation is now found to be another independent risk factor and therapeutic target of the disease. Recent research has led to the approval of anti-inflammatory drugs in CAD patients to prevent heart attacks. As treatments are usually guided by an individual's therapeutic targets, assessing inflammation status in coronary lesions is useful for guiding anti-inflammatory drugs. However, there is currently no complete knowledge of the inflammatory pathways in human coronary lesions. As previous histopathology studies have indicated that advanced coronary lesions are more likely to cause heart attack compared to early lesions, we hypothesize that more inflammatory pathways are enriched in advanced coronary lesions when compared to early coronary lesions.

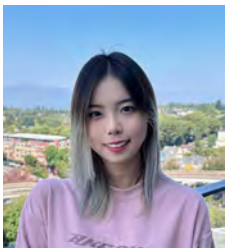
Methods:
To understand the differences in inflammatory pathways enriched in early and advanced coronary lesions, we will need to characterize the activated pathways in the coronary lesions of CAD patients. For characterization, we obtained 341 coronary arteries from 100 CAD patients from the Bruce McManus Cardiovascular Biobank. Tissues were sectioned and stained with H&E and Movat's pentachrome stain, reviewed by a pathologist and classified into different pathologic stages according to the Virmani classification. RNA was extracted by Qiagen RNeasy Mini Kit. Samples with Agilent DV200 value greater than 70% (meaning that more than 70% of RNA are longer than 200 nucleotides) were included for library construction using the ribodepletion method. RNA samples were then sequenced using the Illumina NovaSeq6000 system at Genome Sciences Centre (GSC) to a median depth of 30M reads per library. By comparing genes with differential expression in early lesions (with lower inflammation) and advanced lesions (with higher inflammation), molecular pathways related to inflammation were identified.

Results:
Coronary arteries were categorized into early lesions (pathologic intimal thickening (PIT)) and advanced lesions (fibroatheroma). We found that RNA yield was doubled by proteinase K treatment during RNA extraction, and RNA quality was not compromised by this additional digestion step. Gene ontology analysis showed that neutrophil degranulation and mTOR pro-inflammatory pathways are enriched in advanced lesions.

Conclusions:
Proteinase K digestion improved the efficiency of RNA extraction of coronary lesions. RNA sequencing data showed that pro-inflammatory pathways are enriched in advanced lesions, which are correlated with heart attacks. Future studies could focus on designing inhibitors to selectively block the neutrophil activation and mTOR pathway to reduce the risk of heart attacks.

#67

POSTER PRESENTATION - GRADUATE



Vivian Zhu

Supervisior: Dr. Jacqueline Quandt

Title: Evaluating the impact of an Nr1h3 risk variant identified in multiple sclerosis families on neuroinflammation in preclinical models

AUTHOR(s)

Vivian Zhu1 , Emily Kamma1, Pierre Becquart1, Jacqueline Quandt1

AFFILIATION(s)

1 Department of Pathology & Laboratory Medicine, Faculty of Medicine, University of British Columbia, Vancouver, Canada.

ABSTRACT

Background/objectives: Multiple sclerosis (MS) is a chronic inflammatory neurodegenerative disease of the central nervous system (CNS). Hallmarks include neurodegeneration and demyelination impacting myelin-rich white matter (WM), but also grey matter (GM), particularly in progressive MS. To determine why some individuals develop more severe disability without recovery or progressive MS, we developed knock-in mice carrying a missense mutation (R413Q) in the Nr1h3 gene homologous to that identified in two high-incident MS Canadian families who developed severe and rapidly progressive MS. Nr1h3 encodes liver X receptor alpha (LXRA), critical for lipid/cholesterol homeostasis and inflammation control by macrophages. We induced experimental autoimmune encephalomyelitis (EAE), an autoimmune-mediated demyelinating model of MS where CNS damage causes ascending paralysis. Preliminary results showed Nr1h3 mutant mice had slightly less severe acute disease but consistently failed to recover over chronic disease. The impact of the mutation on CNS histopathology in the acute stage of disease was unknown, thus we aimed to characterize pathological changes associated with the Nr1h3 mutation and acute attacks.

