BIG Research Day

2024

Event Booklet

BIG24

BIOINFORMATICS★INTERDISCIPLINARY ONCOLOGY★GENOME SCIENCE AND TECHNOLOGY

Table of Contents

About

The BIG Research day is an annual tradition of the Bioinformatics, Interdisciplinary Oncology and Genome Science + Technology graduate programs at the University of British Columbia and Simon Fraser University. Over 100 graduate students from all three programs gather to present their research projects and attend seminars and workshops by academic and industry professionals. Every year the BIG Research Day explores different research areas. Our theme for this year is:

“Novel Techniques And Limitations To Modern Methods”

About

Program Directors

Bioinformatics (BIOF)

Dr. Steven Jones
Director
UBC

Dr. Paul Pavlidis
Associate Director
UBC

Dr. Fiona Brinkman
Associate Director
UBC

Interdisciplinary Oncology (IOP/IOGS)

Dr. Wan Lam
Co-Director (IOP)
UBC

Dr. Kevin Bennewith
Co-Director (IOP)
UBC

Dr. Sharon Gorski
Director (IOGS)
SFU

Genome Science and Technology (GSAT)

Dr. Peter Stirling
Co-Director
UBC

Dr. Martin Hirst
Co-Director
UBC

Sharon Ruschkowski
B.I.G. Program Coordinator
UBC

Meet the Team

BIG24 Team Leaders


Manideep Pachva


Annalena Renner


Rachel Hausman


Ana Shahidi


Ali Mirabadi


Jiyoung Han


Andy Jia


Rituparna Banerjee


Eric Brace

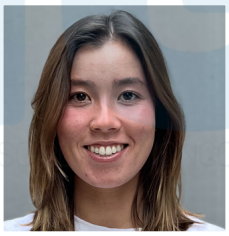

Sarthak Garg


Sara Singh

BIG24 Organizing Committee


Ali Balapour

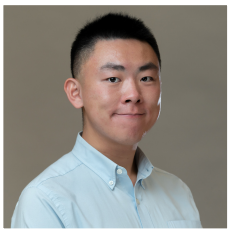

César Ávila


Nicole Howes

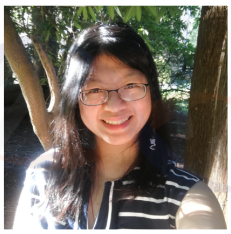

Ian Janzen


Itzel Astiazarán


Jessica Trejo


Joe Huang

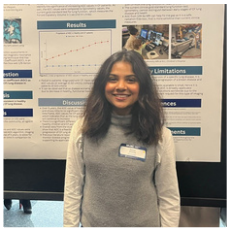

Justin Long


Serena Chuang


Madison Chapel


Maedeh Mirzazadeh


Makoto Kishida

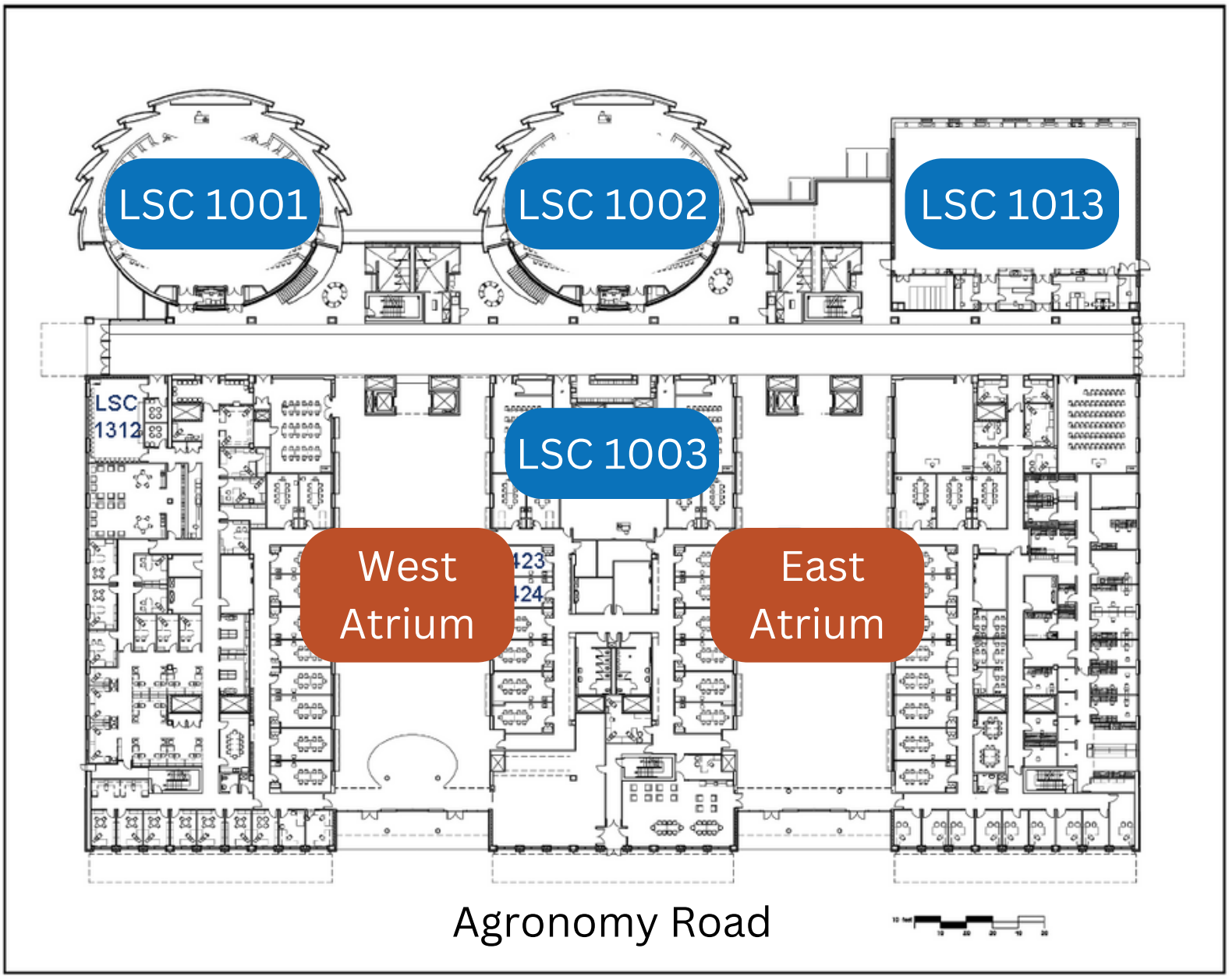

Namya Sharma

Schedule

Time	Event	Location
8:00-8:50	Registration + Breakfast	East Atrium
8:50-9:00	Musical Performance + Opening Remarks	LSC 1001
9:00-10:00	Keynote	LSC 1001
10:00-10:30	Coffee Break	East Atrium
10:30-11:15	Alumni Talks	LSC 1001
11:15-12:15	Poster Session #1 (Evens)	West Atrium
12:15-1:15	Lunch and Sponsor booths	East Atrium
1:15-2:15	Poster Session #2 (Odds)	West Atrium
2:15-2:30	Coffee Break	East Atrium
2:30-3:45	Concurrent sessions	LSC 1001, 1002, 1013
3:45-4:00	Coffee Break	West Atrium
4:00-4:45	Student speed talks	LSC 1001, 1002, 1003
4:45-5:30	Student Workshops	LSC 1001
5:30-6:00	Closing Remarks + Awards	LSC 1001
6:00-9:00	Social Mixer	West Atrium

Conference Map

Life Sciences Institute, UBC



Registration will take place at the East Atrium by the Agronomy Road entrance

Registration: 8:00 AM - 9:00 AM

Keynote

LSC 1001

Dr Daniel De Carvalho
Co-founder and Chief Scientific Officer at Adela & Professor at
Princess Margaret Cancer Centre/University of Toronto

Cancer Early Detection, Classification, and Monitoring through Plasma Cell-Free DNA Methylomes

Dr. De Carvalho is a renowned expert in cancer epigenomics and leads a research group at the Princess Margaret Cancer Centre and University of Toronto. He is a pioneer in the use of cancer epigenetics combined with advanced computational approaches for developing novel liquid biopsy tools applied to cancer early detection, classification and monitoring therapy response.
Dr. De Carvalho has published over 85 high profile research papers, many of those featured in prestigious scientific journals such as Nature, Science, Cell, Nature Medicine, Cancer Cell among others and was an invited speaker to over 160 talks worldwide, including such prestigious venues as the Opening plenary lecture at AACR annual meeting. For his scientific contributions, Dr. De Carvalho received numerous awards including the AACR-Waun Ki Hong Award, Canadian Cancer Society Bernard and Francine Dorval Prize; Canadian Institutes of Health Research (CIHR) Early Career Award in Cancer. He received the Canada Research Chair in Cancer Epigenetics, the Helen M Cooke Endowed Professorship and was elected membership in the 2019 cohort of The Royal Society of Canada. Dr. De Carvalho founded and currently serves as CSO of Adela, a biotech company developing cell-free DNA methylation liquid biopsy technology for cancer early detection, classification and monitoring therapy response.

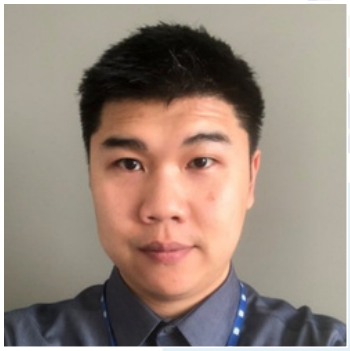
Alumni Talks

10:30-11:15AM
LSC 1001

Dr. Emilia Lim
Assistant Professor,
Department of Biochemistry & Molecular Biology, UBC

Tangled Arrows: Navigating the Complicated PhD path

A PhD is a wonderful time to learn and grow professionally and personally. Everyone's path through their PhD is distinct, but there is one common thread: it is never uncomplicated. Sometimes, the best opportunities are cloaked in adversity. As such, it is important to hear from many different perspectives, and surround yourself with a positive environment that will encourage you in this endeavour. In this session, I will share how I have navigated through the ups and downs of my educational journey. It is but one perspective, but I hope it will serve as an inspiration to all.



Dr. Joseph Lau
Assistant Professor,
Department of Radiology, UBC

Crossroad? Research in Academia and Industry

Navigating graduate studies is undoubtedly challenging, yet it is crucial to recognize that completing your degree also signifies the start of a new chapter. For some, it entails continuing research in academia, while for others; it involves pursuing a career in industry. In this session, we will look at the differences, goals, and motivations inherent in conducting research in academia versus industry. We will discuss transferable skills and attributes that enable success in both settings. Through this analysis, we aim to offer insights for researchers grappling with the decision between academia and industry, while fostering a deeper understanding of the dynamic interplay.

Dr. Zeid Hamadeh
Scientist at Vancouver General Hospital, Cytogenomics Laboratory, Clinical Instructor at UBC, Department of Pathology and Laboratory Medicine

When will my work "translate" into healthcare?

With translational research, it often feels like "translating" into healthcare can be slow or even uncertain. In this talk, I explore the necessary challenges of bringing research from the academic PhD realm to the clinic through key examples that highlight common hurdles, while also discussing how key breakthroughs can happen with the diverse skill set acquired during a PhD. This talk can hopefully pave the way for those looking to bridge the gap between academia and healthcare.

Concurrent Session 1

Novel techniques and limitations to modern methods

2:30-3:45PM

LSC 1001

Dr. Amina Zoubeidi
Senior Research Scientist,
Vancouver Prostate Centre

Unraveling the Phenomenon of Lineage Plasticity in Prostate Cancer: Insights and Implications

At the BIG24, Dr Zoubeidi will be discussing why is treatment-induced lineage reprogramming biased toward particular lineage? How the change in the chromatin architecture can permit an opportune environment for transcription to be “reprogrammed” and, in turn, facilitate tumour cell plasticity and therapy resistance? Does this inherent epigenetic plasticity can be therapeutically re-direct cell fate using epigenetic or transcription factor inhibitors?

The Future of T Cell Engineering

Over the past several decades methods to genetically modify T cells in order to improve their cancer-killing capabilities have been developed. These methods have led to dramatically improved clinical outcomes for relapsed and treatment-refractory leukemia and lymphoma patients, and have the potential to revolutionize therapy for other cancers, autoimmune disorders, and many other conditions. However, current T cell engineering approaches are hampered by the need for ex vivo manufacturing, testing and reinfusion of personalized T cell products. We and others are now exploring alternative approaches where gene delivery to T cells is accomplished in vivo, as gene therapy, rather than ex vivo as cell therapy. Challenges and opportunities associated with this new direction will be discussed.

Dr. Robert Holt
Co-director, BC Cancer
Immunotherapy Program

Concurrent Session 2

Clinical Translations and Patient Experience

2:30-3:45PM

LSC 1002

Dr. Stephen Yip
Clinician-Scientist/Consultant
Neuropathologist, BCCA

Cancer Diagnostics in the Era of Molecular Advances – The long and short of it

Aberrant alterations of the genome/epigenome are the basis of vast majority of cancers. The identification of these molecular biomarkers is critical in this era of precision oncology to improve diagnosis and the identification of treatment targets. I will review these changes and discuss recent implementation of advanced molecular technologies into the clinical realm. Importantly, I will discuss exciting research projects that strive to integrate epidemiology, clinical and genomic features, and deep learning to advance molecular diagnosis.

Challenges and Rewards of Clinical Research

While basic scientific advances ultimately underlie most medical progress, clinical research is a key component in bringing discoveries into practice. Specifically, clinical trials are generally the only way to know if a new treatment will be safe and effective, and are the standard on which regulatory agencies rely when they make decisions about licensing new products and determining their medical indications. For clinical trial results to be valid, strict guidelines must be followed, to avoid pitfalls that can lead to type I or Type 2 error, or in some cases completely invalidate a study. Moreover, international standards require that clinical trials be done within an ethical framework, to avoid exposing study participants to unnecessary risk and to preserve their autonomy and privacy. Sometimes it requires a complex dance to balance the responsibilities to participants, investigators, sponsors, and the global medical community to make a clinical trial successful. In this talk, I will discuss some of the key features of good clinical practice in clinical trials, and some of the challenges that researchers face.

Dr. Theodore Steiner
Medical Director,
Clinical Research Unit

Concurrent Session 3

Professional Development

2:30-3:45PM

LSC 1013

Dr. Christine Genge

Associate Director, Global Talent Acquisition at STEMCELL Technologies

Career Paths for Science Grads

Wondering what to do with your science degree? Christine will share personal stories of transitioning from academic research into the biotechnology industry after a PhD, as well providing insight into industry opportunities and career paths. This session will also provide tips and advice from a recruiter perspective on securing that next career position.Christine Genge is the Director, Global Talent Acquisition at STEMCELL Technologies, a privately-owned biotechnology company based in Vancouver that helps power leading-edge life science research around the world. Christine first joined STEMCELL Technologies as a Scientific Inside Sales Representative after completing her PhD in molecular cardiac physiology at SFU in 2016. Having transitioned from an academic research background into industry, she is passionate about helping STEMCELL grow through recruiting talented individuals committed to moving science forward.

Designing your Grad School/Career Strategy

Graduate students today have a plethora of academic and professional obligations as well as opportunities they may engage in during their graduate journey. There are also many ways to define success both within and after graduate school. How do you choose which opportunities to take advantage of? Are you setting yourself up to succeed in your intended career path after graduate school? Do you even know that path?
Join this interactive workshop to take the lead of your education and hone your career intentions! The session will help you to:

- Develop or enhance your academic plan for completing your graduate degree,
- Explore possibilities and design your career path,
- Build a road map for navigating the many opportunities available, and
- Establish strategies for attaining your professional objectives.

Jacqui Brinkman

Director, Graduate Student Professional Development

Workshops

4:45-5:30PM
LSC 1001

Student Workshop

Cameron Herberts
Genome Science and Technology PhD Candidate, UBC

A Roadmap for Navigating Paper Writing, Peer Review, and Publication as a Graduate Student in Science

Publishing papers drives scientific knowledge dissemination and is the currency of conventional academic achievement. For graduate students, embarking on this process can be challenging as we are seldom exposed to manuscript writing and publishing during routine academic coursework. This presentation aims to demystify the process of paper writing and provide actionable insights on the following topics: when to initiate writing your first paper, individualistic versus collaborative paper writing, writing style and clarity, how to improve your writing skills (and why this is important), what to expect during journal submission, strategies for navigating Peer Review, and the role of ChatGPT for writing assistance.

Student Workshop

Optics and You - Illuminating the Inner Workings of Microscopy

Microscopy plays a critical role in visualizing and assessing samples for biomedical research, however it can be challenging to troubleshoot a suboptimal image. In this interactive workshop, we will explore the principles of optics with a focus on the mechanisms of microscopy and fluorescence. Building on this foundation, we will look at examples of various lighting techniques, polarization, common optical aberrations, and methods to ensure uniform sample illumination.

Eric Brace
Engineering Science PhD Candidate, IOGS, SFU

Student Speed Talks

LSC 1001

Hans Ghezzi - PUPpy: a fully automated primer design pipeline for substrain-level microbial detection and absolute quantification

Erick Navarro-Delgado - Modeling the genome and exposome contribution to newborn DNA methylome variability with the RAMEN package

Rachel Hausman - Overcoming chemotherapeutic resistance by targeting ferroptosis in cancer cells surviving apoptotic caspase release

Lisa Zhan - Unlocking the potential of CAR-T cell therapy in solid tumours through TME-targeted radiation priming

Sarah Anna Okun - Characterizing the Interactome of the MET Exon 14 Oncogene in Lung Adenocarcinoma

Ali Khajegili Mirabadi - GRASP: GRAph-Structured Pyramidal Whole Slide Image Representation

Kevin Sun - Harnessing the potential of nanomedicine: development of a liposomal formulation of irinotecan to enhance immunotherapy treatment outcomes

Jessica Felix - Investigating Autophagy-related Cysteine Protease Atg4a in Drosophila melanogaster Models of Cancer

Student Speed Talks

LSC 1002

Ho Jung Yoon - Decoding the Effects of Air Pollution on Older Adults with COPD: A Comprehensive Transcriptomics Study

Credo Casmil - Expanding the repertoire of self-amplifying ribonucleic acid vectors for next-generation RNA therapeutics

Thilelli Taibi - RNA Deadenylation factor CNOT3 is Required for Hematopoiesis and Maintenance of Hematopoietic Stem Cells

Jordan Yu - Brain-Age Prediction: Systematic Evaluation of Site Effects, and Sample Age Range and Size

Franziska Mey - Utilizing an IPSC-dervied bone marrow-like organoid model to investigate drug resistance mechanisms in acute myeloid leukemia

Rocky Shi - Integrin-linked kinase mediates epithelial-mesenchymal transition and promotes drug-tolerant-persister cell survival during osimertinib treatment in EGFR-mutant lung adenocarcinoma

Eric Brace - Optical coherence tomography and autofluorescence guided biopsy of small airways using a suction-snare biopsy tool

Haley MacDonald - Improving cell cycle resolution in scRNAseq datasets

Student Speed Talks

LSC 1003

Juliana Sobral de Barros - Copy Number Signatures identify therapeutic opportunities for p53 abnormal Endometrial Carcinomas

Andrew Galbraith - Detection of Mitochondrial 8oxoG using Nanopore Sequencing

Andy Murtha - Impact of androgen deprivation therapy (ADT) on circulating tumor DNA (ctDNA) detection in de novo metastatic castration-sensitive prostate cancer (dnmCSPC)

Pakruti Uday - Altered chromatin patterning over the IGH locus as a predisposing factor for MYC translocation in B cell lymphomas

Meredith Clark - Repurposing Telmisartan as an Immunotherapy Adjuvant: Modulating CD8+ T Cell Activation in the Tumour Microenvironment

Lorenzo Lindo - Lymphodepletion enables successful BCMA CAR-T cell engraftment and tumour control in the syngeneic Vk*MYC model of aggressive myeloma

Serena Chuang - The Role of SHPRH in Lung Adenocarcinoma Initiation and Development

Ian Janzen - Predicting Advanced NSCLC Treatment Response with Combination Radiomics and Clinical Features in a Machine Learning Framework

Student Posters

Atrium 2

11:15 AM- 12:15 PM - Poster Session 1 (evens)

EVEN NUMBER POSTERS			
#	NAME	PROGRAM	TALK TITLE
2	Meingold Chan	BIOF	Analytical consideration of cell type heterogeneity in pediatric saliva for DNA methylation analyses
4	Irvin Ng	BIOF	Leveraging ML to Integrate Microbiome-Metabolome Reveals Host Disease Phenotype
6	Saber Hafezqorani	BIOF	ntEmbd: Deep learning embedding for nucleotide sequences
8	Alejandro Aguirre	BIOF	Identification of relationships between transcription factors and target genes from scientific documents using a fine-tuned PubMedBERT model
10	Tony Liu	BIOF	Time-resolved fosmid library pool selection for hydrocarbon tolerance traits in Escherichia coli
12	Alexander Adrian-Hamazaki	BIOF	Why does coexpression predict gene function?
14	Rituparna Banerjee	BIOF	Exploring B cell repertoire evolution post-vaccination via mathematical modelling and phylogenetic trees
16	Sarah Dada	BIOF	Use of long read whole genome sequencing for precision diagnosis and treatment of individuals with Autism Spectrum Disorders
18	Yolanda Yang	BIOF	Spatial transcriptomics deconvolution using marker-gene-assisted topic models
20	Shaocheng Wu	BIOF	Single-cell Characterization of Genomics and Transcriptomics in the Hodgkin and Reed Sternberg Cells
22	Sean Formby	BIOF	Biomarker prediction in wheat for Leaf rust resistance and susceptibility, a RNA-seq batch effect aware classification approach
24	Parham Kazemi	BIOF	AIEdit: polishing genome assemblies using machine learning and spaced seeds
26	Berkay Altintas	BIOF	Decoding the Epigenetics and Chromatin Loop Dynamics of Androgen Receptor-Mediated Transcription
28	Abhijit Chinchani	BIOF	Effects of tACS on electrophysiological signals are task-dependent
30	Faeze Keshavarz	BIOF	Identifying Active and Druggable Pathways in Primary and Metastatic Cancers through Application of Machine Learning Algorithms
32	Brooks Perkins-Jechow	BIOF	Predicting Autoinhibitory Protein States with AlphaFold2
34	Yukai Wang	BIOF	ChemSightTransformer (CST): a transformer architecture to achieve chemical structure de novo generation and clustering from MS/MS data
36	Denitsa Vasileva	BIOF	Identification of Sex-Specific DNA Methylation in Cord Blood
38	Alexander Morin	BIOF	Meta-analysis strategies for inference of transcriptional regulatory targets
40	Kairel Edwards	CHEM	Investigation of molecular oxygen-, pyridoxal-5'-phosphate-dependent oxidases
42	Jiahua Tan	CHEM	Attribute-Weighted Aggregation of MS/MS Reporter Ion Intensities for Protein Quantification Using Isobaric-Labelling
44	Brett Kiyota	GSAT	A scalable computing framework for whole-body mouse cell lineage reconstruction
46	Ren Takimoto	GSAT	A new highly sensitive retrospective cell clone isolation technology
48	Andras Szeitz	GSAT	Developing a volatilome detection platform for functional metagenomic screening and microbial cell factory engineering
50	Karen Ip	GSAT	Revisiting the unipolar brush cell during cerebellar embryonic development through in-silico perturbation
52	Herbert Yao	GSAT	Toward A Large-Scale Gene Regulatory Network Inference for Human Cells through Divide-And-Conquer Approach
54	Tian Liu	GSAT	Investigating the Potential Drivers of Aberrant Splicing in Acute Myeloid Leukemia
56	Nicole Howes	GSAT	Tracking microbial microplastic transformations in marine waters using stable isotope informed metaproteogenomics
58	Rutuja Pattanshetti	GSAT	Development of a high-throughput genome-wide method to assess Ty1 retrotransposon insertion upstream of tRNA genes in Saccharomyces
60	Marco Ho	GSAT	Modeling Group 4 Medulloblastomas (G4MBs) with Mouse Cerebellar Organoids
62	Jackson Moore	GSAT	Construction of a barcoded collection of wild and domestic Saccharomyces cerevisiae strains for competitive fitness assays using CRISPR-Cas9
64	Nick Mateyko	GSAT	Assessing the uniformity of plasmid library amplification by different culturing methods
66	Mingming Zhang	GSAT	Using Blood-based mRNA To Detect Allergen-induced Late Phase Asthmatic Response
68	Naila Adam	GSAT	Three-dimensional in situ mapping of intratumor heterogeneity
70	Ella Beraldo	GSAT	Sex Differences in Cell Composition and Epigenetic Age Acceleration Associated with Prenatal Maternal Stress in the Placenta

Student Posters

Atrium 2

11:15 AM- 12:15 PM - Poster Session 1 (evens)