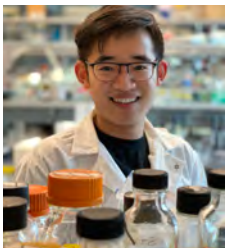
Methods: Wildtype (WT) (n=5), Nr1h3 heterozygote (HET) (n=6), and homozygote (HOM) mutant mice (n=4) were induced with EAE to drive inflammatory demyelination. Naïve WT mice (n=5) acted as controls. The mice were scored for clinical disability until peak disease (day 17), and spinal cord cryosections were evaluated for % coverage by phenotype markers for macrophages/microglia (Iba1) and reactive astrocytes (GFAP) across spinal cord levels. We compared analysis between conventional thresholding with ZEN and pixel classification using Qupath trained with supervised machine learning.

Results: Compared to WT mice, HET and HOM mice tended to have milder disease from onset. Unlike conventional thresholding, pixel classification of GFAP and Iba1 coverage in GM and WM aligned with clinical disease severity across genotypes. Pixel classification also resulted in lower variability within control tissues and genotypes between EAE mice. Iba1 coverage in GM and WM and GFAP coverage in GM was elevated across all EAE tissues compared to healthy controls at thoracic-lumbar levels (TL). However, GFAP coverage in the WM was relatively consistent across all tissues, with only small increases at TL levels, those typically most disrupted in EAE. Across EAE samples, histopathology corresponded with clinical disease: compared to WT mice, we measured reduced GFAP GM coverage in HET ($p<0.0001$) and HOM mice ($p<0.0001$) and reduced Iba1 GM and WM coverage in HET only (GM $p=0.047$, WM $p=0.0195$) at TL levels.

Conclusions: A significant reduction in GFAP and Iba1 GM coverage was observed in HET and HOM mice, across multiple levels using pixel classification and its higher sensitivity. This approach offers better accuracy and regional specificity than conventional thresholding. Our findings underscore the potential of advanced software in characterizing the role of astrocytes and macrophages/microglia in Nr1h3 mutation-related MS pathology.

#68

POSTER PRESENTATION - GRADUATE



Yuchen Ding

Supervisior: Dr. David Huntsman

Title: Exploring the therapeutic effectiveness of EO3001 in the treatment of clear cell ovarian cancers with ARID1A mutations

AUTHOR(s)

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ABSTRACT

Introduction:
Clear Cell Ovarian Cancer (CCOC) is a chemoresistant ovarian cancer subtype, constituting 10% of North American and up to 30% of Japanese epithelial ovarian carcinoma (EOC) cases. Evidence suggests hematogenous spread is key in CCOC metastasis, but overlooked due to a lack of suitable models. ARID1A, a SWI/SNF complex subunit, mutated in ~ 65% of CCOC cases, is a key driver in CCOC progression. Loss of ARID1A induces reactive oxygen species (ROS) accumulation, making CCOC cells dependent on oxidative phosphorylation (OXPHOS) for energy. EO3001, a synthetic drug that selectively transports extracellular Cu(II) to mitochondria, induces mitochondrial ROS, thereby triggering cuproptosis. Recent studies have shown enhanced sensitivity of ARID1A-deficient cells to EO3001. In this study, we employed an ex vivo model, the Pulmonary Metastasis Assay (PuMA), to study EO3001's therapeutic effectiveness for ARID1A-deficient CCOC. PuMA provides a richer microenvironment than traditional 2D and 3D culture models.

Methods:
Fluorescent-labelled isogenic CCOC cell lines (RMG-1, OVCA429, and JHOC-5 +/- ARID1A) were generated using CRISPR-Cas9, and therapeutic effects of EO3001 were evaluated on the tumorigenic potential and cell growth of these cells in vitro and ex vivo using fluorescent microscope for signal intensities and colony size, cell viability, proliferation, total and mitochondrial ROS levels, OXPHOS activities, migration, and invasion assays under ambient and stress conditions. Both early-and late-harvest PuMA were employed to investigate the effects of EO3001 on cancer cells within the lung environment.

Results:
ARID1A-mutant CCOC cell lines show significant sensitivity to EO3001 in vitro, while displaying comparatively reduced sensitivity under hypoxia. Interestingly, in the PuMA model, both +/- ARID1A cells exhibited the same trend as observed in the in vitro assay. Moreover, late-harvested PuMA samples showed enhanced visualizability and required minimal optimization.

Conclusions:
While EO3001 showed promising therapeutic value in ARID1A-deficient cells, both in vitro and ex vivo models, hypoxia significantly diminishes the effects of EO3001, potentially attributed to alterations in glycolysis and OXPHOS. By exploiting the dependence of ARID1A-deficient CCOC on OXPHOS, the use of EO3001 could offer a viable therapy approach. While EO3001 showed promising therapeutic value in ARID1A-deficient cells, both in vitro and ex vivo models, PuMA provides a robust ex vivo for research. While early-harvesting PuMA provides a comprehensive understanding, late-harvesting PuMA, characterized by its enhanced visualizability, ease of quantification, minimal optimization requirements, and broader applicability, stands out as a versatile and efficient choice across diverse research domains.

#69

POSTER PRESENTATION - GRADUATE

Yuqin Li

Supervisior: Dr. David Huntsman

Title: cystathionine gamma-lyase as a therapeutic target for clear cell ovarian cancer

AUTHOR(s)

Yuqin Li1,2, Amal El-Naggar1,2, Yuchen Ding1,2, Grace Wei1,2, Artem Cherkasov1,3, Jason Smith3, David Huntsman1,2

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ABSTRACT

Background/objectives: Clear cell ovarian cancer (CCOC) accounts for 5-11% of ovarian cancers in North America. Previous research has identified cystathionine gamma-lyase (CTH) as a biomarker overexpressed only in CCOC but not other subtypes, and its critical role in tumor invasiveness and metastatic ability. The preliminary mouse model using CTH knockout (KO) CCOC cell line showed a significant reduction in CCOC metastatic ability, yet higher proliferative potential and apoptosis. Further, upon treatment with cisplatin, CTH KO cells showed remarkably reduced cell viability, suggesting inhibition of CTH may sensitize cells for chemotherapy. This study aims to identify potent CTH inhibitors, and we hypothesize that CTH inhibitor treatment combined with chemotherapy, radiotherapy, or antiangiogenics presents a potential therapeutic strategy for CCOC. Commercially available CTH inhibitors have off-target effects as they target the PLP cofactor. Due to the lack of clinically applicable CTH inhibitors, our group used a computer-based screen with the ZINC22 database of 4 billion commercial compounds and found 233 compounds that may bind to the active site of CTH. This study focuses on the inhibitors that increase proliferation to compare with the CTH KO effect.

Methods: The change of morphology and proliferation of CCOC cell culture will be assessed by the IncuCyte live-cell analysis system for the 233 CTH inhibitors using CCOC cell lines. The difference in growth rate will be measured using the Hill slope coefficient by GraphPad Prism, and significance will be assessed by student t-test with the Hill slope coefficient of commercially available CTH inhibitor aviglycine hydrochloride. The most promising compounds across various cell lines will be considered for subsequent in vitro validations such as RNA sequencing and other cell-based assays. The therapeutic potential for targeting CTH lead compounds, either alone or in treatment combinations, will be investigated using cell lines and the ex-vivo pulmonary metastatic assay (PuMA), with results compared to genetic manipulation.

Results: The first round of screening has been done with 233 compounds. The compounds selectively induced greater growth rates in CTH-containing cell lines relative to aviglycine hydrochloride are selected for further validation. The compounds that also induced growth rates in CTH KO cells were excluded from this initial screening. In total 19 compounds that induced greater growth rates were selected. Two compounds also showed a significant selective reduction in growth rate in CTH-containing cells, so were also selected for further validation.

Conclusions: At the advanced stages, CCOC is resistant to chemotherapy and has the worst outcome compared to other subtypes of epithelial ovarian cancer. The lack of molecular mechanisms for tumor recurrence and metastasis presents a major barrier to the development of effective treatment strategies. In this current study, there are 21 compounds out of the 233 compounds screened that showed significant selective effects on CCOC cell lines, and these compounds may present novel therapeutic approaches for people with CCOC.

#70

POSTER PRESENTATION - GRADUATE

Zhihan Wang

Supervisior: Dr. Honglin, Luo

Title: Unraveling the Genetic Factors Influencing Susceptibility and Resistance to Viral Myocarditis

AUTHOR(s)

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3 Department of Anesthesiology, Pharmacology and Therapeutics, The University of British Columbia, Vancouver, BC

ABSTRACT

Background/objectives:
Myocarditis is an inflammatory disease of the heart, usually caused by viral infections, with enteroviruses being among the most prevalent etiological agents associated with this disease. Between 5-15% of patients with viral infections may develop myocarditis, with 10-20% progressing to dilated cardiomyopathy. Notably, the pathogenesis of viral myocarditis shows a strong sex difference, with the most severe symptoms occurring in males. I have recently conducted a genetic analysis on the GSE35182 Gene Expression Omnibus (GEO) dataset, comparing gene expression in enteroviral myocarditis between male and female mice, by which an exciting novel candidate named circadian associated repressor of transcription (CIART) was identified as a potential contributing factor to the observed sex difference. CIART exhibits unique upregulation in males compared to females and plays a key role in the transcriptional repression of genes involved in regulating circadian rhythms. However, the role of CIART in viral myocarditis has not been explored. This study aims to investigate the role and regulation of CIART in viral myocarditis and assess the therapeutic potential of targeting CIART in its treatment. The hypothesis is that increased CIART expression in males contributes to the pathogenesis of viral myocarditis.