72	Mariah Lumpa	GSAT	The role of condensin in Ty1 retrotransposon targeting in Saccharomyces cerevisiae
74	Axel Hauduc	GSAT	Cell-type specific genetic-to-epigenetic relationships in the human breast
76	Ainiah Rushdiana Raquib	IOP	IDENTIFICATION OF A NOVEL INTERACTOR OF ENDOGENOUS SS18::SSX THROUGH MASS SPECTOMETRY-BASED ANALYSIS IN
78	Lauralie Short	IOP	In Vivo Generation of CD19 CAR T Cells by Lipid Nanoparticle Mediated mRNA Delivery
80	Christopher Dusek	IOP	Unlocking the Potential of Prostate Organoid Culturing Systems
82	Mona Orangi	IOP	Role of Innate Lymphoid Cells in Alcohol-HF Diet-Induced Chronic Steatohepatitis and Fibrosis
84	Melika Bakharzi	IOP	Defining the origins and metabolic pathways of osteoclasts in multiple myeloma
86	Leo Escano	IOP	Elucidating the immunomodulatory role of miR-210 in Acute Myeloid Leukemia
88	Lei Wang	IOP	[68Ga]Ga-ProBOMB5 - a novel 68Ga-labeled [Leu13ψPro14]bombesin analog for imaging gastrin-releasing peptide receptor expression with positron
90	Claire Dourieu	IOP	Elucidating the role of IL-33 in prostate cancer following androgen deprivation therapy
92	Devon Heroux	IOP	Repurposing disulfiram for cancer: a drug delivery and population-based approach
94	Cassandra Cui	IOP	Characterizing and targeting the interplay between the SWI/SNF chromatin remodeling complex and ASCL1 in prostate cancer lineage plasticity
96	Sabrina Skyba-Lewin	IOP	Sunflower Trypsin Inhibitor as Novel Scaffold For Development of Hepsin Inhibitor (Not set on Tittle and May change)
98	Elahe Shenasa	IOP	Immune Biomarkers on Tissue Microarray Cores Support the Presence of Adjacent Tertiary Lymphoid Structures in Soft Tissue Sarcoma
100	Charu Sankaran	IOP	Telomerase Activity Corresponds with T cell expansion
102	Lan Valerie Tao	IOP	Elucidating the Role of the IR-A:IR-B Ratio in Pancreatic Ductal Adenocarcinoma
104	Michelle Pewarchuk	IOP	A subset of development-associated PIWI-interacting RNAs show prognostic potential in lung cancer
106	Liam MacPhee	IOP	Overcoming aggressive EMT-driven phenotypes in t(9;11) acute myeloid leukemia through the modulation of microRNA-204
108	Betty Yao	IOP	Increased Glut1 Expression Improves Adoptive T Cell Therapy
110	shunsuke ishige	IOP	Center Detection of Overlapping Nuclei in Micrographs
112	Debajeet Ghosh	IOP	Interplay between miR-146a downregulation and high TP53 activity is associated with DNMT3A CHIP
114	Taras Shyp	IOP	STEAP1 facilitates iron transport in Ewing sarcoma to support mitochondrial activity
116	Jennifer Chan	IOP	GABARAPL2: Grim Reaper and GATEkeeper of viability in pancreatic ductal adenocarcinoma
118	Katie Baillie	IOP	Investigating dominant negative mutations in DNA2 as a model for targeted cancer therapy.
120	Jana Jajarmi	IOP	Identifying Modifiers of EGFR Induced Tumourigenesis to Develop New Therapeutic Strategies for Lung Cancer
122	Davit Khijakadze	IOP	The effect of chronic allergic stimulation on group 2 and group 3 innate lymphoid cells
124	Peipei Wang	IOP	Investigating the biological effects of outdoor air pollution on lung cancer in patients who have never smoked using an integrated genomics approach
126	Namya Sharma	MBIM	Elongation control of mRNA translation drives Group 3 medulloblastoma adaptation to nutrient deprivation
128	Yue Li		CPSF1 drives cell motility via alternative polyadenylation
130	Ho Jung Yoon	BIOF	Decoding the Effects of Air Pollution on Older Adults with COPD: A Comprehensive Transcriptomics Study
132	Jordan Yu	BIOF	Brain-Age Prediction: Systematic Evaluation of Site Effects, and Sample Age Range and Size
134	Ace Shi	IOP	Investigating the role of epigenetic regulator SUV420H2 in Neuroendocrine prostate cancer development and aggressiveness.
136	Erick Navarro-Delgado	BIOF	Modeling the genome and exposome contribution to newborn DNA methylome variability with the RAMEN package
138	Sarah Anna Okun	IOP	Characterizing the Interactome of the MET Exon 14 Oncogene in Lung Adenocarcinoma
140	Cindy Shi		Ovarian Cancer Subtype Classification and Outlier Detection

Student Posters

Atrium 2

1:15-2:15 PM - Poster Session 2 (odds)

ODD NUMBER POSTERS			
#	NAME	PROGRAM	TALK TITLE
1	Katherine Rich	BIOF	Deep Learning-Based Risk Stratification of Low Grade Gliomas: Insights Beyond Molecular Markers
3	Nicole Knoetze	BIOF	Elucidating the regulatory logic of T-cell gene expression
5	Angela McLaughlin	BIOF	Estimation of HIV cases averted in phylogenetic clusters through pre-exposure prophylaxis
7	Meltem Eda Omur	BIOF	Characterization of gene regulatory networks and combinatorial transcription factor interactions during pancreatic β-cell differentiation
9	Ryan McLaughlin	BIOF	Recovery of a novel lineage of sulfur oxidizing denitrifiers in the Saanich Inlet water column using single-cell scaffold-anchored binning
11	Mayur Mallya	BIOF	Automatic Bevacizumab Response Prediction for Ovarian Cancer using Histopathology Images
13	Derek Tam	BIOF	Tracking clonal structure from leukemia diagnosis to relapse using mitochondrial variants
15	Nairuz Elazzabi	BIOF	Comparative Single-Nucleus Transcriptomic Analysis of Gene Co-Expression in Alzheimer's and Healthy Brains
17	Karanvir Singh	BIOF	Genetic Risk for Anorexia Nervosa and Association with General Dimensions of Psychopathology in Childhood
19	Jeffrey Tang	BIOF	CytokineFinder: benchmarking methods and databases for identifying cytokines
21	Johnathan Wong	BIOF	Fast and Memory Genome Completeness Assessment
23	Patty Ye	BIOF	Tree-structured topic modelling of single-cell gene expression data uncovers hierarchical relationships between immune cell types
25	Madison Chapel	BIOF	Simulating the Evolution of Regulatory Complexity in Eukaryotic Populations
27	Neera Patadia	BIOF	Examining Differential Expression Patterns of Drug Treatment Conditions in a Large Data Corpus
29	Amirhossein Afshinfard	BIOF	Phylter: Physlr read filter improves GoldRush genome assemblies
31	Caralyn Reisle	BIOF	Fact-Checking in Cancer Knowledge Bases Using Large Language Models
33	Ishika Luthra	BIOF	Regulatory activity is the default DNA state in eukaryotes
35	Sishir Sishir	BIOF	ASAPP - Annotation of Single-cells by Approximate Pseudo-bulk Projection
37	Tony Liang	BIOF	Nextflow Pipeline for Benchmarking Integrative Multi-Omics Methods for Disease Classification
39	Hans Ghezzi	BIOF	PUPpy: a fully automated primer design pipeline for substrain-level microbial detection and absolute quantification
41	Katie Lyle	CHEM	Biosynthesis of Pyrazole
43	Jason Wong	DUS	Precise assessment of cancer cell growth and survival by artificial intelligence
45	Jonathan Chiang	GSAT	De novo genome assembly and annotation of Aedes togoi, a saline-tolerant coastal rock pool mosquito
47	Yan Chen	GSAT	Using colonoids grown under Air-Liquid Interface (ALI) conditions to model bacterial pathogenesis at the intestinal mucosa
49	Asli Munzur	GSAT	PLASMA CELL-FREE DNA HISTONE METHYLATION ENABLES PHENOTYPIC AND CLINICAL SEGMENTATION OF METASTATIC
51	Eully Ao	GSAT	Exploring the impact of histone variant H2A.Z depletion on nascent transcription regulation during DNA replication stress
53	Rui Wang	GSAT	Exploring the impact of SNCA overexpression on mouse hippocampal DNA methylome and transcriptome during early and midlife
55	Desirée Kelshall	GSAT	A Bioengineered Plant Production System for the Antidiabetic Compound Montbretin A
57	Alex Marr	GSAT	Linking Genomic Structural Variations and Phenotypic Diversity of Saccharomyces cerevisiae Strains: Insights from Vineyards and Wineries
59	Rawnak Hoque	GSAT	Candidate and Genome Wide Pathway Analysis of Super Seniors
61	Yifan Yin	GSAT	Dynamic Changes in the Classical Hodgkin Lymphoma Tumor Microenvironment Using Single Cell Technologies
63	Asfar Salaudeen	GSAT	Decoding Promoter Regulatory Logic in Cancer through Random Mutagenesis Using CRISPR-Cas9 Base Editors
65	Makoto Kishida	GSAT	Characterization of CXCR5-CXCL13 axis in relapse/refractory classic Hodgkin lymphoma
67	Jiyoung Han	GSAT	Sex-influenced DNAm profiles of isolated human placental cell types
69	Megan Wolf	GSAT	Characterization of a cytochrome P450 that catalyzes the O-demethylation of lignin-derived benzoates

Student Posters

Atrium 2

1:15-2:15 PM - Poster Session 2 (odds)

71	Maggie Fu	GSAT	Germline biallelic ASXL1 variants drive T-cell epigenetic and immunological dysfunction, causing combined immunodeficiency and Epstein-Barr virus-
73	Hannah-Ruth Engelbrecht	GSAT	Tick-tock Goes the Epigenetic Clock: Explorations of Biomarkers of Biological Age in the Blue Zone in Costa Rica
75	Shengsen Ding	IOP	Comparative analysis and tumorigenesis of normal human mammary cells from male and female donors
77	Cesar Ulises Monjaras Avila	IOP	Utilizing adipose-derived stem cells on decellularized bladder scaffolds for functional bladder mucosa regeneration
79	Grace Bernard	IOP	Engineering a novel CAR-T cell targeting the solid tumour target podocalyxin
81	Dylan Farnsworth	IOP	Potentiating ERK hyperactivation by targeting the proteostasis network
83	Liam Brockley	IOP	Identifying irreversible molecular changes associated with lung cancer in former smokers
85	Andy Jia	IOP	Enhancing Early Relapse Detection in Testicular Cancer through Rolling Circle Amplification of microRNA Biomarkers
87	Tsz Yin Lam	IOP	Development of PDX humanized mice model for HGSOC
89	Itzel Astiazaran	IOP	Albumin binders to improve tumor uptake of CXCR4-targeted radiopharmaceuticals in advanced prostate cancer
91	Jasper Wong	IOP	Plasmablastic lymphoma (PBL) does not depend on B-cell receptor signaling and the NF-κB pathway
93	Fumi Inaba	IOP	Large-scale DNA Organization Identifies Aggressive Prostate Cancer in Low and Intermediate Risk Patients
95	Jessica Trejo	IOP	THE IMPACT OF EXTRACELLULAR VESICLES DERIVED FROM LUNG ADENOCARCINOMA CELLS ON CAF ACTIVATION
97	Jalal Choupani	IOP	Characterizing the role of NNMT in modulating metabolism and epigenetic reprogramming in prostate cancer lineage plasticity
99	Ariene Cabantog	IOP	Optimizing Culture Conditions of Patient-Derived Multiple Myeloma Cells
101	Kouther Noureddine	IOP	Investigating Intra-tumor Heterogeneity in Non-small Cell Lung Cancer Using Multiplexed ImmunohistoCHEM and Deep Learning
103	Marissa Foo	IOP	DNMT3A Limits Myeloid Signaling Responses In Committed T Cells During Normal And Leukemic Development
105	Zakir Tahiry	IOP	Bloody Sweet: The Role of Chondroitin Sulfate Glycocalyx in Prostate Cancer Vascularization
107	Michael Hall	IOP	Pyruvate supplementation alters metabolism and improves effector molecule expression in CD8+ T cells
109	Yingying Liu	IOP	Single-cell multi-omics profiling of Chronic Myeloid Leukemia stem and progenitor cells across disease stages
111	Hamideh Sharifi Noghabi	IOP	Integrative Analysis of Germline and Somatic DNA Repair Gene Variants in Prostate Cancer Metastasis: Identification and Functionalization of Lead
113	Sara Singh	IOP	Non Invasive trtDNA early detection screening in Pancreatic Ductal Adenocarcinoma
115	Che-Min Lee	IOP	Telmisartan-mediated myofibroblast inhibition in the tumour
117	Cathy Cozma	IOP	Leveraging mutational screening to uncover dominant Rev3 alleles as a novel synthetic lethal therapeutic strategy
119	Junbum Im	IOP	O-GlcNAc Transferase (OGT) is a novel therapeutic approach for EVI1+ AML through increased mitochondrial priming.
121	Jamie Kwon	IOP	Investigating the clinical relevance of pre- and post-treatment serum biomarkers in oropharyngeal cancer
123	Panahi	IOP	Identification of misclassified multiple myeloma patient risk subgroups with a novel biological disease stratifier
125	Yu-Chi (Serena) Chuang	IOP	The Role of SHPRH in Lung Adenocarcinoma Initiation and Development
127	Madeline Lauener	PATH	Expansion and characterization of immune suppressive CD56brightCD16- regulatory natural killer cells for chronic graft-versus-host disease (cGvHD)
129	Jessica Felix	IOP	Investigating Autophagy-related Cysteine Protease Atg4a in Drosophila melanogaster Models of Cancer
131	Juliana Sobral de Barros	GSAT	Copy Number Signatures identify therapeutic opportunities for p53 abnormal Endometrial Carcinomas
133	Andrew Galbraith	BIOF	Detection of Mitochondrial 8oxoG using Nanopore Sequencing
135	Sarthak Garg	IOP	Development and Characterization of a Novel Topotecan Liposomal Formulation
137	Meredith Clark	IOP	Repurposing Telmisartan as an Immunotherapy Adjuvant: Modulating CD8+ T Cell Activation in the Tumour Microenvironment
139	Alireza Omidi		AlphaFold-Multimer captures interactions and dynamics of intrinsically disordered protein regions

Acknowledgements

Speed Talk Abstracts

PUPpy: a fully automated primer design pipeline for substrain-level microbial detection and absolute quantification

Hans Ghezzi

Characterizing microbial communities at high resolution is crucial to unravel the complexity of microbial ecosystems. Advances in bulk sequencing assays such as 16S rRNA and shotgun sequencing have enabled unparalleled qualitative and quantitative microbiota investigations. However, these methods generally do not provide accurate resolution beyond the genus level and lack insights into absolute microbial abundance. Here, we introduce Phylogenetically Unique Primers in python (PUPpy), an automated pipeline to design microbe- and group-specific primers in given microbial communities. PUPpy-designed primers detect individual microbes and quantify absolute microbial abundance in a defined community below the species level, requiring only coding sequence files of the community members as input. We experimentally evaluated the performance of PUPpy-designed primers using two bacterial communities as benchmarks. Each community was comprised of 10 members, exhibiting a range of genetic similarities that spanned from different phyla to substrains. PUPpy-designed primers also enabled the detection of groups of bacteria in an undefined community, such as the detection of a gut bacterial family in a stool microbiota sample. Taxon-specific primers designed with PUPpy showed 100% specificity to their intended targets without unintended amplification, independently of community composition and complexity. Lastly, we show absolute quantification of microbial abundance using PUPpy-designed primers in droplet digital PCR, benchmarked against 16S rRNA and shotgun sequencing. Our data shows that PUPpy-designed microbe-specific primers can be used to quantify substrain-level absolute counts, providing more resolved and accurate quantification in defined communities than 16S rRNA and shotgun sequencing. Altogether, PUPpy enables highly accurate perspectives into microbial ecosystems, supporting the characterization of bacterial communities in both in vitro and complex microbiota settings.

Modeling the genome and exposome contribution to newborn DNA methylome variability with the RAMEN package

Erick Navarro-Delgado

DNA methylation(DNAme) is an epigenetic mark that can regulate the genome, and its variability has been associated with potentially long-term phenotypic changes. Studies suggest that genetics(G) and environmental(E) factors jointly best explain DNAme variability in most of the newborn epigenome(75-89%). Limitations of previous works are that the prenatal environment's influence could be underestimated due to the small set of variables analyzed(<20) and that past methodologies do not address the genome-exposome variable-number imbalance. We aimed to address these gaps and 1)identify Variable Methylated Regions (VMRs) at birth, and 2)analyze the contribution of G and E to VMR's DNAme. Using cord blood samples of CHILD (n=699, 94 E variables across four dimensions of the prenatal environment: parental psychosocial, maternal health, built environment, and maternal nutrition), we identified 28,480 VMRs using variance as a measure of variability. We conducted an E and G variable selection procedure for each VMR using LASSO, and then fitted single-variable G, E, pairwise additive(G+E) and interaction(GxE) linear models. After selecting the winning model using AIC, and labelling VMRs with non-conclusive models identified through a permutation analysis(41.2%), we found G+E to be the predominant best explanatory model(27%), followed by G(18.4%), GxE(13.4%) and E(0.1%). Furthermore, we found that the SNP terms explain a higher proportion of variance across all winning models at birth (mean partial R2=0.22) compared to the environment (mean partial R2=0.008) and interaction terms (mean partial R2=0.007). Finally, we developed an R package: Regional Association of Methylome variability with the Exposome and geNome (RAMEN; github.com/ErickNavarroD/RAMEN), which provides the community an easy-to-use pipeline to conduct integrative genome-exposome analyses with DNAme data. Overall, our work highlights the role of G in DNAme and the importance of addressing it in epigenetic studies.

Speed Talk Abstracts

Overcoming chemotherapeutic resistance by targeting ferroptosis in cancer cells surviving apoptotic caspase release

Rachel Hausman

The survival of cancer cells post chemotherapy continues to be a barrier to effective cancer treatment. The majority of chemotherapeutics kill cells by inducing apoptosis, and resistance to this mechanism of cell death can cause recurrent tumors. Executioner caspase release was previously thought to be the point of no return from apoptotic cell death, however, it has been shown that removal of the reagent causing caspase release can lead to recovery of cells from apoptotic signaling, a phenomenon which has been termed ``Anastasis``. We have hypothesized that when treated with chemotherapeutics, some cells will evade cell death even when executioner caspases have been activated. By identifying surviving anastatic cells and pathways involved in their evasion of cell death, we hope to propose novel therapeutic strategies to prevent their survival. To identify and isolate a population of anastatic cells surviving caspase-3 activation as a result of chemotherapeutic treatment, the CasExpress system developed by the Denise Montell lab group was used to permanently label surviving cells with GFP. Analysis of these cells demonstrates they have increased resistance to chemotherapy, decreased levels of caspase-3, a mesenchymal phenotype, decreased levels of GPX4, and are more sensitive to ferroptosis. To complement this, a down-regulation of GPX4 was also shown when EMT is induced in NMe normal mouse epithelial cells, indicating a potential link between ferroptosis and EMT. Further investigation is currently underway to elucidate the mechanism behind this vulnerability to ferroptosis, acquisition of a more mesenchymal phenotype, and evasion of cell death. We also hope to phenotype these anastatic cells by performing proteomic and metabolomics analysis to gain an overall perspective on how they differ from cells that are susceptible to apoptotic cell death. Using these techniques, we hope to be able to propose novel therapeutic targets for the treatment of recurrent cancers.

Unlocking the potential of CAR-T cell therapy in solid tumours through TME-targeted radiation priming

Lisa Zhan

The solid tumour microenvironment (TME) presents formidable challenges towards efficient CAR-T cell therapy, marked by the presence of immunosuppressive cells, low oxygen, low pH, and an aberrant vascular system. Modifying these factors hold the key to improving immunotherapy outcomes for solid tumours. Patients often undergo ionizing radiation therapy alongside or preceding other treatment modalities, such as immunotherapy. As such, our research focuses on the potential between CAR-T cell therapy and irradiation doses to prime the TME for enhanced CAR-T cell infiltration and anti-tumour activity. We hypothesize that a specific radiation schedule prior to infusion of CAR-T cells will improve their infiltration and anti-tumour activity. B16 melanoma tumour-bearing mice exposed to various ionizing radiation doses ranging from 2 Gy to 12 Gy will undergo various assessments, including measurements of changes in vascular density, perfusion, and the infiltration of immune effector and suppressor cells. These assessments will be conducted through immunofluorescence microscopy and flow cytometry following the tumours for up to 3 days post-irradiation. We are currently testing how various radiation treatment schedules influence the infiltration of CAR-T cells synthesized to target EGFRvIII expressed on the tumour cells. These data will provide insights into the potential synergy between TME-priming-radiation and CAR-T cell therapy. In summary, our research endeavors to elucidate an irradiation dosage and timing capable of improving immune cell infiltration within the TME. The observed increase in tumour perfusion following specific irradiation regimens, coupled with the planned introduction of CAR-T cells, offers a promising avenue for further investigation. We anticipate that this tailored approach will significantly improve the efficacy of CAR-T cell therapy in the challenging landscape of solid tumours, fostering advancements in cancer treatment modalities.

Speed Talk Abstracts

Characterizing the Interactome of the MET Exon 14 Oncogene in Lung Adenocarcinoma

Sarah Anna Okun

Splice site mutations in the MET receptor tyrosine kinase leading to skipping of exon 14 (METex14) are established driver mutations in non-small cell lung cancer (NSCLC), of which lung adenocarcinoma (LUAD) is the most common subtype. Targeted therapies for METex14 have been developed, however patient response is poor, and development of better inhibitors or combination therapies is necessary. The oncogenic potential of METex14 has been attributed to its increased half life and subsequent hyperactivation of downstream signaling pathways. Recent evidence from our lab suggests that it also preferentially upregulates the RAS/MAPK pathway, indicating that METex14 may display altered receptor affinity for its binding partners relative to its wildtype (WT) counterpart. We therefore aim to characterize the METex14 interactome to allow for better understanding of the pathways and mechanisms contributing to its transforming potential, and consequently identify possible therapeutic targets. To this end, V5-tagged constructs will be developed for MET WT, METex14, and MET Y1003F, the latter of which should partially phenocopy METex14's transforming ability. V5-tagged MET will then be pulled down and tandem mass tag mass spectrometry will be performed on co-immunoprecipitated binding partners to identify those which preferentially interact with METex14. Functional impacts of interactions of interest will then be elucidated through a pooled CRISPR-Cas9 screen utilizing a targeted library; enrichment levels of sgRNA before and after several population doublings will elucidate the impact of knocking out target proteins before individual effectors are validated through knockdown cell lines and transformation assays. This information will ultimately help uncover mechanisms through which METex14 drives LUAD oncogenesis and identify potential therapeutic targets, contributing to the design of more effective treatment strategies for lung cancer patients harbouring METex14 mutations.

GRASP: GRAph-Structured Pyramidal Whole Slide Image Representation

Ali Khajegili Mirabadi

Cancer subtyping is one of the most challenging tasks in digital pathology, where Multiple Instance Learning (MIL) by processing gigapixel whole slide images (WSIs) has been in the spotlight of recent research. However, MIL approaches do not take advantage of inter- and intra-magnification information contained in WSIs. In this work, we present GRASP, a novel graph-structured multi-magnification framework for processing WSIs in digital pathology. Our approach is designed to dynamically emulate the pathologist's behavior in handling WSIs and benefits from the hierarchical structure of WSIs. GRASP, which introduces a convergence-based node aggregation instead of traditional pooling mechanisms, outperforms state-of-the-art methods over two distinct cancer datasets by a margin of up to 10% balanced accuracy, while being 7 times smaller than the closest-performing state-of-the-art model in terms of the number of parameters. Our results show that GRASP is dynamic in finding and consulting with different magnifications for subtyping cancers and is reliable and stable across different hyperparameters. The model's behavior has been evaluated by two expert pathologists confirming the interpretability of the model's dynamic. We also provide a theoretical foundation, along with empirical evidence, for our work, explaining how GRASP interacts with different magnifications and nodes in the graph to make predictions. We believe that the strong characteristics yet simple structure of GRASP will encourage the development of interpretable, structure-based designs for WSI representation in digital pathology. Furthermore, we publish two large graph datasets of rare Ovarian and Bladder cancers to contribute to the field.