Methods:
We analyzed differential gene expression in 3 normal and 3 CVB3-infected male and female mice at 10 days post-infection from GSE35182 using the “limma” R package. CIART showed the most significant sex-based expression difference between pre- and post-infection. To further test CIART’s function, we will examine its expression in male and female mice with Coxsackievirus B3 (CVB3)-induced myocarditis using CRISPR/Cas9 gene editing and immunohistochemistry, aiming to link CIART levels with disease severity. The research will explore molecular pathways affected by CIART through transcriptomic and proteomic analyses and validate its regulatory role via in vitro models and genetic engineering. Finally, we'll test CIART modulation's therapeutic potential in a cardiac-specific CIART-deficient mouse model, assessing its impact on myocardial inflammation, immune response, and cardiac function.

Results:
Our research uncovered a notable difference in CIART expression between males and females following Coxsackievirus B3 (CVB3) infection. Specifically, we observed an upregulation of CIART expression in males (n=3), contrasted with a downregulation in females (n=3). We also identified hub genes, including Laptm5, Cfp, and Myo1g, in CVB3 infection using online protein-protein interaction network tools.

Conclusions:
These initial findings provide a valuable foundation for further exploration into the sex-based differences in susceptibility to CVB3-induced myocarditis. Identifying CIART as a therapeutic target could offer personalized treatment for viral myocarditis, especially in males, and may revolutionize the management of this disease.

#71

POSTER PRESENTATION - RESIDENT

Kevin Shopsowitz

Supervisior: Dr. Sakara Hutspardol

Title: Global hemostatic monitoring with ROTEM for managing coagulopathy in ECMO patients: a retrospective study.

AUTHOR(s)

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ABSTRACT

Background/objectives: Critically ill patients treated with extracorporeal membrane oxygenation (ECMO) are at increased risk of bleeding due to anticoagulant exposure and other factors. Unlike conventional coagulation tests (CCTs), global hemostatic assays like ROTEM are generally unaffected by the unfractionated heparin (UFH) typically used during ECMO. We compared commonly used ROTEM parameters with conventional coagulation tests (CCTs) and assessed ROTEM's utility in guiding transfusion management in bleeding patients on ECMO.

Methods: All patients treated with ECMO were included from Apr 1, 2020, to Sep 30, 2022, at the Intensive Care Unit at Vancouver General. Anticoagulation was managed using an institutional protocol. Data were collected retrospectively on patient demographics, ECMO type, anticoagulants, bleeding events, ROTEM parameters (EXTEM A10, EXTEM CT, and FIBTEM A10), CCTs (APTT, INR, Clauss fibrinogen), and platelet count. Institutional ROTEM cut-offs were used to define coagulopathy and guide transfusions. For CCTs, Clauss Fibrinogen level < 1.5 g/L was used to determine fibrinogen deficiency. For categorical variables, p-values were derived from the chi-square test or McNemar test. For continuous variables, between-group differences were analyzed using an independent-sample t-test.

Results: Eighty-eight of 132 patients (67%) treated with ECMO during the study period had both ROTEM and CCTs performed while experiencing WHO grade > 2 bleeding. Of these, 93% received UFH. Prolonged APTT and low fibrinogen were identified in 71 (81%) and 34 patients (39%), respectively. Sixty-six patients (75%) had ROTEM abnormalities, of which low FIBTEM A10 was the most common (n = 53, 60%), followed by low EXTEM A10 (n = 50, 57%), and prolonged EXTEM CT (n = 39, 44%). There was discordance between the proportion of patients with low fibrinogen identified by FIBTEM A10 and the Clauss assay (21/88 discordant, $p < 0.001$). Of the discordant cases, 20/21 had low FIBTEM A10 with normal Clauss fibrinogen while only one case had low Clauss fibrinogen with normal FIBTEM A10. Patients with any of the ROTEM-based transfusion triggers more frequently received plasma and fibrinogen concentrate (FC) compared to those without [41/62 (66%) vs 7/26 (27%) and 52/62 (84%) vs 5/26 (19%), all p-values < 0.001]. Patients with normal Clauss fibrinogen but low FIBTEM A10 received FC more frequently than patients with normal FIBTEM A10/Clauss fibrinogen [18/20 (90%) vs 8/34 (24%), $p < 0.001$]. The median FC in grams, plasma, and red blood cell units transfused in patients with abnormal ROTEM were significantly higher than those without [15 vs 2, 9 vs 1, and 20 vs 5, all p-values < 0.001].