Speed Talk Abstracts

Harnessing the potential of nanomedicine: development of a liposomal formulation of irinotecan to enhance immunotherapy treatment outcomes

Kevin Sun

Irinotecan (CPT-11) is a topoisomerase I inhibitor that is currently used in first and second-line chemotherapy treatments for multiple cancers. The use of CPT-11 has been associated with side effects like gastrointestinal (GI) toxicity which limits its use in metastatic colorectal cancer (mCRC). It is possible that an appropriately designed liposomal formulation of CPT-11 may reduce this GI toxicity. More importantly, the liposomal formulation may be ideally suited for use in combination with immunotherapeutic because CPT-11 can induce immunogenic cell death (ICD), an effect that may best be obtained using a drug delivery system such as liposomes. The ICD effect should enhance response rate when using immunotherapy treatments. A novel liposomal formulation of CPT-11 (referred as Irinosome C) is described. In pharmacokinetic study, this formulation improved the half-life and serum concentration of CPT-11 significantly. It is hypothesized that this formulation will enhance the therapeutic effects of immunotherapeutics through the induction of ICD. ICD inducing effect were also assessed by measuring the release of damage associated molecular patterns such as high mobility group box-1 (HMGB-1). Murine and human colorectal cancer cell lines was treated with Irinosome C in vitro and the treated cell culture media harvested and analyzed to assess the change of HMGB-1 levels (using an established ELISA assay kit). CT26 and MC38 cells treated with Irinosome C was better recognized by antigen presenting cells (APCs) and stimulate them in a co-culturing phagocytosis assay. The ability of Irinosome C to induce ICD was also evaluated in a whole cell vaccination study in CT26 and MC38 models. Cells were treated with Irinosome C ex vivo and inoculated into syngeneic mice. The mice were then challenged with parent (untreated) CT26 and MC38 cells and monitored for tumor growth. The formulation can induce a vaccine-like effect in vivo in mice inoculated with CT26 and MC38 cells

Investigating Autophagy-related Cysteine Protease Atg4a in Drosophila melanogaster Models of Cancer

Jessica Felix

Pancreatic ductal adenocarcinoma (PDAC) accounts for over 90% of all pancreatic cancer cases with a worldwide five-year survival rate of a mere 9%. There is an urgent need for further research into the molecular and cellular mechanisms that contribute to PDAC progression and treatment failure in PDAC. One advancing idea is that an upregulation of autophagy contributes to PDAC tumor survival and resistance to therapy, suggesting autophagy as a potential therapeutic target. Our recent work in human PDAC cell lines suggests that the autophagy related cysteine proteases ATG4A and ATG4B are key players in the progression of PDAC. Knocking out ATG4A and ATG4B in PDAC cells resulted in growth inhibition and a decrease in Atg8-family processing. Further research into the mechanisms of ATG4A/B and their interactions with cancer-related cell signaling pathways in an in vivo model may help provide insight into potential ATG4A/B-related treatments for cancer. Therefore, we will investigate ATG4A/B using Drosophila and its single ortholog for both ATG4A and ATG4B, Drosophila Atg4a. Drosophila is a well-studied and widely used in vivo model with conserved cell signaling pathways, making it a powerful study tool for the complex interactions that occur in cancer host-tumor interplay. Our preliminary data shows that a mutant line Atg4aMB03551 has a decrease in Atg8 processing, as well as an accumulation of p62 and ubiquitin. These phenotypes indicate that autophagy is compromised. This mutant line also shows a delay in an ovary degeneration phenotype, which is known to be mediated by autophagy and is typically observed upon starvation of wild-type flies. A ubiquitous RNAi knockdown of Atg4a also exhibits a decrease in Atg8 levels, as well as a decrease in motor function as measured by climbing assays. Characterization of potential genetic interactions between Atg4a and pancreatic cancer-related mutations in vivo is currently in progress.

Speed Talk Abstracts

Decoding the Effects of Air Pollution on Older Adults with COPD: A Comprehensive Transcriptomics Study

Ho Jung Yoon

Chronic obstructive pulmonary disease (COPD) is a debilitating lung disease, affecting over 384 million individuals worldwide. Key features of COPD include chronic inflammation, airflow obstruction, chronic cough, and alveolar destruction. A significant risk factor contributing to COPD development includes exposure to particulate-rich air pollution. Air pollution exposure may trigger respiratory flare-ups, termed exacerbations, that require in-hospital treatment. To date, studies characterizing the impact of air pollution on COPD using omics approaches have been in cross-sectional population cohorts. To complement and strengthen these population cohorts, we conducted an integrative transcriptomic analysis in a controlled human exposure study. We employed a systems biology approach involving the integration of transcriptomic datasets across lung and blood to uncover novel biomarkers to better comprehend the interplay between COPD and air pollution. For our investigation, we recruited thirty research participants aged 40-80, with and without COPD. Each participant was exposed to diesel exhaust and filtered air (control) for 2 hours, during 2 visits randomized to order and separated by a washout period. Peripheral blood and endobronchial brushings were collected after each exposure for bulk-RNA sequencing. Differential gene expression was assessed by linear mixed-effects models, accounting for repeated measures. We performed integration for novel biomarker discovery using latent components utilizing the R package mixOmics. Differential gene expression analysis revealed that genes related to immune function were altered following exposure. These genes included HSPA1, MTND4P1, MICB, TRIM39, and GPX2. Blood-lung omics integration showed shared clusters of dysregulated genes in the blood that are reflective of the response of the airways to air pollution. This gene set signature in blood is a candidate biomarker for determining response to acute air pollution exposure.

RNA Deadenylation factor CNOT3 is Required for Hematopoiesis and Maintenance of Hematopoietic Stem Cells

Thilelli Taibi

Adult mammalian hematopoietic stem cells (HSCs) constitute a heterogenous population responsible for generation of various cell types in the blood throughout adulthood. Gene expression program underlying regulation of self-renewal and differentiation of HSCs are tightly regulated. However, how post-transcriptional regulation of gene expression influences HSCs and hematopoiesis remains less explored. CNOT3, a subunit of the CCR4-NOT deadenylation complex and RNA biology regulator, maintains human HSCs in an undifferentiated state and plays a critical role in acute myeloid leukemia tumorigenesis. Here, we report the critical role of CNOT3 in regulating hematopoietic stem cells (HSCs) function in murine adult hematopoiesis. We observed that Cnot3 is highly expressed in HSCs and CNOT3 ablation in the murine Cnot3 conditional knockout mouse model resulted in anemia, reduced bone marrow cellularity and enhanced extramedullary hematopoiesis in spleen. Deletion of Cnot3 resulted in the early expansion of immunophenotypic HSCs which were then progressively lost over time. CNOT3 knockout hematopoietic stem/progenitor cells (HSPCs) failed to reconstitute hematopoietic systems of recipient animals in transplantation assays. Single-cell RNA sequencing (scRNA-seq) analysis of HSPCs revealed disruptions in lineage development, loss of HSCs and formation of a CNOT3 knockout specific population exhibiting decreased stem cell gene expression program. Transcriptomic profiling and cell cycle analysis demonstrated that Cnot3 deletion led to increased cycling activity in HSCs. Our results indicate that CNOT3 is critical for maintenance of homeostasis in HSCs and the hematopoietic system.

Speed Talk Abstracts

Brain-Age Prediction: Systematic Evaluation of Site Effects, and Sample Age Range and Size

Jordan Yu

Structural neuroimaging data have been used to compute an estimate of the biological age of the brain (brain-age) which has been associated with other biologically and behaviorally meaningful measures of brain development and aging. The ongoing research interest in brain-age has highlighted the need for robust and publicly available brain-age models pre-trained on data from large samples of healthy individuals. To address this need we have previously released a developmental brain-age model. Here we expand this work to develop, empirically validate, and disseminate a pre-trained brain-age model to cover most of the human lifespan. To achieve this, we selected the best-performing model after systematically examining the impact of site harmonization, age range, and sample size on brain-age prediction in a discovery sample of brain morphometric measures from 35,683 healthy individuals (age range: 5-90 years; 53.59% female). The pre-trained models were tested for cross-dataset generalizability in an independent sample comprising 2,101 healthy individuals (age range: 8-80 years; 55.35% female) and for longitudinal consistency in a further sample comprising 377 healthy individuals (age range: 9-25 years; 49.87% female). This empirical examination yielded the following findings: (1) the accuracy of age prediction from morphometry data was higher when no site harmonization was applied; (2) dividing the discovery sample into two age-bins (5-40 years and 40-90 years) provided a better balance between model accuracy and explained age variance than other alternatives; (3) model accuracy for brain-age prediction plateaued at a sample size exceeding 1,600 participants. These findings have been incorporated into CentileBrain [https://centilebrain.org/#/brainAGE2], an open-science, web-based platform for individualized neuroimaging metrics.

Utilizing an IPSC-Derived Bone Marrow-Like Organoid Model to Investigate Drug Resistance Mechanisms in Acute Myeloid Leukemia

Franziska Mey

Acute myeloid leukemia (AML) is a severe blood cancer, originating from leukemic stem cells (LSC). AML outcomes are poor, with five-year survival rate under 35% overall and □10% in patients above 60 y.o.1. Although classification and patient risk stratification have improved due to increased genomic characterization, progress in treatment approaches has lagged, largely due to the complexity, multiplicity, and heterogeneity of LSCs in a single patient2. LSCs are known to escape current therapies in a quiescent stage localizing in the hematopoietic niche3. Comprehensive modelling of this mechanism remains challenging due to a lack of physiological models. Organoids are of rising interest across different disciplines and seen as bridge between 2D in-vitro studies and animal models. Recent breakthroughs in scaffold technologies, hydrogel engineering and improvements in induced Pluripotent Stem Cells (iPSCs) culture formed the basis for the development bone-marrow like organoid models4.
Immunofluorescence imaging on laser scanning confocal and 3D Thunder microscopes as well as fluorescently activated cell sorting (FACS) and single cell RNA sequencing (scRNA-seq) are utilized to validate the final composition of organoids and to ensure reproducibility compared to the published model. Utilizing an innovative differentiation protocol, we modulate the bone marrow niche to investigate drug resistance mechanism in LSCs. Observing the interplay of different bone marrow cell subtypes in a system which allows us to modulate their composition as well as following their differentiation trajectory, will lead to a better understanding of key players in LSC quiescence and new insides in AML therapy escape and relapse. Showing the engraftment potential and proliferation of AML patient samples in the established organoids as well as co-transplantation potential, will contribute to a future reduction of animal numbers for blood cancer research.

Speed Talk Abstracts

Integrin-linked kinase mediates epithelial-mesenchymal transition and promotes drug-tolerant-persister cell survival during osimertinib treatment in EGFR-mutant lung adenocarcinoma

Rocky Shi

Lung cancer is the leading cause of cancer-related deaths in Canada. Mutant epidermal growth factor receptor (EGFR) is a major driver of lung adenocarcinoma (LUAD). This led to the development of osimertinib, a third-generation tyrosine kinase inhibitor that specifically targets mutant EGFR, thereby greatly improving clinical outcomes. However, patients inevitably develop resistance against osimertinib. Epithelial-mesenchymal transition (EMT) is one known resistance mechanism that also increases the invasiveness and metastatic potential of tumours. In the progression towards EMT-mediated resistance, cancer cells enter an intermediate, transitory drug-tolerant-persister (DTP) state, in which increased phenotypic plasticity permits survival during drug treatment. Integrin-linked kinase (ILK), an important regulator of integrin signaling, has been implicated in the pathogenesis of other cancers through the promotion of EMT. Additionally, a recent study in patients treated with EGFR-TKIs found that high ILK expression was correlated with worse prognosis. Therefore, we hypothesize that ILK may be important for DTP survival and EMT-mediated osimertinib resistance in EGFR driven LUAD. Using publicly available databases, we found that high ILK expression was significantly correlated with an EMT expression signature in EGFR mutant LUAD patients and cell lines. Genetic and pharmacological suppression of ILK in HCC4006 cells reduced EMT induction and improved osimertinib sensitivity, identifying the cytoprotective role of ILK during osimertinib treatment. Importantly, ILK knockdown reduced the viability of osimertinib DTP cells by suppressing YAP activation. Overall, our results suggest that ILK expression is an important factor during osimertinib treatment and potentially a target for combination therapy.

Optical coherence tomography and autofluorescence guided biopsy of small airways using a suction-snare biopsy tool

Eric Brace

MOTIVATION: Collection of biopsies from the most pathologically advanced region is critical for histopathological assessment of potentially cancerous sites in the lung. However, current applications are limited in their ability to simultaneously image and collect samples in subsegmental airways. Alternatives, such as computed-tomography guided transthoracic needle biopsies and cryobiopsies, increase biopsy yield but increase risk of complications.

Optical coherence tomography combined with autofluorescence imaging (OCT-AFI) has been shown to provide good specificity in identification of potentially malignant lesions within the lungs. Biopsy collection using an OCT-AFI guided tool may improve diagnostic yield while minimizing complications.

HYPOTHESIS: Image guided biopsy using a suction-snare device is feasible in small airways.

METHODS: We demonstrate a prototype OCT-AFI-guided biopsy tool capable of simultaneous imaging and sample collection designed for the narrow airways of the lung. The biopsy tool is 3D printed with biocompatible resin and utilizes suction to draw tissue into the housing to be severed with a nitinol snare. Device performance is assessed by collecting biopsies in an ex-vivo porcine airway, which is inflated using a negative pressure chamber.

RESULTS: The device is demonstrated to be feasible in an ex-vivo animal model. Images show that the tool is capable of structural and functional assessment prior to collection of a biopsy. Tissue is successfully drawn into the device using suction and severed through snare retraction.

Speed Talk Abstracts

Improving cell cycle resolution in scRNAseq datasets

Haley MacDonald

Single cell RNA sequencing (scRNAseq) is growing in popularity as a method of investigating heterogeneity in mixed cell populations. One source of heterogeneity in these datasets is the cell cycle, and cell cycle phase assignment is a standard step of scRNAseq workflows. Several popular cell cycle identifying algorithms exist but have not been benchmarked on ground truth single cell datasets, and our results demonstrate their poor performance.

The cell cycle is understudied in the single cell space, and recent studies have identified differences in cell cycling processes, particularly between stem cells and differentiated cells. Cell quiescence (G0) is also poorly understood, though its role in tumours as a mechanism of drug resistance has recently been identified. Despite these findings, none of the existing algorithms currently account for alternative stem-like patterns of cycling, or differentiate between G0 and G1. We hypothesize that the poor performance of existing algorithms is due in part to these factors, and we aim to create a novel algorithm flexible enough to account for heterogeneity in cycling processes and capable of distinguishing between G0 and G1.

One existing algorithm (DeepCycle) successfully uses spliced-unspliced count ratios to order cells along a pseudotime trajectory representing the cell cycle, but consistently fails to assign the correct phase to cells using the expression of reference genes heuristically. Our results have also demonstrated that using spliced-unspliced count ratios rather than gene expression results in better separation of ground truth phases in FACS-sorted datasets. We aim to design a random forest model capable of accurately identifying cell cycle phase using spliced-unspliced ratios and DeepCycle pseudotime as inputs. We hypothesize that using these novel inputs will allow our model to more accurately resolve cell cycle phase, and will allow for future scRNAseq studies of cell cycling and G0 to be performed.

Copy Number Signatures identify therapeutic opportunities for p53 abnormal Endometrial Carcinomas

Juliana Sobral de Barros

Endometrial carcinoma (EC) is the most common gynecologic cancer in North America, with p53 abnormal (p53abn) having the worst outcomes, responsible for 50-70% of EC mortality. Therapeutic advances are urgently needed to improve outcomes for these patients. Shallow whole genome sequencing (sWGS) has been successfully used to derive copy number (CN) signatures in high grade serous ovarian cancer (HGSOC). p53abn ECs share genomic features with HGSOC, supporting applying sWGS to this EC molecular subtype. Identifying CN signatures may allow prognostic stratification and identification of therapeutic options. Tumor DNA was extracted from 187 formalin-fixed paraffin-embedded p53abn ECs. sWGS and targeted panel sequencing was performed. The raw data were aligned and treated to correct CG content and discard low-quality reads. The samples were exposed to the HGSOC signatures and our own custom generated signatures and then compared. Our p53abn EC signatures were composed of 5 distinct groups. Signature 5 was associated with homologous recombination deficiency (HRD) due to CN loss of BRCA1/2 and these were also associated with the HGSOC HRD signature. Both signatures 3 and 4 were associated with a high ploidy state, CCNE1amp, ERBB2amp and MYCamp. Signature 3 is differentiated from signature 4 by an enrichment for PIK3CA mutations. We found no molecular associations for signature 1 and 2, although signature 2 was associated with endometrioid histotype. In this p53abn EC cohort the most common mutations beyond TP53 were PIK3CA (32%), PPP2R1A (29%), PIK3R1 (18%), FBXW7 (16%) and PTEN (16%). sWGS is a relatively inexpensive tool that has been successfully used to derive CN signatures. In this study we identified opportunities for targeted therapy such as PARPi for HRD ECs, anti-HER2 therapy for ERBB2amp and targeting Wee1 inhibitors for CCNE1amp ECs. CN signatures derived from sWGS can provide a clear path to clinical implementation and open new avenues for clinical trials.

Speed Talk Abstracts

Detection of Mitochondrial 8oxoG using Nanopore Sequencing

Andrew Galbraith

Reactive oxygen species can result in oxidative stress, potentially leading to neurodegenerative disorders and tumorigenesis. Due to its low redox potential, guanine is particularly susceptible to oxidation resulting in 8-oxoguanine (8oxoG). The syn conformation of 8oxoG base pairs with adenine resulting in G > T transversion mutations. Further, 8oxoG can regulate gene transcription through a variety of epigenetic and posttranscriptional roles such as by interference with CpG island methylation. To detect 8oxoG, we sequenced 11 conserved mitochondrial oligonucleotide sequences across mice and humans, 8 with 8oxoG at a given sequence motif and 3 without. We then compared the signal of oligonucleotides with and without 8oxoG to develop neural network models for 8oxoG detection. Further, we finetuned a base caller to correctly call the nucleotide sequence even with 8oxoG present. These models were found to differentiate modified oligonucleotides with a greater than 98% accuracy and false positive rate of less than 0.5%. Additionally, the models could correctly predict a significantly higher rate of 8oxoG in PolG mutant mice in comparison to wildtype. These mice are more vulnerable to oxidative damage due to having impaired mitochondrial replication. One of the models also showed higher rates of 8oxoG in cancers with mutation signatures associated with oxidative damage such as SBS 18 and 36. This project presents the first methodology to detect and quantity 8oxoG at single base pair resolution without the need of chemical conjugation or enzymatic treatment. Levels of 8oxoG can be profiled alongside standard nanopore sequencing analysis to do in-depth genetic and epigenetic screening of samples. This will facilitate 8oxoG analysis and help illuminate its role in pathogenesis. Future development of models will be needed to detect 8oxoG in more contexts, improve specificity of models, and profile nuclear and telomeric DNA 8oxoG.

Impact of androgen deprivation therapy (ADT) on circulating tumor DNA (ctDNA) detection in de novo metastatic castration-sensitive prostate cancer (dnmCSPC)

Andy Murtha

Plasma ctDNA is detected in most patients with metastatic castration-resistant prostate cancer, enabling minimally-invasive tumour genotyping that informs prognosis and therapy decisions. Many patients with dnmCSPC will commence ADT before referral for genomic testing, reducing ctDNA fraction (ctDNA%), potentially limiting its clinical utility. Here, we identify predictors of ctDNA% and assess its association with clinical outcomes in patients with dnmCSPC. We examined 188 samples from 179 patients enrolled in a provincial wide prospective biobank. Cell-free DNA and patient-matched WBC samples underwent deep targeted sequencing. All clinical factors were consistent with typical dnmCSPC. ctDNA was detected in 66% (29/44) of ADT-naïve samples compared to 26% (37/144) of ADT-exposed samples. The strongest predictor of ctDNA detection was duration of ADT exposure. ctDNA% reduced after ADT commencement, most evident >7 days (1-7 days: 88% detected; 8-14: 31%, 15-50: 15%; p<0.001). Associations for ctDNA detection were also observed for abnormal hemoglobin (OR 3.5, p<0.001) and visceral disease (OR 3.0; p=0.012), but not other clinical characteristics. ctDNA detection and high ctDNA% were associated with shorter time to castration resistance (TTCR) irrespective of ADT exposure (ADT-naïve: HR 3.6, 95% CI 1.2-11, p=0.02; ADT-exposed: HR 1.9, 95% CI 1.2-3, p=0.01), with outcomes poorest in ADT-exposed patients with high ctDNA% (median TTCR 32 vs 8.4 mo. in <2% and >30% ctDNA groups, respectively). Only ADT-exposed ctDNA detection and ctDNA% were associated with overall survival (HR 1.9, 95% CI 1.1-3.1, p=0.02). ADT exposure is the strongest predictor of ctDNA non-detection in dnmCSPC. However, despite receiving up to 7 days of ADT, 72% of patients had detectable ctDNA, indicating a window for complementing tumour tissue-based genotyping with ctDNA tests. Persistent ctDNA after ADT administration is a potential biomarker to identify patients for treatment intensification.

Speed Talk Abstracts

Altered chromatin patterning over the IGH locus as a predisposing factor for MYC translocation in B cell lymphomas

Pakruti Uday

Chromosomal translocations are frequent genetic events in non-Hodgkin B-cell lymphoma (B-NHL) and commonly involve IGH, MYC or other partner loci. Oncogenic translocations can occur at two main stages of B-cell ontogeny: the pro/pre-B-cell stage in the bone marrow or in the mature B-cell stage in the germinal centre (GC) of secondary lymphoid organs such as lymph nodes. Translocations such as IGH::MYC that are gained at the mature B-cell stage are associated with inferior outcomes in B-NHL such as diffuse large B-cell lymphoma. B-NHL are enriched with loss of function (LOF) mutations in chromatin modifying genes (CMGs) such as KMT2D and ARID1A. The goal of my project is to functionally characterize the role of recurrently mutated genes in human B-NHL and their contributions to increasing the frequency of aberrant DNA translocation events. To accomplish this, I propose to model these mutations in cell line and primary human B-cells. We hypothesize that double-stranded DNA breaks (DSBs) introduced by activation induced deaminase (AID) to facilitate class-switch recombination (CSR) during the GC reaction, in the presence of LOF CMG mutations, could remain unresolved and drive illegitimate recombination events such as IGH::MYC. Preliminary data indicates that KMT2D and ARID1A knockdowns lead to reduced CSR efficiency. In addition to refining PCR- and sequencing-based assays to detect low frequency illegitimate translocation events, we are also interrogating the epigenetic and chromatin accessibility landscape of aberrant B-cells via ChIP- and ATAC-seq. By investigating how recurrently mutated CMGs may alter disease severity and the B-cell epigenome and biology, we can devise new therapeutic strategies that reverse the lymphoma-causing effects of these mutations and improve patient outcomes with effective, less toxic therapies that are based on a deeper understanding of the underlying disease biology.

Repurposing Telmisartan as an Immunotherapy Adjuvant: Modulating CD8+ T Cell Activation in the Tumour Microenvironment

Meredith Clark

Immune checkpoint blockade (ICB) has emerged as a ground-breaking therapy for many cancer types. While effective in immunogenic cancers such as triple-negative breast cancer, enhancing response rates remains a challenge. This project explores repurposing the blood pressure drug Telmisartan (Tel) as a potential adjuvant for ICB. We have previously shown that while Tel decreases collagen deposition by cancer-associated fibroblasts and improves radiotherapy response, it does not affect xenograft growth in an immunodeficient mouse model. However, Tel treatment significantly delayed tumour growth in an immunocompetent murine mammary carcinoma model; suggesting an immune-mediated mechanism of tumour suppression. We hypothesize that Tel improves CD8+ T cell activation and anti-tumour activity through its role as a partial PPARγ agonist, and that Tel treatment will improve ICB response. We have identified that Tel treatment promotes a more activated CD8+ T cell phenotype in E0771 tumour-bearing mice, demonstrated by the increased expression of T cell activation markers, CD44 and PD-1. Further, ex vivo CD8+ T cells treated with Tel exhibited increased expression of effector molecules such as TNFα and granzyme B, suggesting that Tel may increase CD8+ T cell anti-tumour activity. Currently, we are investigating the tumour killing capacity of Tel-treated CD8+ T cells ex vivo and further characterizing the mechanism of Tel-mediated T cell modulation. We are also working to combine Tel treatment with ICB in tumour-bearing mice, as well as investigating other immune cell populations that are modulated by Tel treatment in the tumour microenvironment. This research highlights Tel's potential as an ICB adjuvant, providing a rationale for further exploration of its impact on T cell function and its viability as a complementary approach to cancer immunotherapy. Ultimately, combining Telmisartan with current ICB therapies may lead to more durable clinical responses for cancer patients.

Speed Talk Abstracts

Lymphodepletion enables successful BCMA CAR-T cell engraftment and tumour control in the syngeneic Vk*MYC model of aggressive myeloma

Lorenzo Lindo

Chimeric antigen receptor (CAR)-T cells directed against B-cell maturation antigen (BCMA), have yielded impressive results in clinical trials for relapsed/refractory multiple myeloma (MM). However, progression-free survival is short, demonstrating a need for improvements. While advances have been made to improve this treatment modality in preclinical models, these have not always translated to superior clinical outcomes. A major limitation is that novel therapies are often not tested in a relevant in vivo model, rather, they've been largely tested only in immunodeficient models. To better recapitulate the host-tumour-effector cell interactions, particularly in the TME which is known in MM to affect response to therapy, we sought to develop a system to study CAR-T cell activity in an immunocompetent model of MM. Here, we report on the first known successful model of BCMA-directed CAR-T cells in the Vk*MYC immunocompetent model of MM. We developed a murine CAR-T cells capable of binding to both human and mouse BCMA, herein referred to as sdBCMA2 CAR-T cells. In co-culture assays, sdBCMA2 CAR-T cells effectively lysed target cells, proliferated, and released inflammatory cytokines. To assess the in vivo activity of these CAR-T cells, we injected mice with Vk*MYC cells, and then administered sdBCMA2 CAR-T cells. In the absence of lymphodepletion, CAR-T cells failed to persist and thereby failed to control disease. Lymphodepletion prior to CAR-T cell administration was found to significantly improve CAR-T cell engraftment, reduce disease burden, and induce remission. We report on the first model of BCMA CAR-T cells in the Vk*MYC mouse model. As our construct is capable of binding to both human and mouse BCMA, we are therefore able to fully characterize our lead assets in a relevant model system. Our strategy will enable further research to dissect host-tumour-effector cell interactions, to better inform the design and characterization of novel CAR-T cell therapies in MM.

The Role of SHPRH in Lung Adenocarcinoma Initiation and Development

Serena Chuang

Late-stage diagnosis of lung cancer (LC) is associated with poor prognosis and survival, highlighting a need for increased understanding of risk factors to support early screening and treatment strategies. While environmental factors play a significant role, genetic factors can greatly enhance LC risk in smokers and non-smokers. Using whole exome sequencing of never-smokers with lung adenocarcinoma (LUAD), our lab identified a candidate gene which could be linked to LC susceptibility: SHPRH. Previous work demonstrated that SHPRH acts as a tumour suppressor gene in the context of LUAD, but its mechanism has yet to be elucidated. This project aims to investigate the mechanism of SHPRH-mediated tumour suppression in LUAD cells and identify the key pathways involved with its phenotype. RNA-sequencing and immunoprecipitation-mass spectrometry (IP-MS) will be performed to identify transcriptomic and proteomic changes with SHPRH expression. This will help identify major biological processes and interactors associated with SHPRH, which can increase understanding of SHPRH's function and contribution to tumorigenesis. Genes found to be associated with SHPRH-mediated tumour suppression will be genetically manipulated via lentiviral transduction to be either conditionally expressed or knocked down. This will help validate the contribution of these genes to SHPRH's tumour suppressive phenotype. Increased understanding SHPRH's tumour suppressive function and contribution to LC initiation and development may help identify at-risk patients and increase opportunities for early intervention.

Speed Talk Abstracts

Predicting Advanced NSCLC Treatment Response with Combination Radiomics and Clinical Features in a Machine Learning Framework

Ian Janzen

Lung cancer is the leading cancer-caused death globally, mainly due to most diagnoses being made at incurable advanced stages. More than 75% advanced stage LC patients have no driver mutation therefore cytotoxic chemotherapy is the primary treatment option. Recent trials showed immunotherapy (IO; e.g., pembrolizumab, Pem) is superior to 1st-line chemotherapy. Identifying patients most likely to respond to IO is a challenge as no clinicopathological features or biomarkers -including PDL1 scores- are convincing predictors. Patient pre-selection is more critical in the cohort of similar high PDL1 level (≥50%), as half of this sub-cohort can benefit best with Pem monotherapy and others may respond to a combination Pem plus chemotherapy. The objective of this study is to develop a machine learning model trained on radiomic features extracted from baseline CT to predict response to pembrolizumab in advanced NSCLC with PDL1≥50%. This study included 97 stage IIIB/IV NSCLC patients with PDL1≥50% that received 1st-line Pem (56F:41M, 73±6 yo). Response was assessed using RECIST v1.1 standard definitions (n=60 “disease control, DC”; n=37 “progressive disease, PD”). A radiomic feature extraction pipeline was used to extract 2D radiomic shape, texture, and intensity features of lung tumor lesion. 5-fold cross validated ML model (Linear Regression) were trained on a combination of patient’s baseline clinical CT features and radiomic features to predict response (i.e., DC vs. PD group). Model performance was analyzed with ROC analysis (AUC: 0.87±0.03). The predicted response probability was used to classify patients into “high- vs. “low-probability responder” groups. Survival analysis indicates a separation between the two responder groups using the prediction model (log rank $p<0.001$). Therefore, a ML model framework can serve as a decision-making tool in choosing the optimal 1st line treatment for advanced NSCLC with PDL1 ≥50%, addressing a currently unresolved clinical dilemma.

Expanding the repertoire of self-amplifying ribonucleic acid vectors for next-generation RNA therapeutics

Credo Casmil

Self-amplifying RNA (saRNA) is structurally similar to mRNA and encodes a replicase enzyme that copies the transcript once delivered into the cell. Due to the auto-amplification properties, saRNA requires a lower dose (~100-fold) than typical mRNA. Historically, three viral backbones from the alphavirus genus have been used, including the Venezuelan Equine Encephalitis Virus (VEEV), Semliki Forest Virus and Sindbis Virus. We hypothesized that novel saRNA designs will result in different replication and protein expression profiles optimal for vaccine and therapeutic applications. Therefore, we designed multiple saRNA vectors based on other alphaviruses that can replicate in mammalian cells. To characterize these novel saRNA designs, we first tested their cytotoxicity levels by measuring mammalian cells' viability levels after transfection and their translational efficiency by quantifying the amount of green fluorescent protein (GFP) or firefly luciferase produced in vitro. We found that our novel saRNA designs (CC1, CC2, CC4 and CC6) had higher protein production than the state-of-the-art VEEV_TrD control by a 2- to 5-fold difference in mean fluorescent intensity of GFP+ cells. All constructs with higher protein expression in mammalian cells had similar toxicity levels as the control saRNA except CC6, with a drop of ~5% viable cells as determined through flow cytometry analysis. We then evaluated the saRNA constructs encoding luciferase in a preclinical murine model. Twenty-five days after intramuscular injection of saRNA lipid nanoparticles into female Balb/C mice, CC1, CC2, CC4 and CC6 saRNA had 8.6-, 7.6-, 8.6- and 1.4-fold higher total flux relative to the VEEV control saRNA. Additionally, different expression kinetics were observed for each saRNA construct. These results are a proof-of-concept that other alphaviral backbones can serve as saRNA vectors, and their performances can be predicted based on their evolutionary relationships.

Poster Abstracts

1.

Deep Learning-Based Risk Stratification of Low Grade Gliomas: Insights Beyond Molecular Markers

Katherine Rich

The current diagnostic framework for lower grade gliomas (LGG) focuses on the use of molecular biomarkers, most importantly IDH1/2 mutations and whole arm codeletion in chromosomes 1p and 19q, to predict patient prognosis and guide clinical care. However, LGGs are both morphologically and clinically heterogeneous. A subset of patients, despite having favourable clinical markers, will inevitably progress to glioblastoma and experience poor outcomes. We present a machine learning based biomarker that can stratify IDH mutant patients, who have the best prognosis, into high and low risk cohorts. The high risk cohort is shown experience significantly worse outcomes across a variety of datasets. Our training dataset consists of 844 H&E-stained WSIs from the The Cancer Genome Atlas Low Grade Glioma (TCGA-LGG), as well as an external validation set of 88 H&E-stained WSIs collected from a local site. We trained a self-supervised feature extractor on 256x256 pixel patches extracted at 20x magnification. Three-fold cross validation was then used to train the cox proportional hazard model, with 33% of the TCGA-LGG dataset held out for testing. Hazard scores for each patch were averaged to generate a single hazard score per patient. A pathologist then compared patches with high and low hazard scores to determine the histological features that were associated with high risk, and found a pattern of higher cellularity and pleomorphism. We achieved a c-index of 0.64 on the TCGA-LGG test dataset, and 0.66 on the external validation set. Further analysis of the IDH mutant cases revealed a significant difference in overall survival between the high hazard and low hazard cases. This was independent of both 1p19q codeletion status, as well as other clinical markers such as CDKN2A/B status. Our findings suggest that deep learning-based features can be used to predict outcome, as well as identify morphological biomarkers associated with high risk.

2.

Analytical consideration of cell type heterogeneity in pediatric saliva for DNA methylation analyses

Meingold Chan

Saliva samples, a popular choice in pediatric cohorts for measuring biomarkers due to its minimally invasive collection, has been shown to be an effective sample for measuring DNA methylation (DNAm). Cell type (CT) heterogeneity in bulk samples like saliva has been known to drive DNAm variation given that different cell types have distinct DNAm profiles, hence CT composition should be accounted for in DNAm analyses to prevent confounded study results. Reference-based CT deconvolution method can be used to estimate CT proportions from DNAm data, yet estimations can differ depending on the reference panels used, especially those developed based on samples from different ages and tissues. These issues hold particular significance in pediatric saliva samples given the high CT heterogeneity across individuals and ages. Therefore, the current study addresses important analytical considerations related to CT heterogeneity in pediatric saliva sample by characterizing the estimated CT compositions with two available references for buccal epithelial cells (BECs) and its impact on epigenome-wide association study (EWAS) using 529 saliva samples of 7-year-olds. CT deconvolution was conducted with the EpiDISH R package using robust partial-correlation methods and the two available references. BEC proportions estimated by the child reference has low median and high IQR (median = 80.7%, IQR = 42.3%) than that estimated by the adult reference (median = 51.9%, IQR = 70.8%), t=31.81, p < 2.2*10-16. The DNAm profile of the child reference aligned better with the measured DNAm profile in our samples than adult reference. EWAS models on socioeconomic status perform better when adjusting for CT proportions estimated with child than adult references. The results of this study supported the application of the child reference in cell type estimation and DNAm analyses with pediatric saliva.

Poster Abstracts

3.

Elucidating the regulatory logic of T-cell gene expression

Nicole Knoetze

Restricting transgene expression to specific cell types is an unmet need for many cell and gene therapies that could be addressed by encoding cell-type-specific regulatory logic within synthetic promoters. Toward this goal, we are searching T-cell open chromatin regions (OCRs) for the presence of recurrent DNA subsequences with T-cell-specific regulatory activity. We first integrated publicly available DNase-seq, RNA-seq, and Hi-C interaction data to obtain a set of putative T-cell OCRs associated with genes expressed in T cells. Next, we developed a Monté Carlo statistical framework to determine if pairs of DNA subsequences are enriched in T-cell OCRs linked to genes with enriched expression in T-cells, compared to T-cell OCRs from background genes. Specifically, the framework calculates the number of T-cell-enriched genes an OCR-embedded pair of DNA subsequences is linked to and compares this to the expected value from equally sized, randomly resampled sets of non-enriched T-cell genes. Using our framework, we identified nominally significant co-enriched DNA sequences that may interact with each other to regulate T-cell-specific gene expression. These co-enriched pairs are comprised of many uncharacterized DNA sequences and transcription factor (TF) binding sites for known T-cell TFs. To experimentally validate our findings and to decipher the specific combinations and orientations of these sequences that may be capable of driving T-cell-specific expression, we selected 18 candidate DNA subsequences representing top-ranked co-enriched pairs to create 101,306 synthetic T-cell regulatory elements. In upcoming work, we will validate the regulatory activity of these regulatory elements in a panel of T-cell and non-T cell-based cell lines using the STARR-seq assay. This experiment will also provide information on the regulatory logic (the copy number, order, and orientation of DNA sequences) controlling the expression of genes in T cells.

4.

Leveraging ML to Integrate Microbiome-Metabolome Reveals Host Disease Phenotype

Irvin Ng

The microbes and the metabolites that they produce form a dynamic and physiologically important environment known as the microbiome. It is well established that the microbiome is associated with a multitude of host diseases such as inflammatory bowel disease (IBD) but understanding and predicting associations of microbial taxa to particular diseases have historically been a difficult task. This is likely due to the complex and dynamic nature of the microbiome. Here, we leverage machine learning, namely a multi-layer perceptron neural network, to learn the complex patterns and interactions between the microbiome, metabolome, and IBD. My approach determines and extracts feature attribution scores between all microbe-metabolite pairs which allows me to investigate the complex microbiome-metabolome interactions in the gut and IBD status. We also show that we can predict the gut metabolome profile with just the taxonomic or functional features from a microbiome sample using multivariate learning. This knowledge not only offers insights into disease mechanisms but also facilitates the development of targeted interventions, leading to innovative and cost-efficient therapeutic strategies.

5.

Estimation of HIV cases averted in phylogenetic clusters through pre-exposure prophylaxis

Angela McLaughlin

Pre-exposure prophylaxis (PrEP) prevents HIV acquisition and is a key pillar to end the HIV epidemic. In BC, PrEP has been provided free of charge since 2018, but its population-level effectiveness is largely unknown. We estimated HIV cases in BC averted through PrEP, which we hypothesized were heterogeneously distributed across phylogenetic clusters. 42,043 HIV partial pol sequences from 10,740 individuals were aligned to HXB2 reference using minimap2 and drug resistance mutations were masked. Bootstrap maximum likelihood phylogenies were inferred in FastTree2 to identify clusters with at least 5 members with pairwise patristic distance <0.02 substitutions/site in >90% of bootstraps. We calculated clusters' growth rates and effective reproduction numbers (Re) using new diagnoses over time, then averaged over periods preceding (Jan 2016–Dec 2017) and following (Jan 2018–Feb 2020) widespread PrEP. Counterfactual models fit to growth rates preceding PrEP were used to quantify cases averted. Of 84 clusters with new cases since 2018, 44% of 52 clusters predominantly comprised of men who have sex with men (MSM) had significantly lower growth rates following PrEP compared to 17% of 30 clusters comprised of people who inject drugs (PWID); and Re was reduced in 45% and 31% of gbMSM and PWID clusters. PrEP averted 57-125 HIV cases between 2018 and February 2020, which were concentrated in four clusters of young (median age first ART: 27-35), gbMSM (79-92%), and included previous PrEP users who were later diagnosed, indicating PrEP use among cluster contacts. Rapid growth of clusters of PWID and older median age indicated missed opportunities for PrEP. Cluster-level modeling of interventions' effectiveness revealed PrEP differentially reduced clusters' growth, but cumulatively may have averted 2.2-4.8 new HIV cases per month. Combining phylogenetics and counterfactual models is a pragmatic approach to inform public health policy.

6.

Deep learning embedding for nucleotide sequences

Saber Hafezqorani

Enabled by the explosion of data and substantial increase in computational power, deep learning has transformed fields such as computer vision and natural language processing (NLP) and it has become a successful method to be applied to many transcriptomic analysis tasks. A core advantage of deep learning is its inherent capability to incorporate feature computation within the machine learning models. This results in a comprehensive and machine-readable representation of sequences, facilitating the creation of advanced classification and clustering models. However, compared to machine translation problems in NLP, feature embedding is particularly challenging for transcriptomic studies as the sequences are strings of arbitrary lengths and long-term dependencies between features from different parts of the sequence are difficult to capture. This highlights the need for expressive nucleotide sequence embedding methods that are capable of learning input sequence features implicitly. Here we introduce ntEmbd, a deep learning embedding tool that captures both local and global range dependencies between different features of the sequences and learns a latent representation for given nucleotide sequences. The tool as well as the trained models are freely available on GitHub at https://github.com/bcgsc/ntEmbd

Poster Abstracts

7.

Characterization of gene regulatory networks and combinatorial transcription factor interactions during pancreatic β-cell differentiation

Meltem Eda Omur

Generating an infinite source of insulin producing pancreatic β-cells using human pluripotent stem cells (hPSCs) is an active pursuit towards type 1 diabetes (T1D) treatment. T1D is characterized by pancreatic β-cell destruction by the body's immune system. While insulin replacement by injection or pumps is effective, restoration of healthy β-cells could be curative. As transplantation of donor cells is limited by supply, hPSCs could be the source as they can be differentiated into β-like cells under appropriate conditions.

Directed differentiation protocols include a series of transition steps to pancreatic lineage specific progenitor cells that can be further differentiated into β-cells. Cells exhibit diverse characteristics, which are determined, in part, by underlying gene regulatory networks (GRNs). These networks are composed of specific combinations of transcription factors (TFs) and their target genes. Deciphering GRNs underlying hPSCs-derived pancreatic β-cells differentiation offers opportunities to unravel the factors that define cellular phenotypes and improve differentiation protocols.

The presented research will describe the application of computational approaches to single cell datasets from different stages of pancreatic β-cells differentiation to gain insights into the mechanism driving transcriptomic and chromatin level changes during differentiation processes. Data characteristics and quality were assessed, demonstrating presence of cells of diverse characteristics. Focused analyses of the cell populations explored the contributing GRNs, including TFs and their target genes with coordinated expression.

Interactions between the TFs and across the GRNs will be presented, with a focus on combinatorial interactions of TFs in β-cell differentiation. These findings allow us to further develop models for gene regulatory network analysis and are guiding studies towards improved differentiation protocols.

8.

Identification of relationships between transcription factors and target genes from scientific documents using a fine-tuned PubMedBERT model

Alejandro Aguirre

Biomedical publications appear at an increasing rate, making it difficult to track accumulating knowledge. Global investments in genomics over 20 years have focused on the study of gene regulation, as understanding the human genome sequence requires us to understand how information is accessed across diverse cellular contexts. Thus, extensive literature growth in the topic area of gene regulation has accumulated. Natural Language Processing (NLP) and text-mining techniques can help researchers extract greater value from this literature. With the introduction of transformer neural network architectures and the Bi-directional Encoder Representations from Transformers (BERT) model, the state-of-the-art performance in several NLP tasks has significantly improved. BERT-based models for the biomedical field can be pre-trained with either a mix of general-domain and biomedical-specific text or just the latter. These models have then been finetuned for specific tasks, such as Named Entity Recognition (NER) and Relation Extraction (RE). Despite these advances, to the best of our knowledge, there has not been a BERT model fine-tuned for NER and RE tasks in the context of gene regulation. We are creating a new dataset for NER and RE of transcription factor and target gene interactions, and present a proof-of-concept of fine-tuning PubMedBERT for NER of molecular entities on a public dataset.

Poster Abstracts

9.

Recovery of a novel lineage of sulfur oxidizing denitrifiers in the Saanich Inlet water column using single-cell scaffold-anchored binning

Ryan McLaughlin

Saanich Inlet (SI) is a seasonally anoxic fjord on the coast of Vancouver Island, British Columbia, Canada that serves as a model ecosystem for studying microbial community responses to ocean deoxygenation. Intensive time series investigations of the SI water column have identified key microbes participating in coupled biogeochemical cycling of carbon, nitrogen and sulfur. Consistent with this observation, depth-specific population dynamics associated with different stages of stratification and deep water renewal in SI
have been correlated with variable process rates for denitrification, anammox and dissimilatory nitrate reduction to ammonium (DNRA). Unexpectedly, we observed a population of blooming Arcobacteraceae during renewal events reaching up to 30% of total microbial community abundance. We used scaffold-anchored binning combining single-cell genomes and metagenomes from the time series to assemble 14 Arcobacteraceae extended population genomes (xPGs) ranging between 90-97% completeness and 0-5% contamination. Each genome contained a full-length small subunit ribosomal RNA gene and near-complete repertoires of single-copy marker genes enabling genome-resolved comparisons to other low oxygen marine waters. Collectively, these xPGs formed a globally distributed and distinct clade within the Arcobacteraceae family differentiated from a previously described Sulfurimonas lineage in the Baltic Sea. Genes encoding complete denitrification coupled to sulfur oxidation and the reductive TCA cycle were annotated in all xPGs. When combined with the existing compendia of geochemical and multi-omic sequence information from SI, these data enable more in-depth metabolic reconstruction and numerical modeling of the marine nitrogen cycle relevant to both nitrogen loss and retention processes.

10.

Time-resolved fosmid library pool selection for hydrocarbon tolerance traits in Escherichia coli

Tony Liu

Solvent tolerance is an industrially relevant trait integrating the combined activities of multiple gene circuits within microbial cells. While many genes encoding functional and regulatory proteins associated with solvent tolerance have been identified in isolated reference strains, the diversity and function of these genes in natural and engineered environments remains to be constrained with implications for biotechnology innovation. Here, we designed, built, and tested a functional screening paradigm based on large-insert (fosmid) library pool selection to recover clones conferring hydrocarbon tolerance in Escherichia coli from environmental genomes. After establishing a baseline for tolerance, a fosmid library pool sourced from a methanogenic enrichment culture capable of growth on short-chain alkanes, was cultivated in the presence of inhibitory concentrations of the aromatic hydrocarbon toluene. Fosmid pool sequencing indicated a loss of clone diversity over time with relatively few unique clones remaining after three passages. Clones recovered under selection encoded functional and regulatory proteins relevant to toluene tolerance including fatty acid, glycolipid, glycophospholipid, and lipopolysaccharide biosynthesis, and isoprenoid biosynthesis to modulate cell membrane composition, efflux pumps and other transporters, antioxidant enzymes, as well as chaperones and chaperonins involved in protein folding.

Poster Abstracts

11.

Automatic Bevacizumab Response Prediction for Ovarian Cancer using Histopathology Images

Mayur Mallya

Epithelial ovarian cancer is a lethal gynecologic cancer with high mortality and recurrence rates to traditional treatment involving surgery and chemotherapy. Bevacizumab, as an alternative to traditional treatment, is a targeted therapeu-tic drug that has demonstrated efficacy in recurrent disease. However, there is a lack of concrete biomarkers to predict the potential patient-specific prognosis when treated with bevacizumab. In this work, we leverage the digitized tissue slides from the MICCAI ATEC23 challenge that are collected as part of the clinical routine to predict the slide-level bevacizumab treatment effectiveness. Our deep multiple instance learning (MIL) based model validated across mul-tiple combinations of benchmark MIL models and pre-trained encoders, is able to achieve a top prediction accuracy of 85% on the internal test set. Further, for the external test set of TMA cores, we ensemble the best models based on their performance on the internal test set in order to predict the bevacizumab treatment response.

12.

Why does coexpression predict gene function?

Alexander Adrian-Hamazaki

It is widely accepted in genomics that coexpression of RNA transcripts suggests a commonality of function. This observation is explicitly leveraged in machine learning methods for gene function prediction, where it is often combined with other features such as protein interactions and sequence similarity. For example, including data from human tissue expression profiles boosts performance in prediction of Gene Ontology annotations. However, the biological underpinning of this observation have not been well-investigated. Building on earlier results from our group, in my work I show that the gene function is predictable from coexpression substantially because it reflects differences in expression between cell types. Using simulations and analyses of real data, I show that variance in the cellular composition of samples contributes positively to predictive performance, and that increasing homogeneity or removing the influence of individual cell type reduces performance. I further show that this phenomenon is particularly relevant to the prediction of genes involved in cell-type-restricted functions. Finally, I show that cell type profiles, where the relationship between gene expression and cell type is made transparent, is effective for predicting gene function while increasing interpretability. My results have implications for how gene function prediction methods are developed, evaluated and interpreted.

Poster Abstracts

13.

Tracking clonal structure from leukemia diagnosis to relapse using mitochondrial variants

Derek Tam

Acute myeloid leukemia (AML) is the most common form of blood cancer in the adult population, with an incidence of over 1,100 cases per year in Canada. AML is characterised by clonal expansion of immature blood cells that lead to bone marrow failure. AML is a notoriously heterogeneous disease despite its low mutational burden, leading to a relapse rate of over 40% within three years of remission. Relapse is attributed to the persistence of leukemic stem cells which emerge from molecular heterogeneity and eventually repopulate the leukemia. Understanding patterns in clonal structure is essential for comprehending the factors involved in relapse. In this study, we develop a single-cell DNA-sequencing panel that attempts to infer the clonal structure in a blood sample using a combination of nuclear and mitochondrial variants. Mitochondrial DNA is reported to have more than a 10-fold increased rate of mutation relative to nuclear DNA, and variants are similarly inherited at cell division. These properties make mitochondrial variants particularly useful for tracing clonal relationships between individual cells. The developed panel is applied to bone marrow samples collected from patients diagnosed with AML both before treatment and after relapse. Clones are inferred using the variants in each sample, which are then paired across time points to assess how clonal structure changes. Two patterns of relapse emerge. In the first, a clear shift in clonal structure can be observed, where the dominant clone at diagnosis shrinks and is replaced by another clone at relapse. In the second pattern, the dominant clone at diagnosis remains dominant at relapse and is present at a similar frequency. However, using mitochondrial variants to infer the subclonal structure of this population, expansion of a clone that is not defined by nuclear variants is observed, opening the possibility of selective pressure from treatment favouring an epigenetic difference between these subclones.

14.

Exploring B cell repertoire evolution post-vaccination via mathematical modelling and phylogenetic trees

Rituparna Banerjee

The immune system relies heavily on the B cell repertoire to combat pathogens effectively. Through longitudinal studies, it's evident that B cell populations evolve in response to each vaccine dose, fostering increased diversity. This project aims to unravel the mechanisms driving the diversification of the B cell repertoire following a two-dose vaccine regimen under two vaccination systems: homologous (identical strains for priming and boosting) and heterologous (different strains for priming and boosting). By integrating pathways of immune response like the extra-follicular and germinal center pathways into a mathematical model, we seek to elucidate how these systems impact repertoire diversity. Utilising simulated B cell data, we then plan to construct phylogenetic trees to discern differences in immune responses between homologous and heterologous vaccination, specifically investigating whether diversity increases with heterologous vaccination. Additionally, we aim to explore how varying the time between vaccinations influences repertoire diversification. Eventually, we also plan on comparing the phylogenetic trees of simulated data with that of data from longitudinal studies to validate the observations from the former.

Poster Abstracts

15.

Comparative Single-Nucleus Transcriptomic Analysis of Gene Co-Expression in Alzheimer's and Healthy Brains

Nairuz Elazzabi

Alzheimer's disease (AD) is the most common cause of dementia worldwide, pathologically defined by the accumulation of amyloid plaques and neurofibrillary tangles in the brain. Recent advancements in single-nucleus RNA-sequencing have enabled the comprehensive profiling of cell-type-specific transcriptomic alterations within the postmortem frontal cortex of individuals at varying stages of AD pathology. These studies have shown that AD pathogenesis involves the complex interplay of virtually every major brain cell type, identifying gene expression signatures that delineate the transition from healthy aging to cognitive decline. In this project, I aim to assess the functional implications of these reported gene expression changes within the context of AD. Specifically, this project will examine changes in co-expression patterns through meta-analytic analyses. Leveraging large-scale, publicly available single- nucleus expression datasets encompassing prefrontal cortex samples from postmortem individuals spanning various AD pathological and clinical stages, as well as healthy controls and a rare cohort of fresh cortical biopsies, I will curate an extensive dataset. Utilizing this curated dataset, I will construct cell-type-specific gene co-expression networks to perform comparative analyses between AD and healthy samples, thereby shedding light on the underlying molecular mechanisms driving AD progression.