Conclusions: Abnormal ROTEM in patients bleeding while on ECMO was frequently observed and was associated with higher consumption of blood components and derivatives. ROTEM-based FIBTEM A10 was more sensitive in detecting hypofibrinogenemia than the Clauss method, which led to additional fibrinogen transfusions. Since ROTEM is more expensive and not widely available, prospective studies are required to assess its additional benefits to evaluate coagulopathy in the ECMO setting.

#72

POSTER PRESENTATION - PDF

Anne-Sophie Archambault

Supervisior: Dr. Ramon Klein Geltink

Title: Purine catabolism is important in the secretion of IL-1beta in macrophages

AUTHOR(s)

Anne-Sophie Archambault*1,2, Lauar de Brito Monteiro*1,3, Lucas F. Starchuk1,2, Yvonne Pang1,4, Armando Alcazar5, Joshua Dubland1,2,6, Bojana Rakic1,2,6, Annette E. Patterson1,2, Juhee Oh1,2, Emilia Perraso1,2, Susan Menzies1,4, Laura Sly1,4, C. Bruce Verchere1,3, Ramon I. Klein Geltink1,2,7.

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ABSTRACT

Background/objectives: Activation of pro-inflammatory macrophages (M(LPS+g)) leads to the production of pro-inflammatory cytokines, such as IL-1beta, TNFa and IL-6. These cytokines are important in the control of infections, but overproduction can be detrimental and lead to chronic inflammation. To exert their functions, M(LPS+g) macrophages undergo metabolic remodeling, including the upregulation of the pentose phosphate pathway and purine metabolism. Using inhibitors of purine catabolism (small molecule Febuxostat or purine analog Allopurinol), we aimed to identify the role of purine metabolism in M(LPS+g) macrophage polarization and function.

Methods: Mouse bone-marrow-derived macrophages (BMDMs) or human monocyte-derived macrophages (MDMs) were generated over 7 days with L929-conditioned media, or with human M-CSF, respectively. Macrophages were then pre-treated for 30 minutes with purine metabolism inhibitors before adding LPS and IFNgamma

Results: Blocking purine catabolism did not affect the expression of CD80, CD86 or iNOS in M(LPS+g), nor the accumulation of the M(LPS+g) hallmark metabolites itaconate and succinate. However, inhibition of purine degradation with Febuxostat significantly reduced IL-1b secretion. Mature IL-1b release is dependent on both transcriptional activation of the pro-IL1 gene and the NLRP3/Caspase-1 pathway. Blockade of purine degradation with Febuxostat led to sustained pro-IL1 transcription, an accumulation of pro-IL1b and significantly reduced caspase-1 activity. When purine synthesis was inhibited with Allopurinol, we observed reduced IL-1b, TNFa and IL-6 transcription and secretion.

Conclusions: In summary, purine metabolism is dispensable for the phenotype of M(LPS+g) macrophages, but it regulates cytokine production. Targeting specific nodes in purine metabolism in macrophages provides opportunities for immunomodulation in inflammatory diseases affected by pro-inflammatory macrophage-linked cytokines.