16.

Use of long read whole genome sequencing for precision diagnosis and treatment of individuals with Autism Spectrum Disorders

Sarah Dada

Background: Autism Spectrum Disorder (ASD) is the most common childhood developmental disability, affecting 1 in 58 Canadian school-aged children. ASD is defined by deficits in social communication and interactions, as well as restricted and repetitive behaviours. ASD diagnosis is complex with a highly variable pattern of behavioural symptoms. ASD is heterogeneous and can be caused genetically by both inherited and de novo mutations. Structural Variants (SV) represent substantial genomic diversity. Their role in ASD is undetermined, largely due to limitations in the commonly used short read whole genome sequencing (WGS). Similarly, aberrant DNA methylation is known to be associated with ASD. Long read genome sequencing offers previously unseen insight into the genome of individuals with ASD at a reasonable cost, while deriving small variants, SV, and methylation patterns. My project will derive and integrate previously unseen genomic changes identified from long read genome sequencing with phenotypic (symptom-based) data, to improve diagnosis and treatments in participants with ASD.

Methods: From a cohort of 500 participants with childhood ASD I will identify participants who have no definitive genetic abnormalities from pre-existing short read WGS. Using long read WGS I will analyze and determine the impact of participants' structural variants, their methylome and their imprinting. I will look for variant association within cohort and public data, and integrate the genomic data with the participants' clinical phenotype to resolve the implicated behavioural impacts of found variants.

Results: I have discovered novel complex large structural variants and associated methylation patterns among several patients.

Conclusion: This project will increase the clinical utility genomic data by providing a stable and defined ASD view of the patient, which will allow us to provide an individualized and cost-effective treatment in an anticipatory, rather than a reactive way.

Poster Abstracts

17.

Genetic Risk for Anorexia Nervosa and Association with General Dimensions of Psychopathology in Childhood

Karanvir Singh

Eating disorders (EDs) are a class of deadly and debilitating psychiatric conditions best characterized by grave disturbances in eating and/or weight regulation behaviours. 15% of females and 5.5% of males are estimated to be at risk for an eating disorder in high-income countries, which in Canada roughly translates to 7.5 million people experiencing an eating-related illness. Anorexia nervosa (AN) is the most well-studied ED and is characterized by restriction of food intake, which results in a dangerously low body weight. Psychological, social and biological factors all play a role in AN pathophysiology. Heritability estimates for AN are between 0.48 and 0.74, which are on par with those of other psychiatric traits (such as major depressive disorder) and obesity. Genetic susceptibility to AN (as measured by a polygenic risk score, PRS) is associated with lower body mass index (BMI) and lower weight trajectories in the general population. Whether genetic susceptibility to AN is associated with dimensions of childhood psychopathology, including how the genetic overlap with anthropometric and metabolic traits affects these associations, is unknown. We obtained GWAS summary statistics for AN and BMI. To disentangle the genetic correlation between AN and BMI, we ran a "GWAS-by-subtraction", resulting in a set of "conditional AN" summary statistics. We developed PRSs for children in the Canadian Healthy Infant Longitudinal Development (CHILD) study. Our GWAS-by-subtraction results showed that after adjusting for the genetic correlation with BMI, the 8 risk loci for AN had reduced effect sizes, and most were no longer genome-wide significant. Higher AN PRS was nominally associated with lower attention problems at 5 years (IRR=0.89, p=0.005) in a model adjusted for sex, parity and family income. Effects in the opposite direction were observed for BMI PRS. We found no associations between AN genetic risk and physical growth during the first 5 years of life.

18.

Spatial transcriptomics deconvolution using marker-gene-assisted topic models

Yolanda Yang

Spatial transcriptomics (ST) offers valuable insights into gene expression patterns within the spatial context of tissue. However, most ST technologies do not have a single-cell resolution, masking the signal of the individual cell types. In our study, we developed SMART (Spatial transcriptomics deconvolution using MARker-gene-assisted Topic models), a reference-free deconvolution method that simultaneously infers the cell type-specific gene expression profile and the cellular composition at each spot. Unlike most existing methods that rely on having a single-cell RNA-sequencing dataset as the reference, SMART uses marker gene symbols as the prior knowledge to guide the deconvolution process. To evaluate our method, we simulated ST datasets from single-cell ST data. Pearson's correlation r and root mean square error (RMSE) between the predicted and ground truth cell type proportions were used as the evaluation metrics. In multiple simulated and real ST datasets, SMART demonstrated improved performance and lower variability in realistic settings even when compared to some of the best-performing reference-based methods ($P<0.05$ using per-spot RMSE). Both the predicted cell type proportions and the cell type-specific gene expression profile were strongly correlated with the ground truth ($r>0.7$ for each cell type). SMART also provides a two-stage approach to enhance its performance on cell subtypes. Allowing the inclusion of covariates, it provides condition-specific estimates and enables the identification of cell type-specific differentially expressed genes across conditions, which elucidates biological changes at a single-cell-type resolution.

Poster Abstracts

19.

CytokineFinder: benchmarking methods and databases for identifying cytokines

Jeffrey Tang

Cytokines are intercellular signaling molecules that regulate innate and acquired immunity, and inflammation within the body. These responses play a critical role in allergy, asthma, and clinical immunology, influencing cell-cell communication within or between tissue types, which can be insightful for clinicians. Recent advances in high-throughput sequence have allowed for a growing number of cytokine data sets but there are a limited number of methods and databases that shows a systematic approach in identifying cytokines. Thus, developing a tool to identify and rank cytokines based on publicly available datasets and methods can provide insight on a consensus of cytokines that may have clinical utility. We hypothesize that systematic benchmarking of gene expression data sets will allow us to evaluate approaches that can best detect key cytokines. We used publicly available datasets to assess various methods. Five cytokine ranking approaches were used. Cytokine data can be useful for clinicians and the recent popularization of integrating multi-omics datasets have created an influx in cytokine data availability. In this study, we found methods that used Baderman lab annotations were ranked higher than other databases while we found NicheNet and Fantom5 annotations were lower. There was high variability in rankings for the cFGSEA method which could mean that methods may be sensitive to annotation quality. This signifies the need for development of higher quality databases.

20.

Single-cell Characterization of Genomics and Transcriptomics in the Hodgkin and Reed Sternberg Cells

Shaocheng Wu

The characterization of rare malignant Hodgkin and Reed Sternberg (HRS) cells in classic Hodgkin lymphoma (cHL) has made some progress with the bulk sequencing data but complex in-depth single cell characterization of this population in terms of genomics and transcriptomics are still lacking. Here, we applied single cell DNA (scDNA) and single cell RNA (scRNA) sequencing on purified HRS samples. Together with the whole-exome sequencing (WES) data, we characterized the genomics, transcriptomics, and their evolution in HRS cells.

Poster Abstracts

21.

Fast and Memory Genome Completeness Assessment

Johnathan Wong

In recent years, the ever-increasing affordability and throughput of DNA sequencing has resulted in the democratization of whole genome sequencing and, consequently, a surge in the number of publicly available draft genome sequences. One common genome quality evaluation method is measuring the completeness of an assembly in the genic space using a tool such as BUSCO. BUSCO, however, takes a considerable amount of time to run on large genomes, and requires a substantial amount of diskspace and RAM. We developed a utility that addresses these issues with the existing genome completeness assessment tools, while also providing new functionality. To assess the presence and completeness of input proteins in a genome, the tool employs a two-step workflow, building on recent cutting-edge bioinformatics advances, such as fast and efficient hashing (ntHash) and the multi-index Bloom filter (miBf), a space-efficient probabilistic data structure. The utility first generates a database of protein sequences provided by the user. Next, the input genome sequences are six-frame translated into corresponding amino acids. Each frame is shredded into words of length k (i.e. k-merized), and hashed using aaHash, a hashing algorithm derived from ntHash for amino acids, to query database proteins stored in miBf. A given protein is classified as "complete", "partial", or "missing" based on a metric combining the number of k-¬mer hits and the synteny between the translated genomic sequences and hits in the protein database. The tool uses less than 15GB of ram and takes 2 minutes to assess the completeness of a human genome using the human proteome as the database.

22.

Biomarker prediction in wheat for Leaf rust resistance and susceptibility, a RNA-seq batch effect aware classification approach

Sean Formby

Wheat, like other plants, has evolved sophisticated responses to defend against pathogen insult. Pathogenic microbes, evading the plant's preformed defences, can be arrested through induction of PAMP (Pathogen Associated Molecular Pattern)- and/or Effector-Triggered Immunity(ETI). Molecular differences which characterize resistant versus a susceptible phenotype in wheat cultivars are important to understand for the future development of disease resistant wheat. RNA-seq, combined with differential transcript expression (DTE) analysis, is a powerful tool to evaluate the transcriptional changes that occur when wheat is infected with a pathogen. However, most tools for DTE analysis rely on comparing only two conditions, control versus treatment. To glean a more nuanced understanding of the resistance phenotype and to characterize the transcriptional profile involved, machine learning classifiers can be applied to the gene counts generated from these datasets. The result is a model, and a list of features (genes) that, together, can potentially predict a resistant versus susceptible phenotype. Moreover, the selected features provide a short list from the thousands of genes which can then be scrutinized and tested as potential biomarkers for disease resistance or susceptibility. Here, an extensive RNA-seq dataset from >150 leaf rust-infected wheat samples were generated and combined with publicly available data to train a variety of machine learning classifier models resulting in a relatively high predictive set of classifiers. Additionally, from these models, transcriptional differences underpinning the molecular mechanisms of resistant versus susceptible phenotypes in wheat to leaf rust insult were explored and potential candidate biomarkers of disease were found. Such biomarkers could be used for accelerating development of resistant cultivars and early detection of disease before the appearance of symptoms, allowing for more effective treatments.

Poster Abstracts

23.

Tree-structured topic modelling of single-cell gene expression data uncovers hierarchical relationships between immune cell types

Pattie Ye

Immune cells undergo a series of differentiation steps following a lineage-tree structure stemming from hematopoietic stem cells. During differentiation of immune cells in both homeostasis and pathological processes, many gene regulatory mechanisms are shared by fully differentiated immune cell sub-types. In order to characterize these features quantitatively, we propose LaRCH, a tree-structured embedded topic model. In this model, single-cell gene expression profiles are represented by a mixture of topics consisting of latent features that follow an underlying tree structure, mirroring that of cellular differentiation--nested cluster structures. We present findings of our model trained on simulated single-cell RNA sequencing (scRNA-seq) based on cell-sorted bulk RNA-seq data as well as on a scRNA-seq dataset of over 1.2 million cells from healthy individuals and individuals diagnosed with systemic lupus erythematosus (SLE). The cellular topic profiles estimated by our model markedly improve clustering accuracy over traditional latent variable models and illustrate transcriptomic differences between SLE phenotypes, revealing a pivotal role of multiple immune cell types in disease progression and relapse. Ultimately, LaRCH captures the hierarchical context between cellular subtypes by simultaneously identifying shared and distinct latent features amongst subsets of heterogeneous samples of cells.

24.

AIEdit: polishing genome assemblies using machine learning and spaced seeds

Parham Kazemi

High-quality genome assemblies serve as a bedrock for discovering genetic diseases and evolutionary dynamics across diverse organisms. Polishing, a critical stage in assembly pipelines, enhances the accuracy of draft assemblies by correcting mismatches and small indels, which are often prevalent in assemblies obtained from third-generation sequencing technologies. Commonly employed approaches to genome polishing include alignment-based methods (e.g., Pilon, Apollo, and BlockPolish) and k-mer-based methods (e.g., ntEdit, JASPER). Alignment-based techniques run alignment algorithms to build consensus sequences, ensuring robustness, whereas k-mer-based methods rely on frequency analysis of short substrings, offering a less computationally intensive alternative. However, the sensitivity of k-mers to sequencing errors poses a challenge when detecting multiple errors within the span of k bases. Here, we present AIEdit, a polisher that uses spaced seeds, i.e., k-mers with ‘care’ and ‘do not care’ positions, and machine learning (ML) techniques to detect multiple errors in small regions. AIEdit’s ML model, trained once for each parameter set, operates independently of the dataset and assembly, thus allowing the use of pretrained models for polishing any assembly. AIEdit’s polishing time is comparable to non-machine learning k-mer-based polishers, while delivering high accuracy, with 98% and 86% of mismatches and 94% and 70% of indels fixed in assemblies with simulated sequencing data from E. coli and C. elegans, respectively. AIEdit also fixes 64% of the errors in a draft assembly generated with the GoldRush assembler using ONT data from D. melanogaster. In summary, we show AIEdit’s efficacy in polishing assemblies using both short-read and long-read data and underscore its integration into genome assembly pipelines. Additionally, we demonstrate the benefits of utilizing AIEdit and ntEdit together, facilitated by the sharing of Bloom filters between the two tools.

25.

Simulating the Evolution of Regulatory Complexity in Eukaryotic Populations

Madison Chapel

Prokaryotes and eukaryotes regulate their genomes in fundamentally different ways, as demonstrated by the architecutre of their gene regulatory networks (GRNs). Eukaryotic GRNs are complex; a single gene's expression may be influenced by many transcription factors (TFs), while each TF can influence the expression of many genes. In prokaryotes, TFs bind with high specificity to a few target regions, resulting in low-complexity GRNs. The role that different reproductive mechanisms (i.e., sexual vs asexual reproduction) between eukaryotes and prokaryotes plays in the complexity of their regulatory logic has been under-explored. I hypothesize that eukaryotic GRN complexity arises as a form of mutational robustness due to recombination during sexual reproduction. A tight coupling between TFs and their binding sites (i.e., a low-complexity network) would be strongly disfavoured, as recombination could introduce alternate TF alleles from the other parent, changing target expression levels, and potentially decreasing overall fitness.

To explore the evolution of eukaryotic regulatory complexity, individual-based evolution simulations were performed. In these simulations, individuals are represented as directed graphs of regulatory interactions. A biochemically-informed model of transcription factor binding was used to determine gene target expression levels and overall fitness. As expected, populations evolving under conditions of sexual reproduction tended towards higher complexity than asexual populations. Asexual populations also demonstrated lower overall fitness, and an increasing trend in mutational susceptibility – a measure of the robustness of their GRNs – while the mutational susceptibility of sexual populations remained constant over time. Future work will apply this evolutionary model to investigate robustness and complexity in other contexts, such as robustness to gene duplication events.

26.

Decoding the Epigenetics and Chromatin Loop Dynamics of Androgen Receptor-Mediated Transcription

Umut Berkay Altıntaş

Androgen receptor (AR)-mediated transcription plays a critical role in normal prostate development and prostate cancer growth. AR drives gene expression by binding to thousands of cis-regulatory elements (CRE) that loop to hundreds of target promoters. With multiple CREs interacting with a single promoter, it remains unclear how individual AR bound CREs contribute to gene expression. To characterize the involvement of these CREs, we investigated the AR driven epigenetic and chromosomal chromatin looping changes. We collected a kinetic multi omic dataset comprised of steady-state mRNA, chromatin accessibility, transcription factor binding, histone modifications, chromatin looping, and nascent RNA. Using an integrated regulatory network, we found that AR binding induces sequential changes in the epigenetic features at CREs, independent of gene expression. Further, we showed that binding of AR does not result in a substantial rewiring of chromatin loops, but instead increases the contact frequency of pre-existing loops to target promoters. Our results show that gene expression strongly correlates to the changes in contact frequency. We then proposed and experimentally validated an unbalanced multi-enhancer model where the impact on gene expression of AR bound enhancers is heterogeneous, and is proportional to their contact frequency with target gene promoters. Overall, these findings provide new insight into AR-mediated gene expression upon acute androgen simulation and develop a mechanistic framework to investigate nuclear receptor mediated perturbations.

27.

Examining Differential Expression Patterns of Drug Treatment Conditions in a Large Data Corpus

Neera Patadia

In genomics, we are interested in understanding how gene activity is regulated in response to biological conditions. This can be examined by performing differential expression (DE) analyses, where the expression of genes are compared between a baseline and biological condition of interest. Individual DE studies offer insights into gene expression, but the specificity of DE genes to a particular condition remains a question. Aggregating DE studies covering different biological conditions allows for the examination of global patterns of gene DE. The identified DE patterns can then guide downstream analyses to see if they can be grouped into distinct modules, and investigated for their underlying molecular bases. Previous attempts at answering these questions have been limited by quantity and diversity of high-quality DE datasets. To overcome these issues, the Pavlidis lab has developed the Gemma database, which aggregates thousands of DE spanning many biological contexts. In this project, I plan to analyze the DE landscape for drug treatment conditions in Gemma, where experiments examining the same or similar drug treatments are expected to have similar patterns of DE expression. To carry out this analysis, patterns of DE among experimental contrasts will be investigated by performing co-differential expression analyses. Identified co-differentially expressed genes will be interpreted through functional annotation analysis. Understanding relationships between biological conditions based on similarities between gene expression profiles can allow for the identification of molecular similarities between biological conditions. This can help guide our understanding of the molecular dynamics which underlie the DE landscape.

28.

Effects of tACS on electrophysiological signals are task-dependent

Abhijit Chinchani

Transcranial alternating current stimulation (tACS) is a non-invasive technique that delivers low-intensity alternating currents intending to affect neural activity and behavior. Recent research has shown that the effects of tACS are often inconsistent and not replicable. In this study, we investigated the effects of 10Hz alpha (vs 41Hz gamma) stimulation on alpha oscillations during a vigilance-oddball paradigm.

Participants (n=38) underwent occipital alpha (10Hz) and gamma (41Hz) stimulation, on separate days, where they performed three blocks of a vigilance-oddball task: pre-stimulation, alpha or gamma stimulation (STIM), and post-stimulation. During the task, they responded to one of 2 color changes to the fixation cross (DEFAULT for 80% and ODDBALL for 20% of the trials). Half the participants used their left index finger for the DEFAULT color change and the other half their right. Simultaneous EEG is recorded from 256 electrodes.

We observed that enhancement in alpha power (Δ = POST - PRE) was greater for alpha stimulation than gamma stimulation but only for the contralateral electrodes ($t(37)=-2.55$, $p=0.015$) to the dominant response hand and not for the ipsilateral electrodes ($t(37)=1.45$, $p=0.156$).

Our findings reveal a lateralized effect of tACS though our tACS electrode montage wasn't lateralized. This implies that the effect is likely driven by the motor planning aspects involved during the task paradigm. Thus suggesting that the effects of tACS on electrophysiological signals depend on the nature of the task being performed.

Poster Abstracts

29.

Phylter: Physlr read filter improves GoldRush genome assemblies

Amirhossein Afshinfard

GoldRush, a cutting-edge de novo genome assembly tool developed in our lab and introduced in Nature Communications (2023), presents a novel approach to long-read genome assembly. Leveraging a linear-time algorithm, GoldRush initiates assembly by selecting 1x coverage of the read dataset, followed by downstream scaffolding, correction, and polishing. While GoldRush demonstrates superior resource efficiency and comparable contiguity and correctness metrics against existing methods, the ultimate assembly quality is inherently constrained by the initial read selection. Addressing this limitation, we propose an enhancement to GoldRush by integrating a read dataset filter, courtesy of Physlr, tailored for chimeric and lower-quality reads. Through comprehensive experiments conducted on three human cell lines (CHM13, HG002, and HG01243), our study showcases substantial improvements across key metrics – both at large-scale and base resolution. Notably, enhancements are observed in NGA50 (32%, 19%, and 4%), misassemblies (-6%, -11%, and -15%), local misassemblies (-75%, -6%, and -44%), unaligned contigs (-73%, -24%, and -62%), mismatches (-58%, -3%, and -46%), and indels (-33%, -2%, and 36%). These improvements are achieved while incurring only a marginal increase in runtime (1.3x), underscoring the efficiency of Phylter in enhancing genome assembly accuracy.

30.

Identifying Active and Druggable Pathways in Primary and Metastatic Cancers through Application of Machine Learning Algorithms

Faeze Keshavarz

DNA is exposed to many mutagenic factors that can alter protein functions and the cellular pathways they participate in. In extreme cases, the accrual of these changes can lead to uncontrolled cellular proliferation i.e., cancer. A key to understanding cancer is to determine the impact on the cellular pathways that the repertoire of mutations accrued in a cancer cell cause. Such information will not just improve our understanding of the disease but will help identify tumour vulnerabilities. Advances in the next-generation sequencing technologies have led to the generation of a large volume of data. This wealth of omics data has facilitated numerous advancements in our understanding of cancer. Several machine learning approaches have been designed to investigate the transcriptome of cancerous cells. We could successfully show that a random forest model can identify transcriptional signatures of disease that are associated with the loss of wild-type activity of some cancer-related genes. Our investigations have also demonstrated that such transcriptional patterns can exist either at a pan-cancer level or tumour type specific level. The findings from this work aids in identifying tumour vulnerabilities and opens avenues for the development of novel targeted therapies for patients with cancer.

Poster Abstracts

31.

Fact-Checking in Cancer Knowledge Bases Using Large Language Models

Caralyn Reisle

Tailoring treatment to an individual's genetic profile through precision oncology has played an increasing role in cancer treatment over the last decade. However, interpretation is currently one of the most significant bottlenecks during the analysis of genomic results due to the very high level of expertise in cancer biology, genomics and bioinformatics required. Efforts to streamline this process include creating knowledge bases (KB) to store annotations of individual genes and variants. The popular open-data cancer KB CIViC (https://civicdb.org) adopted a crowd-sourcing method to curate structured content efficiently. However, these submissions still require expert review. Accurate KBs are essential to interpreting genomic findings. Recent advances in computing have given rise to large generative text models, such as GPT-4, commonly referred to as large language models (LLMs). LLMs show impressive performance but are prone to errors, emphasizing the importance of fact-checking, especially in high-stakes applications such as precision oncology. Several fact-checking models and datasets exist but are out of domain (political facts, celebrity news, etc.) or limited to scientific abstracts. By contrast, the claims that will need to be verified in the precision oncology domain are complex and require scientific expertise as well as the integration of highly heterogeneous knowledge ranging from basic functional experiments to advanced clinical trials. To accomplish this we have created a fact-checking companion dataset for the CIViC KB, CIViC-Fact. We have used CIViC-Fact to train language models. Preliminary results are promising, with accuracy of 93.5% and 79.5% with BERT-based models and LLMs, respectively. This accuracy will continue to improve as the dataset grows. Future work includes open-access publication of this dataset so that the scientific community may use it to further the field of fact-checking and ultimately improve KBs used for precision oncology.

32.

Predicting Autoinhibitory Protein States with AlphaFold2

Brooks Perkins-Jechow

Autoinhibition is a self-regulatory mechanism in cells where proteins can switch themselves “on” or “off” in response to certain stimuli. It is used in myriad processes from signaling, to transcription, to degradation, making it an ideal drug target and an important component of multiple biomolecular disciplines. Previous understanding of autoinhibitory structures was challenged by obtaining experimental protein structures. Current methods can take months, or even years, to deliver results. AlphaFold2 may change that. AlphaFold2, developed by Google Deepmind, is a machine learning model used to predict protein structures that approaches or equals empirical structures in accuracy. Users input a protein sequence and AlphaFold2 produces a possible structure. However, like many other AI algorithms, it is a “black box”, meaning that the reason for which a particular protein structure and conformation is predicted is unknown. The information AlphaFold2 uses to predict protein structures comes from a multiple sequence alignment (MSA) of the given sequence and structural “templates” that AlphaFold2 pulls from the Protein Data Bank (PDB). Several studies have shown that altering the multiple sequence alignment depth of AlphaFold2’s input leads to differing structural outputs for the same protein. This suggests that there is an evolutionary basis for AlphaFold2’s decisions. By comparing predicted structures of varying inputs with solved experimental structures, I will determine whether a desired conformation of a predicted protein structure can be actively selected for. Such a possibility would allow us to generate structures of both “on” and “off” autoinhibitory proteins intelligently, affording insights that could be used in drug development and applied to many fields of research.

Poster Abstracts

33.

Regulatory activity is the default DNA state in eukaryotes

Ishika Luthra

Genomes encode for genes and the regulatory signals that enable those genes to be transcribed, and are continually shaped by evolution. Genomes, including those of human and yeast, encode for numerous regulatory elements and transcripts that have limited evidence of conservation or function. Here, we sought to create a genomic null hypothesis by quantifying the gene regulatory activity of evolutionarily naïve DNA, using RNA-seq of evolutionarily distant DNA expressed in yeast and computational predictions of random DNA activity in human cells and tissues. In yeast, we found that >99% of bases in naïve DNA expressed as part of one or more transcripts. In humans, we found that, while random DNA is predicted to have minimal activity, dinucleotide content-matched randomized DNA is predicted to have much of the regulatory activity of evolved sequences. Naïve human DNA is predicted to be more cell type-specific than evolved DNA and is predicted to generate co-occurring chromatin marks, indicating that these are not reliable indicators of selection. Our results indicate that evolving regulatory activity from naïve DNA is comparatively easy in both yeast and humans, and we expect to see many biochemically active and cell type-specific DNA sequences in the absence of selection.

34.

ChemSightTransformer (CST): a transformer architecture to achieve chemical structure de novo generation and clustering from MS/MS data

Yukai Wang

We introduce ChemSightTransformer (CST), a novel approach for elucidating molecular structures from MS/MS spectra. CST uniquely incorporates a transformer model that simultaneously learns from molecular fingerprints and SMILES representations, capturing structural details at varying levels of granularity. This dual learning strategy allows for effective exploration of the chemical space, facilitating both the identification of known structures and the discovery of novel ones. Our evaluations using the CASMI benchmark demonstrate that CST delivers competitive performance. Significantly, we show that CST's approach of combining fingerprint regression with structure generation outperforms methods focusing on a single aspect. These findings indicate that employing a mixed-granularity approach for probing chemical space is an effective strategy for molecular structure elucidation.

35.

ASAPP - Annotation of Single-cells by Approximate Pseudo-bulk Projection

Sishir Subedi

Large-scale multimodal studies profiling single-cell have increased in recent years, generating unprecedented amounts of omics data and enormous opportunities to gain insights into biological processes. However, the growth rate in data generation has yet to be matched by the development of powerful and robust computational methods capable of handling large-scale datasets on a million scale. Traditional methods of analyzing single-cell datasets include dimension reduction methods such as principal component analysis (PCA), which is not scalable to large datasets due to cubic time computational complexity. Here, we propose a highly scalable single-cell annotation tool, ASAPP (Annotation of Single-cells by Approximate Pseudo-bulk Projection), capable of analyzing million-scale cells within an hour using modest computational resources. We show that ASAPP generates unbiased, interpretable cellular topics using simulated and real datasets. Further, we extend the applicability of our method using transfer learning to analyze multimodal datasets, including bulk-RNAseq and spatial data. ASAPP is a new scalable tool that provides a robust alternative approach for clustering scRNA-seq data with interpretable results, a crucial step in the bioinformatics analysis pipeline.

36.

Identification of Sex-Specific DNA Methylation in Cord Blood

Denitsa Vasileva

DNA methylation (DNAm) is a dynamic epigenetic modification at Cytosine-p-Guanine (CpG) dinucleotides. Differences in autosomal DNAm between males and females have been reported and may explain the sex-specific prevalence of diseases such as asthma, autism and scleroderma. Asthma exhibits changes in prevalence and course by age and sex with most (65%) of childhood-onset cases seen in boys and resolved over time while adult asthma is chronic and predominantly female (65%). Autism (70% male) and scleroderma (75% female) both demonstrate sex-associated incidence and prevalence. We sought to identify CpGs with sex-specific DNAm in cord blood and annotate the associated genes.

The Canadian Asthma Primary Prevention Study (CAPPS) is a prospective longitudinal birth cohort of children at high risk of asthma. Cord blood was obtained for 144 CAPPS newborns (76 male) and DNAm profiled at 3.3 million CpGs with the Illumina TruSeq sequencing library. Quality control tests (e.g. principal component analysis (PCA), identity by state, sex check) and cell type deconvolution were performed. DNAm was arcsine-transformed and regressed on gestational age and cell type proportions in a weighted linear model (weight: read depth). Residuals were extracted and the association with biological sex evaluated by logistic regression. Differentially methylated CpGs were annotated using EWAS catalogue and NCBI resources. There were 109,901 CpGs significantly associated with sex. Differentially methylated sites were found in ZPBP2 (p value=1.08x10-9) – a gene involved in spermatogenesis that is also part of the ORMDL3 locus linked to childhood asthma and scleroderma - and in autism-related genes (PDE4DIPP1, p value= 1.24x10-8; NHIP, p value= 4.57x10-7). In summary, we found differential DNAm in genes implicated in diseases with sex-specific prevalence. Further analysis of sex-related methylation in relation to genetic variants and the molecular basis of these phenotypes is needed.

Poster Abstracts

37.

Nextflow Pipeline for Benchmarking Integrative Multi-Omics Methods for Disease Classification

Tony Liang

Advancements in profiling diverse molecular data (genes, proteins, etc.) from identical samples (multiomics) offer potential for understanding disease mechanisms and developing early detection, prevention, and treatment strategies. However, with increasing multiomics data, the optimal method for classification and driver identification remains unclear. We propose Multiomics Experiments with SysStematic Interrogation (MESSI) , an automated framework to systematically benchmark and analyze multiomics data. MESSI uses a workflow pipeline to test various methods across diverse datasets and compare results using relevant classification metrics. Initially, MESSI will benchmark existing methods like DIABLO, MOGONET, and GOAT using simulated data. Later, it will incorporate other methods and real-world data types (bulk, single-cell, spatial). The blends of technologies in Nextflow make pipeline development easier, enhance resource efficiency and allow for portable workflows and easy sharing. We anticipate the benchmarking results to identify methods with variable performance on the simulated datasets as different simulations were designed for specific methods. The best performing methods on the real-world data will provide insights into why certain methods are better than others with respect predictive performance and capturing the correct underlying biological processes. Currently, multiomics data resides in repositories like Gene Expression Omnibus, The Cancer Genome Atlas. We are building a first-of-its-kind data portal with around 100 curated studies across various diseases, accessible in standard formats like R's MultiAssayExperiment and Python's MuData. This research will inform the development of a new integrative method applicable to diverse multiomics data types, ultimately contributing to advancing our understanding and treatment of diseases.

38.

Meta-analysis strategies for inference of transcriptional regulatory targets

Alexander Morin

Despite the availability of genetic tools, linking transcription regulators (TRs) to direct gene targets is hindered by multiple factors. These include the cost and difficulty of collecting experimental data implicating direct regulation, such as TR binding information from chromatin immunoprecipitation sequencing (ChIP-seq), and the inherent complexity of the underlying biology. Furthermore, it has been demonstrated that there is often poor overlap in the gene targets identified by distinct methodologies. Cumulatively, this suggests the need for comprehensive data compilation to seek reproducible regulatory interactions. I re-analyzed and aggregated hundreds of ChIP-seq, TR perturbation, and single cell RNA-seq datasets in both mouse and human in search of TR-target interactions supported by multiple lines of evidence. Keeping with the theme of “Limitations to Modern Methods,” my work strives to keep a pragmatic outlook on the biological signals that can be revealed by each methodology, to provide insight into potentially “generic” signals, and to create organized collections of TR-gene rankings for community use.

Poster Abstracts

39.

PUPpy: a fully automated primer design pipeline for substrain-level microbial detection and absolute quantification

Hans Ghezzi

Characterizing microbial communities at high resolution is crucial to unravel the complexity of microbial ecosystems. Advances in bulk sequencing assays such as 16S rRNA and shotgun sequencing have enabled unparalleled qualitative and quantitative microbiota investigations. However, these methods generally do not provide accurate resolution beyond the genus level and lack insights into absolute microbial abundance. Here, we introduce Phylogenetically Unique Primers in python (PUPpy), an automated pipeline to design microbe- and group-specific primers in given microbial communities. PUPpy-designed primers detect individual microbes and quantify absolute microbial abundance in a defined community below the species level, requiring only coding sequence files of the community members as input. We experimentally evaluated the performance of PUPpy-designed primers using two bacterial communities as benchmarks. Each community was comprised of 10 members, exhibiting a range of genetic similarities that spanned from different phyla to substrains. PUPpy-designed primers also enabled the detection of groups of bacteria in an undefined community, such as the detection of a gut bacterial family in a stool microbiota sample. Taxon-specific primers designed with PUPpy showed 100% specificity to their intended targets without unintended amplification, independently of community composition and complexity. Lastly, we show absolute quantification of microbial abundance using PUPpy-designed primers in droplet digital PCR, benchmarked against 16S rRNA and shotgun sequencing. Our data shows that PUPpy-designed microbe-specific primers can be used to quantify substrain-level absolute counts, providing more resolved and accurate quantification in defined communities than 16S rRNA and shotgun sequencing. Altogether, PUPpy enables highly accurate perspectives into microbial ecosystems, supporting the characterization of bacterial communities in both in vitro and complex microbiota settings.

40.

Investigation of molecular oxygen-, pyridoxal-5'-phosphate-dependent oxidases

Kairel Edwards

Pyridoxal-5′-phosphate (PLP) is a versatile organic cofactor that catalyses ~ 4% of all enzymatic activities. It primarily utilizes amino acid substrates and among the more common reactions are transaminations, decarboxylations, racemizations, β-substitutions, and β-eliminations. Consequently, PLP-dependent enzymes are especially pertinent in natural product biosyntheses. During catalysis, the deprotonated or decarboxylated amino acid-PLP adduct forms a carbanionic intermediate which is usually sequestered in the active site of the enzyme and thus protected from reaction with O2. However, recent studies have identified a small number of PLP-dependent enzymes in multiple protein families that employ molecular oxygen as a cosubstrate. This emerging group of oxygen-, PLP-dependent enzymes oxidize L-arginine. Two types of oxidases are known: hydroxylases and desaturases. The present study aims to expand the library of arginine oxidases through in vitro biochemical assays, product analysis, steady-state kinetics, and stoichiometric analyses.

Poster Abstracts

41.

Biosynthesis of Pyrazole

Katie Lyle

Nitrogen-nitrogen (N-N) bonds are found in over 300 natural products produced by various plants, bacteria, and algae. These compounds contain a vast degree of structural diversity, including hydrazines (R1R2N-NR3R4), hydrazones (R1N-N=R2R3), nitrosamine (R1R2N-N=O), and N-N bond heterocycles. Due to their structural diversity, these compounds have various applications in clinical drugs with anti-bacterial, anti-tumor, and analgesic effects. For example, kutzneride exhibits anti-fungal activity, and celecoxib targets the COX-2 enzyme. Synthetically, multiple routes produce N-N bonds, but nature creates N-N bonds using three main reactions: the formation of hydroxylamine, the generation of nitrite, and the formation of radicals. Although research into N-N bond formation has expanded, many biosynthetic mechanisms and pathway details are still unknown. This work aims to increase our understanding of N-N bond biosynthesis through transcriptomics and metabolomics approaches. Understanding the biosynthesis of N-N bonds can lead to biocatalytic opportunities in drug development and synthesis.

42.

Attribute-Weighted Aggregation of MS/MS Reporter Ion Intensities for Protein Quantification Using Isobaric-Labelling

Jiahua Tan

Isobaric labelling, such as tandem mass tag labelling, is a commonly used technique in quantitative proteomics. Because quantification is done at the peptide-spectrum match (PSM) level in bottom-up proteomics, protein abundance estimation requires combining reporter ion intensities from all the corresponding PSMs, a process referred to as aggregation or summarization. It is usually assumed that different PSMs represent protein abundance equally in aggregation, but due to the differences in ionizability and propensity to isolation interference, this assumption is not always valid, resulting in PSMs having different levels of quantitative accuracy. Here, we proposed an attribute-weighted aggregation (AWA) method that leverages PSM attributes to provide a more accurate estimate of protein abundance. First, a random forest model was applied to learn the characteristics of PSMs with high accuracy in spike-in datasets affected by ratio compression and predict the level of quantitative inaccuracy of PSMs in new datasets based on their attributes. PSMs were then aggregated to the protein level by a weighted average value considering the predicted inaccuracy. To facilitate its implementation, an R package AWAggregator was also developed for its potential users. The performance of the AWA was evaluated on three spike-in datasets and two large cancer cohorts by a variety of metrics. The results showed that applying the AWA to different datasets can lead to better recall in the differential expression analyses while maintaining the level of precision, as well as improving reproducibility in protein quantification.

Poster Abstracts

43.

Precise assessment of cancer cell growth and survival by artificial intelligence

Jason Wong

Cancer research requires accurate methods for measuring analytical parameters like cell culture confluence, cell count, colony numbers, viability, and motility. These methods must be unbiased and user-independent for reproducible data. Cell analytics involves manual processes or reagent-based approaches (e.g., viability kits). In recent years, semi-automated systems have been introduced that can either count cells, measure cell growth by density tracking, and/or determine cell viability. However, these methods are often time-consuming, require reagents and labeling, and may involve costly instrumentation. Artificial Intelligence (AI) has made strides in clinical and laboratory research, holding promise for swift integration into cancer research. Here, we present the development and validation of SnapCyte™, an AI performing accurate, unbiased, label- and reagent-free cell analytics from basic cell culture images, independent of specialized instrumentation. After multiple training iterations, SnapCyte™ AI detection models achieved 99% precision for confluency and >95% precision and recall for cell count. SnapCyte™ surpassed standard methods (Crystal Violet, WST1, MTT, Presto blue, CyQuant, Incucyte®, and Bio-Rad TC20 cell counter), displaying high accuracy and smaller standard error variation than reagent-based assays. Compared to IncuCyte® Bio-Rad TC20, SnapCyte™ demonstrated similar accuracy and greater user-independent results. Furthermore, SnapCyte™ acquired data in under 10 minutes, with non-invasive measurements, allowing direct use of cells in downstream assays. We have developed and validated an AI model for advanced cell analytics. Our data show that the SnapCyte ™ AI is at par or better than existing reagent and instrument-based solutions in assessing cell confluency, number, and viability. This technology offers a fast, accurate, and unbiased cell analytics platform that is resistant to user variations, and independent of reagents and costly equipment.

44.

A scalable computing framework for whole-body mouse cell lineage reconstruction

Brett Kiyota

Since the advent of CRISPR-Cas9 genome editing, several cell lineage tracing technologies have been developed. The basic idea employs a CRISPR-Cas9 system to introduce random mutations to chromosome-embedded, synthetic DNA barcode arrays. As these barcodes are continuously mutated and inherited from mother to daughter cells, the lineage of cells sacrificed at the time of observation can be reconstructed from the mutation patterns in their DNA barcodes, analogous to phylogeny estimation in evolutionary biology. However, one of the major drawbacks of current technologies is the limited information decoding capacity. Here we aim to further develop a method capable of reconstructing the phylogeny of hundreds of millions of sequences—a task that far surpasses the capabilities of existing software. We have recently proposed a computing framework for phylogeny estimation at unprecedented scales. A major remaining challenge in cell lineage reconstruction from single cells is dealing with biological biases and sparse measurements of single-cell barcode sequence readouts. To tackle this issue, we have developed orthogonal sampling-based methods to extract robust biological signal from the recovered barcode sequence readout while maintaining scalability throughout the reconstruction process. A scalable computing framework is paramount to realizing the goal of resolving a whole-body mapping of a high-resolution mouse developmental lineage—a feat that would revolutionize our understanding of mammalian development.

Poster Abstracts

45.

De novo genome assembly and annotation of Aedes togoi, a saline-tolerant coastal rock pool mosquito

Johnathan Chiang

The coastal rock pool mosquito (Aedes togoi) is found along coastal areas of east Asia and the Pacific Northwest. It has a rare capability to tolerate high salinity water, whereas most mosquito species require freshwater to breed. This extreme physiological adaptation makes Ae. togoi an ideal organism to study the sensory and ion regulatory systems of mosquitoes. Highlighting significant adaptations within these systems in mosquitoes is crucial to understanding their feeding and oviposition behaviour, geographic range, and host preference—all key factors that inform potential vector control strategies. However, we are currently lacking the genomic resources needed to properly study Ae. togoi's uniquely adapted sensory systems and ion regulatory mechanisms that allow for high salinity tolerance. We present a high-quality de novo genome assembly from a single Ae. togoi mosquito, utilizing PacBio HiFi long-read sequencing and Hi-C data. The genome assembly has a total length of 800.58 Mb, a contig/scaffold N50 of 653.6 Kb, and a (BUSCO) completeness score of 96.7%. The pickpocket (PPK) gene family, which is important in salt-sensing pathways in mosquitoes, was identified as the first of many to be annotated in several broad families of sensory receptors. This work lays groundwork for Ae. togoi to be further studied and opens new avenues for comparative studies to investigate physiological and behavioural adaptations within the diverse Culicidae (mosquito) family.

46.

A new highly sensitive retrospective cell clone isolation technology

Ren Takimoto

The idea of retrospective clone isolation (RCI) has emerged to interrogate heterogeneous cell populations, in which cells are initially tagged with DNA barcodes, propagated, and then split into subpopulations. One of the subpopulations undergoes a given assay, while the other is preserved. Upon identifying a clone of interest with a specific phenotype after the assay, the same clones can be isolated from the preserved population in a barcode-specific manner. This framework offers the interrogation of potential molecular factors that prime cell clones to derive the identified phenotype in the heterogeneous population. We recently established a high-performance RCI method CloneSelect using CRISPR base editing. This method however has limitations in sensitivity and clone labeling speed mainly due to the CRISPR reagent delivery and the time required for genome editing. To overcome these issues, we are developing a new, rapid transcription activator-like effector (TALE) and zinc finger (ZF)-based method. In this LightningSelect system, we barcode cells using unique TALE/ZF peptides projected on their cell surfaces. A barcoded clone of target can then be labeled by a fluorophore-conjugated double-stranded DNA highly efficiently with no intracellular process-related time lag. Using LightningSelect, we will derive elite effector T-cells and visualize cancer stem cell expansions in a mouse xenograft model.

47.

Using colonoids grown under Air-Liquid Interface (ALI) conditions to model bacterial pathogenesis at the intestinal mucosa

Yan Chen

The clinically important bacterial pathogens, enteropathogenic E. coli and enterohaemorrhagic E. coli, target their host's intestinal epithelial cells (IEC), but this requires the subversion of key mucosal defenses, including a protective mucus layer comprised of the mucin MUC2. Citrobacter rodentium, a robust in vivo model for these pathogens that readily infects mice, exhibits increased virulence when infecting Muc2 deficient (Muc2-/-) mice. However, a suitable in vitro model to address how this pathogen interacts with the intestinal mucus layer is lacking. We found that organoids derived from the mouse colon, when grown under Air Liquid Interface (ALI) conditions, generate a monolayer of well-differentiated IEC, including goblet cells which produce an overlying mucus layer. Proteomics and lectin staining confirmed the production of a relevant mucus layer comprised of glycosylated Muc2 in this ALI culture. When C. rodentium was used to infect ALI, it gradually penetrated the mucus layer, leading to direct IEC infection and cell death. To interrogate the role of the mucus layer, we also infected ALI generated from Muc2-/- mice. Muc2-/- ALI cultures experienced exaggerated IEC damage, as C. rodentium infection proceeded more quickly, causing monolayer disruption. We also pre-incubated C. rodentium with sialic acid, a mucus-derived sugar that accelerates in vivo infection. This resulted in pronounced IEC damage and death in both Muc2+/+ and Muc2-/- ALI monolayers, suggesting that sialic acid may enhance C. rodentium's production of virulence factors that facilitate both mucus penetration and IEC infection. Taken together, these experiments emphasize the importance of including a physiologically relevant mucus layer when evaluating the in vitro pathogenesis of enteric bacterial pathogens.

48.

Developing a volatilome detection platform for functional metagenomic screening and microbial cell factory engineering

Andras Szeitz

There are an estimated 10^30 prokaryotic (bacteria and archaea) microorganisms on Earth, including two of three domains of Life and expanding into numerous unknown divisions with no cultured representatives. Microbial life in more abundant than the number of stars in the universe, or the neurons in the human brains, and synapses combined. The combined microbial communities mediate matter and energy conversion processes that lead to creating and sustaining the conditions for life on the planet. For more than 3.5 billion years, microorganisms have annealed diverse metabolic challenges at every level of biological existence and offered solutions to nutrient and energy conversion mechanisms withstanding the test of time and generating a large pool of genomic potential. This potential embraces many metabolic processes, enzyme variants and regulatory systems that adapt to changing environmental conditions. To utilize the metabolic problem-solving potential of this microcosmos, we need new platforms and approaches to explore metabolite expression and regulatory frameworks supporting microbial adaptation and response at every level of biological life. We are proposing an analytical platform using gas chromatography coupled to mass spectrometry with front-end sampling modules for the high-throughput analysis of volatile organic compounds (VOCs) produced by microorganisms. This will facilitate new types of integrated science connecting VOC production with multi-omics approaches within the emerging framework of "volatilome" research. The resulting volatilome datasets will highlight microbial interactions and the generation of value-added compounds in individual microbial isolates and consortia leading to functional metagenomic screening in the quest for genes and gene cassettes controlling VOC production in natural and engineered environments enabling potential biotechnological applications.

49.

PLASMA CELL-FREE DNA HISTONE METHYLATION ENABLES PHENOTYPIC AND CLINICAL SEGMENTATION OF METASTATIC PROSTATE CANCER

Asli Munzur

Epigenomic reprogramming frequently occurs in the context of metastatic castration-resistant prostate cancer (mCRPC), particularly during emergence of treatment resistance and neuroendocrine prostate cancer (NEPC). Circulating plasma cell-free DNA (cfDNA) can remain bound to histones containing cell-of-origin posttranslational modifications—and can be analyzed via chromatin immunoprecipitation followed by sequencing (cfChIP-seq). Here, we tested the potential for cfChIP-seq to inform on biological and clinical subgroups of mCRPC. We examined 63 cfDNA samples derived from 33 patients with mCRPC and 19 controls. Samples underwent H3K4me2 profiling using cfChIP-seq. cfDNA samples and patient-matched white blood cells also underwent low-pass whole genome and deep targeted sequencing. A custom statistical framework was used to account for variable ctDNA% between samples, which can confound comparisons of H3K4me2 activity between genes and/or patients. 51% of patients had >10 bone lesions, 20% had liver, 15% had lung, and 58% had bone metastases without visceral involvement. Transcription factors canonically linked to AR-driven adenocarcinoma (e.g. AR, FOXA1) and NEPC (e.g. EZH2, NANOG, POU5F1) were differentially enriched across multiple separate binary patient subgroups, including patients with bone versus liver-predominant disease, biopsy-confirmed NEPC, and presence of RB1 deletions, suggesting an apparent axis of AR versus NEPC phenotypic segregation. KLK3 (encodes prostate specific antigen [PSA]) promoter H3K4me2 count was strongly correlated with time-matched serum PSA ($p<0.01$). AR binding motif enrichment showed a positive correlation with PSA ($p<0.005$) while NEPC related motifs were negatively correlated ($p<0.05$). cfChIP-seq can capture biological distinctions in clinically stratified patients with mCRPC. cfChIP-seq may complement ctDNA profiling for discovery of predictive and prognostic biomarkers and detection of treatment resistance.

50.

Revisiting the unipolar brush cell during cerebellar embryonic development through in-silico perturbation

Karen Ip

The unipolar brush cell (UBC) is an excitatory interneuron in the cerebellum that facilitates sensorimotor processing for eye, head, and body positioning. Its classic morphology is a single short dendrite brush. UBCs are one of three glutamatergic cell types that arise from the rhombic lip. Previous work from the lab has shown that all three glutamatergic lineages require the transcription factor Pax6—the UBC specifically needs Pax6 to ensure proper cell production. Although recent studies have modeled embryonic cerebellar development, recapitulating the gene regulatory network (GRN) of specific glutamatergic lineages has not been shown. Multiomic approaches to modeling embryonic cerebellar development remain underrepresented. GRNs models how cells arrive at their observed state—an apt approach for studying disease and developmental states. In development, GRN perturbations have been a successful predictive tool for studying human brain organoid synthesis and murine organogenesis. The availability of gene expression and chromatin accessibility data for the UBC's key developmental timepoints (E14.5-E18.5), Tbr2/Eomes as a cell marker throughout development, and the classification of at least two distinct subtypes make this cell type a promising lineage of interest. The present study aims to model UBCs through its key developmental timepoints using integrated scRNA-seq and scATAC-seq analyses. The result will be GRNs that capture the developmental trajectory of UBCs. The GRNs will be perturbed by CellOracle and SCENIC+ to simulate gene of interest knockouts in-silico. The resultant GRN synthesis and perturbation pipeline should recapitulate findings from previous studies, and will be compared with in-house Pax6-null scRNA-seq and Pax6 Cut&Tag data to evaluate its efficacy and agreement. The pipeline provides the foundation for studying other cell types of interest through an inference-based embryonic cerebellar development model.

Poster Abstracts

51.

Exploring the impact of histone variant H2A.Z depletion on nascent transcription regulation during DNA replication stress

Eully Ao

H2A.Z is an evolutionary conserved histone variant. In S. cerevisiae, it is incorporated into chromatin by the SWR1 chromatin-remodeling complex (SWR1-C). While implicated in transcriptional regulation, among many other processes, H2AZ's exact role in this process is still debated as gene expression does not appear to be correlated with its localization at gene promoters. The limited transcriptional changes observed may be due to experimental design. Previous studies utilized 1) H2A.Z knockout models; 2) nutrient-rich conditions; and 3) total cellular RNA. However, these may be limitations because these studies may be overlooking 1) compensatory mechanisms; 2) H2A.Z's potential role during cellular stress, particularly in priming genes for transcription in specific environmental conditions; and 3) exclusive transcriptional activity. To address these limitations, I propose an alternative approach to reveal immediate H2A.Z loss effects and uncover previously undetected changes in gene expression to explore H2A.Z's role in transcriptional regulation. I will be 1) using the Auxin-Inducible Degron system to deplete H2A.Z; 2) adding the replication inhibitor hydroxyurea as a stressor; followed by 3) measuring nascent transcription with the 4-thiouracil labeling technique. These analyses aim to identify the timing and extent of transcriptome alterations post-H2A.Z depletion, pinpoint genes responsive to induced stress and H2A.Z depletion, discern differences between bulk RNA and nascent RNA readouts, and distinguish differences between depleting H2A.Z alone and simultaneously depleting of H2A.Z and Swr1 as it has been shown that SWR1-C without its substrate causes "mischief." Overall, this project will dissect the role of H2A.Z in nascent transcriptional regulation, particularly under stress conditions, contributing to our understanding of its impact on eukaryotic cellular processes and aiding in resolving the debate surrounding H2A.Z's role in transcription regulation.