#73

POSTER PRESENTATION - PDF

Guangze Zhao

Supervisior: Dr. Decheng Yang

Title: CVB3-induced m6A modification of RNA enhances viral replication via suppression of YTHDF-mediated stress granule formation

AUTHOR(s)

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ABSTRACT

Background/objectives: N6-Methyladenosine (m6A) is the most abundant internal mRNA modification. It is regulated by methyltransferases (called writer) and demethylases (called eraser). After modification, the m6A sites are recognized by m6A-binding proteins YTHDF1-3, YTHDC1-2 (called reader) through its YTH domain. These reader proteins play critical roles in a variety of biological processes. Specifically, YTHDF1 promotes cap-independent translation of mRNA lacking a 5'm7G cap structure, YTHDF2 increases RNA decay and YTHDF3 regulates both processes.
Stress granules (SGs) are nonmembranous granular aggregates formed in cytoplasm of eukaryotic cells exposed to a variety of environmental stress conditions. They are the storage place for mRNA-protein complex consisting of m6A-modified mRNAs and proteins/enzymes involved in translation.
Coxsackievirus B3 (CVB3), a member of enteroviruses, is a common pathogen of viral myocarditis, particularly in children and adolescents. Its viral genome translation is initiated by a cap-independent, internal ribosome entry site (IRES)-dependent mechanism, requiring IRES trans activating factors (ITAF). It is well documented that ITAFs, such as HuR, PTB, PCBPs, etc., are key actors of stress response. In addition, enteroviral infection induces SG formation at early phase of infection but suppresses it at later time points, which raises several fundamental questions: what is the mechanism of phase-dependent action of SG formation on controlling CVB3 replication? What is the overall effect of m6A modification and SG production on CVB3 replication? This study aims to address these questions.

Methods: The m6A sites on viral RNA was determined by MeRIP-RT-qPCR using 7 pairs of virus-specific primers. SG was determined by immunostaining. Western blot was used to determine YTHDF reader protein cleavage. RNA-protein co-immunoprecipitation was conducted for detecting interaction of CVB3 RNA and HuR protein. Viral replication was determined by western blot to measure CVB3 VP1 protein and by plaque assay to measure viral particles.

Results: m6A modification in CVB3-infected HeLa cells, HL-1 cardiomyocytes and mouse heart. m6A modifications are mainly located at the 3' untranslated region (3'UTR), stop codon region and the 5'UTR. These modifications enhanced CVB3 replication. Further we found that the suppression of antiviral SG formation plays a pivotal role, which is linked to the cleavage of the three m6A reader proteins YTHDF1-3 during CVB3 infection. By immunofluorescent staining, we found for the first time that CVB3 RNA is stored in SG and co-localized with HuR, an ITAF protein, at early stage of infection but released to cytosol at late stage when SG assembly is inhibited. These observation on altered cytosolic localization of SG components (CVB3 RNA and HuR) directly parallel to the increased viral VP1 protein synthesis and viral particle formation.

Conclusions: CVB3-induced m6A modification of RNA enhances viral replication via suppression of YTHDF-mediated stress granule formation.

#74

POSTER PRESENTATION - PDF

Zhenwei Ma

Supervisior: Dr. Zu-hua Gao

Title: Multifunctional hydrogel bandage device for head and neck cancer management

AUTHOR(s)

Zhenwei Ma, Wena Shi, Hui Xue, Tanwei Destin Du, Victor Ling, Yuzhuo Wang, Zu-hua Gao

AFFILIATION(s)

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Department of Integrative Oncology, BC Cancer Research Institute

ABSTRACT

Background/objectives: Head and neck cancer are lethal diseases of high mortality and morbidity. Incomplete tumor resection and progression of precursors at margins are the major underlying causes. Moreover, tumor resections are often associated with significant complications, such as infection and scarring. We aim to develop a multifunctional hydrogel bandage (MHB) device to (1) accelerating wound healing, and preventing infection and scarring; (2) deliver targeted therapeutics to increase efficacy and reduce systemic toxicity and recurrence; and (3) prevent cancer development by eradicating precancerous lesions. The versatile device design could be adapted to treat all mucosal tumors, such as those in the gastrointestinal tract, cervix, and urinary bladder.

Methods: The MHB device is fabricated with a polyacrylamide/alginate interpenetrating hydrogel with chitosan solution as the bioadhesive layer. Freshly excised porcine skin, buccal mucosa and rat tumor were used as model tissues. The adhesion between the MHB and the tissues were measured using a modified lap shear test. Doxorubicin (0.5-5 mg/ml) was either directly loaded into the hydrogel precursor solution or loaded with nanoclay of various concentrations (0.5-6% w/w). The Doxorubicin release from the MHB device was characterized using a plate reader. The cancer-killing capability of the released therapeutic from the MHB device was characterized in a 2D monolayer and a 3D spheroid model using MBA-MB-231 breast cancer cell line and OI33 esophageal adenocarcinoma cell line. The cell viability was characterized using MTT assay and Live/Dead assay.