52.

Toward A Large-Scale Gene Regulatory Network Inference for Human Cells through Divide-And-Conquer Approach

Herbert Yao

The gene expression landscape, shaped by transcription regulation, is crucial for controlling various aspects of a cell's life, including its growth, differentiation, aging, and death. This intricate control system is often conceptualized as gene regulatory networks (GRNs), which are mathematical models representing how genes regulate themselves and each other. Constructing a comprehensive and simulatable GRN model is a primary objective in modern biology, as it would significantly enhance our understanding of cellular processes and their roles in development and disease within the human body. However, the field of GRN faces challenges due to gaps in knowledge, data availability, and computational complexity. In this project, we propose a novel "divide-and-conquer" methodology to construct large-scale GRNs using data from single-cell RNA sequencing (scRNA-seq). Our approach involves initially sampling gene expression profiles from extensive scRNA-seq datasets to create multiple low-resolution or local GRNs. These smaller GRNs are then assembled into a comprehensive sample-level GRN model. This unified model undergoes a process of simulation and data assimilation to ensure it accurately reflects the scRNA-seq data. Additionally, we aim to extend this framework to incorporate GRNs from various scRNA-seq datasets, constructing a global GRN model. Such method to achieve a simulatable cell model that comprehensively interprets cellular behaviors at the scale of the entire body has not been established previously. We believe our approach marks a significant advancement in biological research, paving the way for groundbreaking developments in whole-cell and tissue-level modeling of mammalian systems.

53.

Exploring the impact of SNCA overexpression on mouse hippocampal DNA methylome and transcriptome during early and midlife

Rui Wang

Parkinson's disease (PD) is a common neurodegenerative disease. Multiplication of SNCA, a gene encoding synaptic protein alpha-synuclein, leads to PD in a dosage-dependent manner. Although the symptomatic phase of PD typically begins in late life, it is usually preceded by a prodromal phase during early to midlife during which many non-motor and pathological signs of PD emerge. However, the precise of role of excessive alpha-synuclein during early to midlife remains elusive. In this study, we described and characterized the epigenomic and transcriptomic landscape during the prodromal phase of PD in hippocampus, a brain region linked to many cognitive impairments of PD, using a transgenic (TG) mouse model overexpressing human SNCA. When comparing TG mice against their age-matched wildtype (WT) counterparts, we observed a more pronounced influence of SNCA overexpression at midlife compared to early life at both DNA methylation and gene expression level. SNCA overexpression also affected the isoform regulation of two distinct sets of genes at early life and at midlife. Comparisons between middle-aged mice and young mice of the same genotype supported that SNCA overexpression affected the early-to-midlife transition of the epigenetic, transcriptional, and isoform regulation pattern of hundreds of genes. Functional characterization of the genes with a significant genotype or age effect implicated a wide array of biological processes crucial for neurological functioning and revealed specific molecular factors that are attractive targets for future validation studies. Taken together, our study confirmed that the molecular consequences of SNCA overexpression began to manifest prior to late life and progressed in an age-dependent manner. These results laid foundation for future PD studies targeting early to midlife and underscored the importance of further investigation into the role of SNCA overexpression during the pre-symptomatic phase of PD.

54.

Investigating the Potential Drivers of Aberrant Splicing in Acute Myeloid Leukemia

Tian Liu

Acute myeloid leukemia (AML) is a fatal hematologic malignancy characterized by the rapid accumulation of myeloblasts in the bone marrow and blood, disrupting normal blood cell production. Alternative splicing, facilitated by the spliceosome, is a ubiquitous cellular process enabling a single gene to generate multiple proteins with distinct functions by splicing pre-mRNAs into various mature mRNAs. While prior investigations have implicated genome-wide splicing variation as a contributor to gene dysregulation in AML and a complementary focus of understanding the disease progression, the prevalence of common splice factor mutations known to induce AML splicing dysregulation is limited to less than 20% of AML patients. This suggests the involvement of other mechanisms in gene disruption contributing to splicing dysregulation. In this study, we analyzed alternative splicing events in AML patients, quantifying each splicing event by splicing junction usages across three AML cohorts: AML-PMP, BEAT-AML, and Leucegene. Comparing gene expressions between high-risk and low-risk AML groups, we observed that down-regulation of the splice factor NOVA1 correlated with poorer survival outcomes. Subsequently, in AML patients exhibiting lower NOVA1 expression, we conducted differential splicing analysis and GO over-representation analysis, identifying common aberrant splicing events in genes associated with lipid regulation, mitochondrion organization, and immune response, such as AUP1, TGFB1, FBXO7, and FIS1. These genes have been previously implicated in influencing AML disease progression. Through this approach, we unveiled the down-regulation of splice factor NOVA1 as a potential driver of splicing dysregulation in AML.

Poster Abstracts

55.

A Bioengineered Plant Production System for the Antidiabetic Compound Montbretin A

Desirée Kelshall

Diabetes mellitus and obesity are significant global health challenges with over 5.7 million adults in Canada alone living with diabetes, primarily type-2 diabetes (T2D). Blood glucose regulation in T2D patients is often achieved by inhibiting starch degradation enzymes. However, current medications predominantly inhibit α-glucosidases, triggering adverse side effects. Montbretin A (MbA) has emerged as a potent, and highly specific inhibitor of the human pancreatic α-amylase (HPA), demonstrating success in efficacy and toxicity tests in animal studies. It is naturally synthesized in montbretia plants, but its production is limited to a short window of time, significantly restricting its pharmaceutical or nutraceutical application. Bioengineering of MbA is now possible due to the previous elucidation of its biosynthesis pathway in montbretia. Research in the Bohlmann Lab is exploring Nicotiana benthamiana (Nb) as a heterologous production system for MbA. One major limitation is the interference of endogenous Nb enzymes when MbA biosynthetic genes are expressed in Nb, leading to undesired side products. Thus, this study aims to produce MbA in Nb at commercially viable levels, by using gene silencing and CRISPR Cas9 gene editing techniques to reduce the effects of these enzymes on pathway output and improve MbA yield. Currently, CRISPR Cas9 is being employed to knock-out interfering Nb genes to generate stable transgenic Nb lines with improved precursor availability. We employ the Golden Gate Modular Cloning system for MGC assembly, agroinfiltration for gene delivery, tissue culturing for plant propagation, and LC-MS for the identification of compounds. Overall, this study holds promise for the development of more efficient and scalable methods of MbA production, and engineered Nb plants with high expression levels of precursors and CRISPR mutant lines are expected to serve as improved production chassis for MbA.

56.

Tracking microbial microplastic transformations in marine waters using stable isotope informed metaproteogenomics

Nicole Howes

Approximately 14 million tons of plastic end up in oceans per year, and slowly breaks down due to physical corrosion and UV exposure to create microplastics. Microplastics are an emerging environmental and health concern where they have been found to threaten marine and terrestrial ecosystems. Understanding the fate of these microplastics that are continuously being cycled through the ocean is crucial to limit their harmful impacts and protect ecosystems. Furthermore, the addition of carbon into the oceans may contribute to global carbon emissions while also negatively affecting marine organisms' ability to absorb environmental carbon dioxide. Currently the fate of these microplastics and the stability of these particles are not well studied. Marine microorganisms likely play a large role in the degradation and/or transformation processes, as they can express and use proteins that metabolize plastic. However due to the low temperature and biomass in the ocean, this biological process likely occurs at a slow pace. To further constrain the extent of microplastics conversion in ocean environments, there is a need to quantitatively measure the carbon flux from microplastics into the biomass of marine microorganisms. The aim of my research is therefore to 1) quantify the amount of marine microplastic carbon incorporated into microbial biomass, 2) further explore the organisms that can breakdown and utilize microplastic polymers 3) investigate the metabolic pathways and/or novel enzymes involved in microplastic transformation. Incorporating microplastics into global geochemical models will allow us to understand their fate and explore ways to mediate their long lifetime and ultimately limit their harmful ecological impacts.

57.

Linking Genomic Structural Variations and Phenotypic Diversity of Saccharomyces cerevisiae Strains: Insights from Vineyards and Wineries

Alex Marr

Saccharomyces cerevisiae (SC) strains in vineyards/wineries are responsible for alcoholic fermentation in spontaneous wine fermentations. The Measday lab has performed genome sequencing for 175 diverse British Columbian and Californian SC strains isolated from spontaneous wine fermentations to investigate these strains' evolutionary origin, domestication patterns, and wine-making potential. Genomic structural variations (SVs) are large rearrangements (>1 kb), including inversions, translocations, and copy number variants (CNVs), and often have drastic phenotypic impacts. To evaluate the influence of SVs on strain phenotypes, high-throughput phenotypic screening was conducted on 270 diverse SC strains (175 from this study and 95 global strains) for 55 conditions on solid media using the Singer Rotor+ pinning workstation. Hits were determined using a two-tailed t-test to analyze strain normalized growth ratios in comparison to standard growth media, with consideration for deviations exceeding 1 standard deviation. CNV was evaluated for 265 strains (with genome sequencing data available) using cnv-kit software. Translocation of the SSU1 gene was identified by SV-calling software Lumpy and multiplex PCR genotyping. Hits from phenotypic screens were used for further gene CNV enrichment analysis using Fisher's exact test. We find that strain central metabolism and stress phenotypes are independent of clade classification. Our CNV enrichment analysis suggests that 54/55 conditions contained at least one significant gene CNV enrichment. Translocation of the SSU1 gene is unique to the European/Wine clade (57/107 strains). Strain TWMY76 contains a novel XVI-t-VII translocation of SSU1 and has increased resistance to SO2. This work elucidates the pivotal role of genomic structural variations in shaping the phenotypic diversity and adaptive traits of SC strains in vineyards and wineries, providing valuable insights into their evolutionary origin and potential for winemaking.

58.

Development of a high-throughput genome-wide method to assess Ty1 retrotransposon insertion upstream of tRNA genes in Saccharomyces cerevisiae

Rutuja Pattanshetti

Background: Transposable elements are DNA elements comprising repeated sequences that can change their location within a genome. Retrotransposons mobilize via RNA intermediates and this phenomenon, in humans, can cause diseases. In the S288c genome of the yeast Saccharomyces cerevisiae, Ty1 is the most abundant retrotransposon, present at ~32 copies. Ty1 typically integrates within a 1kb window upstream of genes that are transcribed by RNA Polymerase III, such as transfer RNA (tRNA) genes. Because the lifecycle and structure of retrotransposons resembles that of retroviruses, studying Ty1 in yeast will help better understand human retrotransposons and retroviruses. Our aim is to develop and validate a high-throughput, genome-wide method to assess Ty1 insertion upstream of tRNA genes in S. cerevisiae. We have selected 14 genomic loci to monitor using Next Generation Sequencing (NGS) of amplicons derived from the unique genomic loci generated by Ty1 transposition. In a proof-of-principle study, a wildtype (BY4741) and a condensin mutant (brn1-9) strain were transformed with a Ty1-donor plasmid containing a Ty1 element under galactose-induced expression. PCR using primers that recognize a barcoded Ty1 sequence and a sequence upstream of a tRNA gene were used to identify and amplify Ty1 insertions. We mapped the insertion patterns and compared frequencies of Ty1 insertion between the wildtype and the mutant. Our sequencing results confirmed that the barcoded Ty1 inserted upstream of the targeted tRNA genes with higher insertion frequencies in the wildtype in comparison to the mutant. We selected 14 loci for NGS quantitation and have validated all 14 using NGS. We have successfully multiplexed up to 6 loci and are testing if all loci can be multiplexed. We will apply the bioinformatic methods developed to assess these Ty1 insertion differences loci between wildtype and mutant strains, including cdc6-1, spt5-194, and eco1-1.

59.

Candidate and Genome Wide Pathway Analysis of Super Seniors

Rawnak Hoque

The Super Seniors Study aims to identify genetic factors that influence healthy aging. 'Super Seniors' are individuals 85 or older who have never been diagnosed with cancer, cardiovascular or major pulmonary disease, diabetes or dementia. We conducted candidate and genome-wide pathway analyses (GWPA) of the Super Seniors' phenotype to identify biological pathways for which genetic variation may contribute to healthy aging. The analysis was performed using 6.6 M imputed variants, in individuals of European ancestry. 541 Super Seniors and 373 population-based mid-life controls without regard to health status were analysed. Pathways from KEGG database, using MAGMA-1.08b, gene-based association tests were performed. The outputs of the gene-based association were used to test for pathway-based associations that compare genes in a specific pathway with rest of the genome. Initially, out of 3 candidate pathways, only insulin signaling (P = 0.008) remained significant after Bonferroni correction for 3 tests, whereas two did not: the mammalian (mechanistic) target of rapamycin (mTOR) signaling (P = 0.58), and AMP-activated protein kinase (AMPK) signaling (P = 0.10). For further exploration, GWPA was performed for 186 gene sets representing all KEGG canonical pathways. No pathway remained significant after Bonferroni adjustment for 186 tests. Top ranked (P<=0.05) pathways included several known to be relevant to healthy aging or longevity, including Alzheimer disease (P = 0.003), P53 signaling (P = 0.004), Insulin signaling (P = 0.008), Type 1 diabetes mellitus (P = 0.01), Huntington disease (0.02), Lysosome (P = 0.02), nicotinate and nicotinamide metabolism (P = 0.02) etc. Though the results are not genome wide significant, the results of candidate pathway analyses and the observation of pathways of known relevance among the lowest p-values supports that multiple genetic variants in these pathways may contribute to healthy aging in this unique cohort of Super Seniors.

60.

Modeling Group 4 Medulloblastomas (G4MBs) with Mouse Cerebellar Organoids

Marco Ho

Medulloblastomas (MBs) are the most common form of malignant brain tumor in children and the leading cause of cancer-related deaths in this age group. MBs can be classified into four molecular subgroups, with "Group 4" MBs (G4MBs) being generally of poor prognosis and constituting 35% of all cases. Even so, the underlying pathology of G4MBs is still not well-understood. Recently, organoids have been utilized as high-throughput, in vitro modeling systems in the fields of medicine, pharmaceuticals and developmental biology. With the use of various external signaling molecules, pluripotent stem cells can be patterned and cultured into these organoids, which have been shown to recapitulate the genetic and cellular characteristics of a target organ. With their relative ease of maintenance and manipulation, organoids are uniquely suited for modeling disease states. Since the early 2000s, researchers have been able to generate cerebellar organoids using protocols that revolve around the key patterning factor FGF2. However, a recent study showed the expression of forebrain genetic markers in these organoids. The same study also demonstrated the generation of cerebellar organoids with significantly lower forebrain marker expression using the patterning factor FGF8, which is a well-established and critical signaling molecule that induces the formation of the isthmic organizer in the developing neural tube. As such, we aim to compare these protocols to determine which one generates cerebellar organoids that more "faithfully" recapitulate the genetic expression and cellular composition of a cerebellum. The resulting organoids will be evaluated using RT-qPCR and immunohistochemistry. With the optimization of the cerebellar organoid protocol, we will further utilize them to study G4MBs. By overexpressing potential cancer drivers, such as Prdm6, Mycn, and Cdk6, in these cerebellar organoids, we hope to understand the genetic causes and cellular origins of G4MBs.

Poster Abstracts

61.

Dynamic Changes in the Classical Hodgkin Lymphoma Tumor Microenvironment Using Single Cell Technologies

Yifan Yin

Lymphoma is a cancer that develops from lymphocytes and is the fifth most common cancer in Canada. Classical Hodgkin Lymphoma (cHL) is a type of lymphoma that is characterized by the presence of malignant Hodgkin and Reed–Sternberg (HRS) cells representing about 1% of the biopsy tissue. HRS cells are surrounded by different types of normal immune cells, such as T cells, B cells, and macrophages. Some findings suggest that HRS cells recruit these immune cells and form a well-organized tumor-supporting microenvironment. Although the treatment of primary cHL has improved significantly with the adoption of combined modality therapies, approximately 10-25% of cHL patients experience relapse or refractory disease. However, the mechanisms of treatment resistance still remain unclear.

Our lab previously performed digital gene expression profiling on 73 cHL biopsies taken at the timepoint of both diagnostic and relapse, and confirmed differences existed in the TME between diagnostic and relapse samples. Moreover, our lab has established a gene-expression profiling based prognostic model to predict response to autologous stem cell transplantation for relapsed cHL

However, gene expression profiling technologies usually process a mixture of all cells, averaging out underlying differences in cell-type-specific transcriptomes, which limits the scope of the published TME studies. The recent development of single cell technologies, such as single cell RNA sequencing and imaging mass cytometry, enables the characterization of the complex cell heterogeneity at the single cell level. In this study, we aim to use single cell technologies to reveal correlates and molecular mechanisms of therapeutic resistance and treatment failure in relapsed cHL.

62.

Construction of a barcoded collection of wild and domestic Saccharomyces cerevisiae strains for competitive fitness assays using CRISPR-Cas9

Jackson Moore

The budding yeast Saccharomyces cerevisiae (S. cerevisiae) is a powerful model system for eukaryotic biology. Experimental designs are often restricted however to a limited set of domesticated laboratory strains, typically derived from S288c. Here we describe a genetically diverse collection of barcoded S. cerevisiae strains, representing multiple phylogenetic clades, that enable pooled competitive fitness assays in non-laboratory strains. Marker-less genetic barcodes were introduced into the genomes of each strain by targeting the HO locus via CRISPR-Cas9 and replacing the gene with single-stranded oligonucleotide donor DNA (ssDNA) consisting of a 20-nucleotide barcode and two short 40-nucleotide homology arms. High-throughput transformation of CRISPR-Cas9 machinery and ssDNA into each strain was achieved in 96-well format, and correct barcode insertion was confirmed via Sanger sequencing and microsatellite genotyping. The barcoded collection will be utilized for high-throughput drug screening via pooled liquid-phenotyping bar-seq assays in response to antineoplastic and antifungal compounds targeting various biological pathways. Examples of compounds include methotrexate, an anticancer agent that inhibits DNA synthesis, and nocodazole, an antifungal that disrupts microtubule dynamics. After drug screening, genotype-phenotype relationships will be investigated via genome-wide association, allele-swap experiments, and bulk-segregant analysis. This study will provide novel gene-trait mapping and increase the power of using S. cerevisiae as a model organism.

63.

Decoding Promoter Regulatory Logic in Cancer through Random Mutagenesis Using CRISPR-Cas9 Base Editors

Asfar Salaudeen

Cancer progression is influenced by both coding and non-coding mutations, with the latter playing a crucial role in the regulation of gene expression. Understanding the regulatory logic of promoter sequences in cancer could be valuable for developing targeted therapies and personalized treatments that modulates the expression level of cancer associated genes. In this study, we present a high-throughput approach to unravel the regulatory logic of cancer-associated promoters using CRISPR-Cas9 base editors through random mutagenesis. We have designed a versatile mutagenesis system employing CRISPR-Cas9 base editors to selectively mutate endogenous regulatory regions in cancer cells. Our approach allows for the introduction of various mutations within targeted promoter sequences, perturbing transcription factor binding sites and subsequently affecting gene expression levels. By sorting cells based on the change in gene expression of the targeted gene and sequencing the mutated promoter regions, we generate a sequence-to-expression dataset that can be used to train machine learning models for understanding gene regulatory logic in promoters. As a proof of concept, we demonstrate the application of our high-throughput random mutagenesis approach on the PD-L1 gene, a critical player in immune evasion within the tumor microenvironment. By systematically mutating the promoter region of PD-L1 and measuring the mutation's impact on gene expression, we showcase the potential of our approach for decoding the regulatory logic in cancer promoters. Our CRISPR-Cas9 base editor-driven random mutagenesis system provides a powerful tool for studying the impact of non-coding mutations in cancer and can be readily adapted to target any regulatory region of interest. The insights gained from this study will facilitate a deeper understanding of cancer biology and inform the development of targeted therapies and personalized treatment strategies.

64.

Assessing the uniformity of plasmid library amplification by different culturing methods

Nick Mateyko

High complexity DNA libraries are used in a variety of modern high-throughput biological assays. These libraries are usually kept in plasmids that are amplified in E. coli to generate sufficient material for an experiment. Uniform amplification is crucial to prevent both drop-out and overrepresentation of sequences, and the choice of medium for culture expansion is thought to influence the uniformity of library amplification. However, to our knowledge, no direct comparison between plasmid library culturing methods has been done. We tested five methods to amplify plasmid libraries that started with equal abundance of ~100,000 unique 80 bp sequences: liquid, semisolid, high and low colony density spread plates, and bead spread plates. All libraries were then sequenced by NGS to determine the uniformity of sequence representation maintained by each culture condition.

65.

Characterization of CXCR5-CXCL13 axis in relapse/refractory classic Hodgkin lymphoma

Makoto Kishida

Lymphoma is a cancer of the lymphatic system, and classic Hodgkin lymphoma (cHL) is a subtype of B-cell lymphoma, characterized by several unique features among all human malignancies. Hallmark features include the presence of rare multi-nucleated, malignant Hodgkin and Reed-Sternberg (HRS) cells, that account for approximately 1% of cells, which are surrounded by a tumor microenvironment (TME) composed of a multitude of non-malignant cell types from both the innate and adaptive immune systems. Previous studies have started to unravel the compositional and spatial complexities of the TME that are linked to pathologic and clinical parameters.

Using cutting edge technologies like single cell RNA-sequencing and imaging mass cytometry, we previously comprehensively characterized spatial architecture of HL at single cell resolution and identified unique subsets like CXCL13+ T cells and macrophages (Aoki et al, PNAS and Aoki et al, JCO). Importantly, spatial analyses have identified a high expression of cognate receptor of CXCL13, CXCR5, on HRS cells in a subset of HL patients, and their correlation with outcome in relapse/refractory HL. In this project, we focus on functional characterization of CXCR5-CXCL13 axis in cHL.

We have generated isogenic CXCR5 KO HL cell line systems. They will be subjected to phenotypic characterization (ie. CXCL13 stimulation, Western blot, migration assay, RNA-seq), in vitro co-culture system, and in vivo model in which we aim to study the complex interactions between HRS cells and the TME. We have demonstrated specific CXCR5-CXCL13 interaction through migration assay and WB, where we observed significantly increased migration and phosphorylation of ERK upon treatment with CXCL13 in WT cell line models. With these systems, we aim to identify the molecular mechanism behind poorer prognosis in CXCR5+ HRS cases in r/r cHL, and potentially exploit this interaction as a novel immunotherapy target.

66.

Using Blood-based mRNA To Detect Allergen-induced Late Phase Asthmatic Response

Mingming Zhang

Background: Late phase asthmatic response (LAR) refers to the phenomena that a second episode of airway irritation arise after the first reaction to certain allergens among people with mild asthma. The symptoms, including airway narrowing, coughing, are usually more severe than the initial ones. The decline in lung function during the late response is very variable, such that some individuals experience significant lung function decline after 3h whereas other individuals may not experience any decline. Current asthma treatments target the late phase response, therefore identifying individuals who will experience a large decline in lung function during the late asthmatic response may improve clinical trial design. The goal of this study is to identify messenger RNA (mRNA) expression biomarkers that are associated with a decline in lung function during the late asthmatic response. Hypothesis: We can identify blood-based mRNA biomarker panel to predict the late phase asthmatic response. Method: Blood samples were collected previously from 36 mild asthma patients and immune response profiling of 770 genes was performed using the NanoString nCounter System. FEV1s were also collected at baseline level, and 5 other timepoints (3 to 7 hours after allergen inhalation). The late asthmatic phase was quantified by computing the area under the FEV1 curve (AUCFEV1) between 3 to 7 hours. We will perform differential expression analysis using the LIMMA R-library, with false discovery rate of 10% to assess statistical significance. We will develop machine learning methods to predict the AUCFEV1using the Glmnet R-library. Expected Result: We expect an association of immune response-related genes with the decline in lung function during the LAR. We also expect to identify biomarker panels associated with the AUCFEV1. Conclusion: A blood-based mRNA biomarker panel can be used to predict the LAR in mild asthmatics.

67.

Sex-influenced DNAm profiles of isolated human placental cell types

Jiyoung Han

Sex differences in function and morphology of the human placenta can lead to sex differences in pregnancy outcomes. X-chromosome inactivation (XCI) is the primary mechanism for dosage compensation between the sexes, and in somatic cells, is strongly associated with X-chromosome promoter DNA methylation (DNAm), but in the placenta a reduction in this promoter DNAm has been reported. The placenta is a complex organ consisting of cells of different cellular origins, but the sex differences in specific cell types have not been investigated.

We investigated the sex-influenced DNAm profiles of 94 term placental samples (XX=50, XY=44) including 18-19 samples each of endothelial, stromal, Hofbauer cells, cytotrophoblasts and chorionic villi from previously collected EPIC DNAm arrays. Sex-stratified X/Y and autosomal chromosomes were analyzed to identify sex differences associated with sex chromosome. DNAm distribution of the X differed by cell type, reflecting differing developmental origins The XX placental cell types derived from the extraembryonic mesoderm (endothelial/stromal cells) and trophectoderm (cytotrophoblast) showed distinct DNAm distributions from each other and from Hofbauer cells, which shared a similar distribution with somatic cells. Surprisingly, the typical DNAm at promoter-associated CpG islands on the X of XX cells was completely absent for endothelial/stromal cells and present only at low levels in cytotrophoblasts, suggesting that XCI escape may occur in the absence of promoter DNAm. Sex-influenced DNAm at autosomal loci was mainly observed in endothelial cells, and included ZNF300.