Results: We developed a family of MHB device with excellent tunable biomechanical and biochemical properties. We demonstrated the sustained release of doxorubicin for 1 week. By further engineering the delivery vehicle with clay nanoparticles, we showed that we can fine-tune the drug release profile of Doxorubicin with various loading capacity, release rate and duration (from days to months). The released drug was not altered during the drug encapsulation and delivery and retain their cancer-killing capabilities. We further discovered that the co-delivery of penetration enhancer (e.g. sodium dodecyl sulfate) and Doxorubicin demonstrated enhanced drug penetration in tissues in an ex vivo model and showed markedly increased anti-cancer efficacy in an in vitro 3d tumor model.

Conclusions: We report a novel medical device that can robustly adhere to various tissues, deliver therapeutics locally with high tissue penetration depth, and eliminate cancer cells with high efficacy. Thus, this new technology holds great promise to manage all mucosal cancers.

#75

POSTER PRESENTATION - STAFF

Ingrid Elisia

Supervisior: Dr. Gerald Krystal

Title: a low carbohydrate diet high in soy protein and fish oil reduces azoxymethane/dextran sodium sulfate-induced colorectal cancer in balb/c mice: role of metabolism, inflammation and the microbiome

AUTHOR(s)

Ingrid Elisia1, Sara Kowalski1, Michelle Yeung1, Gerald Krystal1

AFFILIATION(s)

1Terry Fox Laboratory, BC Cancer Research Center

ABSTRACT

Background/objectives:
Since we established that a low carbohydrate diet containing soy protein and fish oil (15%Amylose/Soy/FO) was effective in preventing nicotine-derived carcinogen-induced lung cancer in A/J mice when compared to a Western diet we asked if this diet might also be effective in preventing colon cancer.

Methods:
To test this we fed Balb/C mice with either the Western or the 15%Amylose/Soy/FO diet and treated them with azoxymethane/dextran sodium sulfate (AOM/DSS) to induce colorectal cancer. We measured tumour numbers in the colon as well as levels of pro-inflammatory cytokines/chemokines in the colon by Mesoscale and Luminex analyses. To evaluate changes in the gut microbiome, 16S sequencing was performed on the feces of these mice.

Results:
The mice that consumed the 15%Amylose/Soy/FO had significantly ($P<0.05$) lower blood glucose and higher plasma β-hydroxybutyrate, indicating an increased use of fats as an energy source rather than glucose. Along with this shift in metabolism, we observed a significant ($P<0.05$) reduction in tumour nodules and burden in mice that consumed the 15%Amylose/Soy/FO diet. There was also a significant reduction in several pro-inflammatory cytokines/chemokines in the colon, including IL-1β, TNF-α, KC/GRO and IL-17, indicating that the 15%Amylose/Soy/FO likely prevented colon cancer, at least in part, by reducing AOM/DSS-induced colitis. This was confirmed by the significantly ($P<0.05$) lower disease activity index in the mice fed the 15%Amylose/Soy/FO diet. We also observed a significant change in the fecal microbiome composition, with the genus Akkermansia being one of the genera most expanded in the 15%Amylose/Soy/FO diet-fed mice.

Conclusions:
Since Akkermansia muciniphila, a well-known species of the genus, has been reported to reduce intestinal permeability that could prevent the translocation of LPS-containing gut bacteria, we suggest that the 15%Amylose/Soy/FO diet potentially reduces AOM/DSS induced colitis by promoting the blooming of Akkermansia muciniphila and this in turn reduces chronic inflammation and the development of colorectal cancer.

#76

POSTER PRESENTATION - STAFF

Ingrid Elisia

Supervisior: Dr. Gerald Krystal

Title: Fish oil enhances the efficacy of a ketogenic diet in lowering tobacco carcinogen-induced lung cancer

AUTHOR(s)

Ingrid Elisia1, Michelle Yeung1, Sara Kowalski1, Jason Tee1, Gerald Krystal1

AFFILIATION(s)

1Terry Fox Laboratory, BC Cancer Research Center

ABSTRACT

Background/objectives:
Since cancer cells typically take up and need more glucose than normal cells, we hypothesized that a ketogenic diet, which is very low in carbohydrates, might slow tumor growth. However, since ketogenic diets are very high in fat, and different fats might have different biological effects, we investigated whether fat types influenced the efficacy of a ketogenic diet to lower tobacco carcinogen-induced lung cancer in mice.

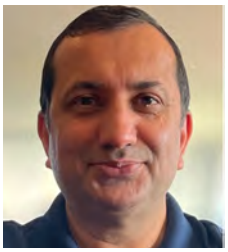
Methods:
We placed 12-week-old A/J mice on a Western diet, or ketogenic diet comprised of either palm oil, olive oil, corn oil, milk fat, fish oil, medium chain triglyceride oil, or a blend of fats typically found in a Western diet. We then intraperitoneally injected the tobacco-specific carcinogen, nicotine-derived nitrosamine ketone (NNK) into these mice twice, one week apart, to initiate lung tumor formation.

Results:
Lung nodules in these mice were then counted five months later. Mice that were fed the ketogenic diets had significantly ($P< 0.05$) lower lung nodule numbers compared to the Western diet, suggesting that glucose restriction is indeed a feasible strategy to slow tumor formation. Comparing the different ketogenic diets, we found the ketogenic diet containing fish oil was significantly ($P< 0.05$) more effective than the rest of the ketogenic diets in reducing lung tumor formation. This increased efficacy was associated with higher plasma ß-hydroxybutyrate, lower blood glucose, and liver fatty acid expression. Taken together, fish oil appears to be unique in its ability to promote ketosis in mice fed a ketogenic diet.

Conclusions:
We conclude that not all fats are created equal in the context of a ketogenic diet. Incorporation of fish oil, which is high in the omega 3 fatty acids, EPA, and DHA, can increase the efficacy of a ketogenic diet in preventing the formation of NNK-induced lung tumors.

#77

POSTER PRESENTATION - STAFF



Jaswinder Khattra

Supervisior: Dr. Shazia Masud

Title: Evaluation of TB Xpert® MTB/Rif Ultra assay on formalin-fixed paraffin-embedded tissues for Mycobacterium tuberculosis diagnosis

AUTHOR(s)

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ABSTRACT

Background/objectives:
GeneXpert MTB/Rif Ultra assay (Cepheid, Sunnyvale, CA, USA) is an approved real-time PCR assay for detecting Mycobacterium tuberculosis (MTB) in sputum specimens. We evaluated the GeneXpert MTB/Rif Ultra assay performance for MTB detection in formalin-fixed paraffin-embedded tissue (FFPET) compared to mycobacterial culture or reference laboratory laboratory-developed MTB PCR test (LDT).

Methods:
Archived FFPET samples with histological features suggestive of tuberculosis (i.e., granulomatous inflammation, with or without acid-fast bacilli) from 2018 to 2023 were selected. Depending on the size of the tissue, 2 to 4 scrolls at 20 microns thickness each were obtained. 500 microlitres of ATL Buffer (Qiagen GMBH, Hilden Germany) was added to the FFPET scroll and incubated for 5-minutes at 75 degrees Celsius to melt paraffin. After centrifugation, 50 microlitres of proteinase K 600 mAU/mL (Qiagen GmbH) was added. The FFPETs were then incubated overnight at 56 degrees Celsius and sample aliquots were processed according to manufacturer's instructions. MTB culture or reference laboratory MTB LDT were used as comparator for sensitivity and specificity calculations.

Results:
Of 51 eligible FFPET samples, 32 were positive for MTB either by culture of concurrently collected fresh tissue specimen or reference laboratory MTB LDT on FFPET. GeneXpert MTB/Rif Ultra detected MTB in 23/32 positive specimens (71.9 percent). Of the 9 discordant specimens, 7 had positive MTB culture from an accompanying specimen and 2 specimens were not submitted for culture but MTB was detected by reference laboratory LDT MTB PCR.

Of the 19 negative samples (predominantly negative by reference laboratory LDT), 5 had concurrent negative MTB culture (16 had reference lab LDT PCR) and all tested negative by Xpert MTB/Rif Ultra assay, for 100 percent specificity.

Conclusions:
Xpert MTB/Rif Ultra assay demonstrated 71.9 percent sensitivity relative to culture and/or reference laboratory MTB LDT. Sampling variability, exhaustion of the tissue block, degradation of DNA during storage and/or formalin fixation, and extraction variability may explain the false negative results. Specificity was found to be 100 percent. Clinical implementation in a hospital laboratory is promising given its significant improvement in turnaround time compared to existing culture and reference laboratory LDT PCR methods, and ability to detect MTB in cases where no tissue sample is available for MTB culture.

Author Index - Abstract Page

WIP- Coming Soon