We demonstrated that X-chromosome DNAm profiles in XX cells are different by cellular origins. The lack of promoter DNAm in association with XCI escape in extraembryonic mesoderm-derived cells suggests an origin distinct from the other cell types. This work may provide insight into the sex differences and origin of cells of the human placenta.

68.

Three-dimensional in situ mapping of intratumor heterogeneity

Naila Adam

Single-cell sequencing of tumors enable detailed understanding of intratumor heterogeneity and the individuality of cells, missing the context. Constructing a 3D picture that include the spatial context of the tumor microenvironment (TME) is a critical factor in understanding selection of malignant cells with proliferative potential at the tumor front, immune surveillance and suppression of malignant immunogenic clones and deciphering spatial modes of growth and dispersal that impact tumor-immune co-evolution. At the IMAXT consortium, we used a 4T1 polyclonal mouse model to map TNBC tumor and its TME at single-cell resolution as a function of immune competency (immunocompetent vs immunosuppressed mice). We employed scRNA-seq and CITE-seq to identify tumor and immune cell states and design protein panels for single-cell spatial imaging methods (IMC and merFISH). Leveraging scDNA-seq, we identified clones within a mixed tumor population. This work specifically tackles multimodal single-cell integration challenges by presenting an analysis framework and devising strategies for tying together different data types using common anchors. Here, we project cell types/states discovered by single-cell sequencing on an accurate map of spatial organization in the TME by integrating CITE-seq and IMC . Moreover, with scDNA-seq, scRNA-seq and merFISH modalities, we create an accurate spatial map of tumor clones and their TME context.

69.

Characterization of a cytochrome P450 that catalyzes the O-demethylation of lignin-derived benzoates

Megan Wolf

Cytochromes P450 (P450s) are a superfamily of heme-containing enzymes that are well known for their broad range of mono-oxygenase activities. One such activity is O-demethylation, an essential and potentially rate-determining step of lignin valorization, the process of converting the abundant yet highly underutilized lignin into higher value compounds. Emerging lignin valorization strategies require the processing of complex mixtures of aromatics, which can be efficiently accomplished by microbial cell factories. These biocatalysts exploit the natural ability of bacteria to funnel diverse aromatics into central metabolism. Thus, the discovery and engineering of O-demethylases active on lignin-derived aromatic compounds is crucial for developing biocatalysts for lignin valorization. We recently identified CYP199A3, or PbdA, a P450 from Rhodococcus jostii RHA1 which catalyzes the O-demethylation of para-methoxylated benzoates and confers growth of RHA1 on these compounds. In this work, we detail the structure and activity of PbdA on several benzoate derivatives. The binding pocket is resolved in high-resolution structures of PbdA-substrate complexes, allowing for the identification of key determinants of substrate specificity. This is complemented by biochemical data, which reveal the relative affinity and specificity of the enzyme for several lignin-derived substrates. We further demonstrated that in addition to O-demethylation, PbdA catalyzes the hydroxylation and dehydrogenation of 4-ethylbenzoate even though RHA1 does not grow on this compound. The detailed characterization of an enzyme that transforms lignin-derived aromatic compounds facilitates the design of microbial cell factories for lignin valorization.

70.

Sex Differences in Cell Composition and Epigenetic Age Acceleration Associated with Prenatal Maternal Stress in the Placenta

Ella Beraldo

In utero exposure to prenatal maternal stress (PNMS) is associated with increased risk of adverse outcomes in offspring. These negative outcomes may also be sex and gestational age (GA) at exposure dependent. The placenta protects the fetus from changes in the maternal environment through several mechanisms, including by inactivating maternal cortisol. DNA methylation (DNAme) has been shown to be responsive to some external influences; however the effect of PNMS on placental DNAme is not well understood. EPIC array data was processed on 105 placentas exposed to the severe 2011 Queensland floods. Participants were surveyed to assess objective and subjective PNMS with the Queensland Flood Objective Stress Scale (QFOSS) and the Composite Scale of Maternal Subjective Stress (COSMOSS) respectively, as well as cognitive appraisal of the flood's consequences (CONSEQ). Fetal sex, GA at birth, and GA at flood exposure were also collected. We observed sex differences in the ratio of cytotrophoblast (CT) to syncytiotrophoblast (ST) proportion in placentas exposed in the first trimester, and at some of the stress exposure levels across QFOSS, COSMOSS, and CONSEQ scores, with males (n=59) having lower predicted CT to ST ratio than females (n=46). Placental epigenetic age was significantly accelerated ($p<0.05$) in cases with more negative CONSEQ scores compared to cases with neutral or positive scores. Significant intrinsic placental epigenetic age acceleration was seen with increasing QFOSS ($p=0.027$) and COSMOSS ($p=0.026$) scores. CT to ST ratio decreased significantly with increasing extrinsic epigenetic age ($p=0.002$), but not intrinsic epigenetic age ($p=0.682$). Our results suggest that neutral or positive maternal cognitive appraisal may reduce epigenetic age acceleration in the placenta. We also find that stress is associated with sex differences in both cell composition and epigenetic age acceleration in the placenta, though the mechanism by which this occurs remains unknown.

71.

Germline biallelic ASXL1 variants drive T-cell epigenetic and immunological dysfunction, causing combined immunodeficiency and Epstein-Barr virus-associated Hodgkin lymphoma

Maggie Fu

Inborn errors of immunity (IEIs) constitute a group of disorders caused by damaging variants in immune-related genes, including some that function as epigenetic regulators. Additional sex combs-like 1 (ASXL1), encoded by ASXL1, is an epigenetic modifier not previously associated with an IEI. Somatic ASXL1 variants are found in clonal hematopoiesis and hematologic neoplasms, while heterozygous germline variants cause the neurodevelopmental disorder Bohring-Opitz syndrome. We have discovered a new IEI caused by biallelic compound heterozygous germline variants in ASXL1. This patient has a complex and highly unusual history of disease progression notable for severe, scarring cutaneous vaccine-strain rubella granulomas initially manifesting at age 3 years that persisted for more than a decade, and chronic macrocytosis and mild bone marrow cellular hypoplasia that progressed to Epstein Barr virus-associated Hodgkin lymphoma in adolescence. Detailed immunophenotyping revealed progressive loss of B-cells, hypogammaglobinemia, and T-cell lymphopenia with severe skewing towards a memory phenotype with high expression of exhaustion and senescence markers. Molecular investigations confirmed ASXL1 protein deficiency in patient T-cells and fibroblasts. Notably, patient T-cells exhibited stark loss of DNA methylation, accelerated epigenetic aging, and CD8 T-cell dysfunction. These aberrations were rescued upon lentivirus transduction of wild-type ASXL1, confirming the ASXL1 variants' pathogenicity. This study delineates a novel human immune disorder caused by ASXL1 deficiency, a diagnosis that should be considered in individuals with chronic viral infections, viral-associated hematologic malignancies and combined immunodeficiency. Furthermore, it offers fresh insights into the mechanisms underlying human ASXL1 in T-cell functionality, as well as in the development and maintenance of lymphomas.

72.

The role of condensin in Ty1 retrotransposon targeting in Saccharomyces cerevisiae

Mariah Lumpa

Retrotransposons are mobile genetic elements that replicate by reverse transcribing their mRNA into complementary DNA (cDNA) which integrates back into the genome via a retrotransposon encoded integrase (IN). Ty1 is a 6kb retrotransposon with 32 copies in the Saccharomyces cerevisiae S288C genome and its research applications include being a model system for human retroviruses, such as Human Immunodeficiency Virus (HIV) and Human Endogenous Retroviruses (HERV). Ty1 cDNA is targeted into the genome upstream of genes transcribed by RNA Polymerase III (RNA Pol III), such as tRNA genes, by the interaction of Ty1-IN with RNA Pol III. The condensin complex is enriched at tRNA genes in multiple organisms, including yeast and humans, and physically interacts with the RNA Pol III transcriptional machinery. Condensin is made up of five proteins and is responsible for condensing chromatin into chromosomes during mitosis. The condensin subunits Smc2 and Smc4 form a hinge joint while Ycs4 and Ycg1 interact with Brn1 to bind DNA. Given that both Ty1-IN and condensin localize to tRNA genes and interact with RNA Pol III, we evaluated the effect of condensin on Ty1 targeting and mobility. These findings show that Ty1 insertion patterns are altered in condensin mutants, most distinctly in brn1-9 and smc2-8 mutant strains. In the brn1-9 mutant, no Ty1 insertion is observed upstream of tGLY genes; however, we find that Ty1 mobility in brn1-9 is 3.4-fold higher than wild type ($p=6.5x10-5$). This indicates that Ty1 in brn1-9 may be targeted to other locations in the genome. Additionally, Ty1 mobility in smc2-8 is 1.9-fold higher than wild type ($p=0.0013$). Ty1 mRNA, Gag protein, and cDNA levels are similar to wild type in the brn1-9 and smc2-8 mutant strains indicating that defects in Ty1 insertion are not due to reduced Ty1 transcription or reverse transcription. Future work could evaluate where Ty1 inserts in brn1-9 and evaluate if Ty1-IN interacts directly with any condensin subunits.

73.

Tick-tock Goes the Epigenetic Clock: Explorations of Biomarkers of Biological Age in the Blue Zone in Costa Rica

Hannah-Ruth Engelbrecht

Healthy aging is difficult to measure quantitatively, with a complex combination arising from a myriad of molecular, physiological, and neurological traits. DNA methylation (DNAm), the addition of -CH3 tags to CpG dinucleotides , has several features that make it an interesting molecular candidate for human aging studies. DNAm can be used to predict an individual's biological age using "epigenetic clocks" (including PhenoAge, GrimAge, DNAmAge, Hannum DNAmAge, and the Pace of Aging), which have been assessed in many aging-related conditions. Additionally, epigenetic drift, the increased randomness of DNAm with age, can be evaluated using the entropy of the methylated sites or the deviation from the mean per site over time, termed the noise barometer of aging. We interrogated the behaviour of these DNAm biomarkers and a physiological index (the HAI) in a Costa Rican cohort from a region of endemic longevity (Blue Zone) and surrounds using a sex-stratified moderation model to ascertain how DNAm can be used to predict healthy aging, moderated by HAI. We identified that a higher HAI was associated with lower biological age according to the GrimAge and PhenoAge clocks in both sexes (Females: est.=-0.290, SE=0.106, p=0.006; Males: est.=-0.285, SE=0.132, p=0.031), while a higher HAI was associated with a lower Pace of Aging score in males (est.=-0.006, SE=0.003, p=0.023). Residence in the longevity region indicated a protective effect in males, for whom an association was found between Blue Zone residence and improved HAI (est.=0.742, SE=0.354, p=0.036). Blue Zone habitation was not associated with any differences in entropy, but males had lower entropy than females (est.=-3.77E-03, SE=1.15E-03, p=0.00115). These results indicate that molecular biomarkers of health do not indicate a marked difference between Blue Zone longevity regions and surrounding areas, but sex differences in aging remain complex and prevalent across molecular indications.

74.

Cell-type specific genetic-to-epigenetic relationships in the human breast

Axel Hauduc

Interplay between the genome and the epigenome is fundamental to cell type specification and disease mechanisms. Most studies that have explored these interactions have leveraged population-scale genotype surveys to associate genetic polymorphisms with epigenetic states in whole blood or other tissues composed of heterogeneous cell types. Epigenetic states differ by cell type, raising the possibility of cell-type specific genetic-to-epigenetic relationships that drive specific functional states and disease features. To address this, I measured the impact of genetic variation on histone modifications across a cohort of eight individuals in four distinct cell types of the normal human breast. I found that cell-type specific histone modification-associated variants were enriched in associations with active histone modification states and predicted transcription factor binding site creation/disruption events normally upregulated in the matched cell types. Correlation of variant-associated histone modification states with nearest-gene expression allowed for the prioritization of functional candidates. One such variant in the first intron of ANXA1 was found in association with H3K4me3 differences uniquely in the breast luminal epithelial cell subset and could be reproduced using CRISPR-mediated homology-directed repair in a cell line model in vitro. Previous work has suggested contradictory effects of ANXA1 expression, with worse outcomes for some triple negative, basal-like, and invasive ductal carcinomas, and improved outcomes in ductal carcinoma in situ. This study thus demonstrates the potential importance of assessing cell type when considering the impacts of genetic variants on regulation and disease mechanisms.

75.

Comparative analysis and tumorigenesis of normal human mammary cells from male and female donors

Shengsen Ding

Men normally develop and maintain mammary glands that similar to female mammary glands, but with reported deficient lobule formation. Men also develop breast cancer, mostly of the ER+ subtype and an age-associated increasing onset, but at a 100-fold lower incidence and overall worse outcome. However, the classification and treatment of male breast cancers are largely based on strategies developed for female patients, despite their known hormonal and other differences. We now report the utility of multiple systems for analyzing normal adult human pre-menopausal and post-menopausal mammary cell properties to freshly obtained and viably cryopreserved gynecomastia human male mammary tissues. Use of procedures created for analyzing pre-menopausal and post-menopausal breast tissue indicates that the human male mammary glands contain the same subsets but in different relative proportions which is consistent with a lack of alveolae. However, the freshly isolated male Luminal Progenitors (LPs) and Basal Cells (BCs) contain a similar frequency of EGF-responsive colony-forming cells (CFCs) as female LPs and BCs. Subcutaneous transplantation of the male mammary cells in male immunodeficient mice resulted in the generation of ER+/PR+/CK14+/p63+/CK18+ hollow mammary structures 4 weeks later as previously shown for female cells, and these were found to still contain clonogenic cells. We have also found evidence from qPCR and RNA-seq analyses of reduced progesterone signaling and alveologenesis in male and post-menopausal mammary cells. Meanwhile, post-menopausal samples have an intermediate differentiation-related gene expression profiles between male and pre-menopausal samples. Interestingly, preliminary experiments also indicate that specific combinations of oncogenes have similar effect on normal human male and post-menopausal mammary cells as on human pre-menopausal mammary cells in terms of tumour sizes and subtypes.

76.

IDENTIFICATION OF A NOVEL INTERACTOR OF ENDOGENOUS SS18::SSX THROUGH MASS SPECTOMETRY-BASED ANALYSIS IN SYNOVIAL SARCOMA CELLS

Ainiah Rushdiana Raquib

The role of the fusion oncoprotein SS18::SSX in synovial sarcoma has been studied extensively to understand how one mutant protein is able to cause and sustain oncogenesis. Prior experiments using mass spectrometry to analyse interactors of SS18::SSX have relied on cell line models engineered to tag the protein for pulldown experiments due to the lack of an antibody specific to SS18:SSX. The current study aims to identify novel interactors of the synovial sarcoma fusion oncoprotein in endogenous cellular backgrounds using a novel, commercially-available SS18::SSX antibody. Whole cell lysates were collected from six synovial sarcoma cell lines containing the canonical SS18::SSX protein. Dynabeads was used to perform IP with the SS18::SSX antibody and a control rabbit IgG antibody, followed by processing of eluted peptides to determine protein abundance in each sample. Protein abundance was compared in each cell line against negative control. Comparison of the top hits in all samples identified a canonical PRC1 complex member, CBX4, as a positive hit in 4 out of 6 cell lines. As part of the canonical PRC1 complex, CBX4 utilises its chromodomain to recognise the epigenetic H3K27me3 mark on histones to mediate transcriptional repression. Co-IP of SS18::SSX with western blot detection showed CBX4 to be present in all 6 cell lines and immunofluorescence imaging confirmed nuclear co-localisation of SS18::SSX with CBX4. Using the fusion-specific antibody for immunoprecipitation of the endogenous SS18::SSX protein helps to detect common interactors amongst different synovial sarcoma cell lines, and uncover new pathways and roles for the protein in sarcomagenesis. The discovery of a novel interaction between SS18::SSX and the PRC1 member, CBX4, presents itself as a druggable target in synovial sarcoma with the use of existing inhibitory molecules.

77.

UTILIZING ADIPOSE-DERIVED STEM CELLS ON DECELLULARIZED BLADDER SCAFFOLDS FOR FUNCTIONAL BLADDER MUCOSA REGENERATION

Caesar Ulises Monjaras Avila

Treatment for Bladder Cancer (BCa) often involves radical cystectomies, requiring bladder reconstruction. Current methods, like enterocystoplasty, have limitations, highlighting the need for better alternatives. Tissue engineering offers potential solutions, including using cellular or acellular scaffolds. Acellular scaffolds, like the bladder acellular matrix, show promise of providing a foundation for native cell growth. However, obtaining normal bladder cells from BCa patients is challenging, prompting the use of alternative sources like patient-derived stem cells. Adipocyte-derived stem cells (ASCs) have shown potential for differentiating into urothelial cells, leveraging this ability this project focuses on transdifferentiating ASCs into functional urothelial cells for bladder reconstruction. The methodology involves isolating ASCs from adipose tissue obtained during liposuction, transdifferentiating them into urothelial-like cells using a co-culture technique and then evaluating their characteristics and functionality. Results indicate successful isolation and characterization of ASCs, displaying positive markers for stem cells as guidelines mandate. The co-culture of ASCs with SV-HUC cells resulted in changes resembling epithelial cells, indicating a potential transdifferentiation process, and is corroborated by the mRNA and protein levels. For the functional assay, urothelial-like cells were seeded onto decellularized bladder tissues. ASCs and SV-HUC cells were used as controls. After 10 days in culture, the urothelium barrier function will be assessed by analyzing uroplakin and tight junction proteins. The outcomes of this project hold promise for advancing bladder reconstruction methods, offering a potential alternative to current approaches. The successful transdifferentiation of ASCs into functional urothelial cells could pave the way for innovative, more effective strategies in BCa treatment and reconstruction.

78.

In Vivo Generation of CD19 CAR T Cells by Lipid Nanoparticle Mediated mRNA Delivery

Lauralie Short

Background: Patients with relapsed or refractory B cell malignancies have seen promising outcomes when treated with CD19-directed chimeric antigen receptor (CAR) T cells. However, the current manufacturing process for CAR T cells is costly, laborious, and time-consuming as a GMP-grade bespoke product needs to be produced for every patient. The need for autologous products limits the accessibility to this immunotherapy and rationalizes the production of an off-the-shelf product to enable the engineering of CAR T cells in vivo. Lipid nanoparticles (LNPs) have proven to be safe and effective delivery vehicles for nucleic acids as seen in the development of the SARS-CoV-2 vaccines. Currently however, LNP formulations have been shown to largely accumulate in the liver. Thus, to better target immune cells in vivo, novel LNP prototypes have been formulated to limit their hepatic uptake. Hypothesis: We hypothesize that novel LNP formulations can deliver CD19 CAR mRNA to T cells and functionalize them to eradicate malignant B cells. Methodology: To evaluate the feasibility of this approach, we will determine the tropism of novel LNP formulations encapsulating for the CD19 CAR mRNA, in vivo across multiple organs by flow cytometry. We will also evaluate in vitro the antigen-specific cytotoxicity, cytokine production and proliferation of the LNP-generated CAR T cells. Furthermore, in vivo lymphoma tumour models in immunocompetent mice are under development, allowing us to understand the therapeutic activity and safety of this approach. Results: Following the administration of LNP-CAR mRNA, we saw that T cells in the blood, and in the bone, marrow expressed the CD19 CAR mRNA. We also noted transfection in other immune effector cell types like macrophage in various immune compartments. Lastly, this novel LNP showed very little tropism to the liver. Conclusion: By creating an off-the-shelf CAR product, we seek to broaden the use of CAR technology by increasing its accessibility.

79.

Engineering a novel CAR-T cell targeting the solid tumour target podocalyxin

Grace Bernard

Chimeric antigen receptor (CAR) – T cells have rapidly become one of the most promising immunotherapeutic approaches for treating cancer. Through the addition of unique scFv sequences, CARs can easily be adapted to target a desired antigen, therefore showing encouraging therapeutic potential for not only cancer treatment but also for the treatment of autoimmune diseases. Although effective responses have been observed in lymphoid cancers, CARs targeted against solid tumours have shown limited efficacy for multiple reasons, among which is a lack of unique tumour-specific targets. Thus far, CAR T-cell development has largely focused on targeting tumour-associated proteins that become overexpressed on malignant cells. However, proteins that are overexpressed on cancerous cells but also found at lower levels throughout the body can cause on-target off-tumour toxicity; ultimately resulting in the destruction of healthy or helpful cells. In an attempt to increase CAR-T cell efficiency against solid tumours and decrease off-tumour engagement, we plan to engineer a CAR that targets the protein podocalyxin (podxl). Podxl is expressed on the surface of various cell types throughout the body and is essential for development, while also actively contributing to cell mobility. In the context of cancer, podxl is highly expressed on a myriad of different cancer types, including ovarian and pancreatic cancers, and has been shown to enhance tumour invasiveness, promote metastasis, and is associated with poor prognosis. Recent studies have demonstrated that podxl acquires a unique glycosylation signature on tumour cells that is no observed in normal tissues; making it an ideal target for CAR-T cells. We aim to develop and test a novel CAR-T cell that targets the tumour-specific podxl glyco-epitope using in vitro and in vivo models. Ultimately, podxl shows great therapeutic promise for CAR-T cell therapy targeting solid tumours while mitigating patient toxicity.

80.

Unlocking the Potential of Prostate Organoid Culturing Systems

Christopher Dusek

There are around 10 parent cell lines in use for prostate cancer research which fail to fully recapitulate the disease seen in clinic. Of those 10, three lines are used disproportionally more (LNCaP, PC-3, and DU-145) and account for ~93% of the models used in published literature from 2010-2019. In-vivo models are costly and lack high-throughput assay capabilities. Primary tissue organoids serve as a model system in between the two current models allowing for better recapitulation of the disease without the high cost and low-throughput of in-vivo models. Prostate organoids (POs), however, have not been a viable research tool for those seeking to study prostate biology due to their historically low in-vitro success rate of 15-20%. Through a MITACS partnership, a collaboration between STEMCELL Technologies and the Ong Lab was formed. In this collaboration we seek to establish a reproducible, effective, and efficient in-vitro organoid culture method to support the growth of human prostate tissue from both non-cancerous and tumor tissue.

Poster Abstracts

81.

Potentiating ERK hyperactivation by targeting the proteostasis network

Dylan Farnsworth

Recent findings have highlighted that cancer cells retain sensitivity to excessive signaling driven through the extracellular regulated kinase (ERK) pathway, referred to as ERK hyperactivation. In normal cells, excessive ERK signaling drives proliferative arrest through activation RB and p53, and induces senescence. The mechanism of proliferative arrest driven by ERK signaling in cancer cells, as well as the effect of different genetic backgrounds on this response, remain unclear. We aimed to characterize both in the context of targeted therapy resistant lung adenocarcinoma (LUAD). We performed a genome wide CRISPR-Cas9 knockout screen paired with transcriptome profiling in models of ERK hyperactivation to identify pathways that could potentiate cell death. We uncovered components of the unfolded protein response and proteostasis network as key dependencies during states of ERK hyperactivation. We found that inhibiting these nodes further sensitized cells to ERK hyperactivation. We next sought to characterize potential for ERK hyperactivation across different genetic alterations detected in the context of targeted therapy resistance. Using dox inducible promoters, we overexpressed genes associated with EGFR inhibitor resistance in EGFR mutant LUAD cells and found several that induced proliferative arrest when co-expressed with mutant EGFR. Finally, we developed models of resistance to EGFR, KRAS and MET inhibitors, respectively. In these models, we found that disrupting proteostasis sensitized cells to a drug holiday, suggesting clinical translation for this novel dependency. Together, this work highlights the various positive mediators of ERK hyperactivation in LUAD as well as the mechanisms by which cancer cells tolerate it.

82.

Role of Innate Lymphoid Cells in Alcohol-HF Diet-Induced Chronic Steatohepatitis and Fibrosis

Mona Orangi

Chronic hepatitis and cirrhosis significantly elevate the risk of liver cancer. Alcoholic liver disease (ALD) and non-alcoholic fatty liver disease (NAFLD) can advance to hepatitis and fibrosis. ILC2s have been shown to be involved in the development of hepatic fibrosis in ConA-induced hepatitis and CCL4-induced liver fibrosis. However, the role of ILCs in ALD and NAFLD is still unclear. We established a murine model of chronic steatohepatitis and fibrosis. B6 mice received a combination of alcohol in drinking water and a high-fat (HF) diet. This model mirrors human conditions, as heavy drinkers often consume fatty foods. Within 6 weeks, alcohol-HF diet induced hepatomegaly, steatosis, and hepatitis, confirmed by triglyceride assays, liver sections, and increased myeloid cell numbers. Fibrosis, evident by picrosirius red staining of collagen, emerged within 9 weeks. RAG1-deficient mice treated similarly developed steatohepatitis and fibrosis, indicating no adaptive immunity involvement. Conversely, NSG mice lacking lymphocytes and ILC2-deficient CD127cKO mice, generated by crossing Rora-IRES-Cre and floxed Il7ra mice, showed no significant increase in liver myeloid cells, suggesting ILCs' role in hepatitis, although RORγt-KO results indicated ILC3s' non-involvement. Single-cell RNA sequencing revealed liver ILC1 heterogeneity, dividing them into four clusters. Although total ILC1 numbers remained unchanged post-treatment, cluster-1 ILC1s, highly expressing granzyme c, expanded. These findings suggest the critical roles of ILC1s and ILC2s in alcohol-HF diet-induced chronic steatohepatitis and fibrosis.

83.

Identifying irreversible molecular changes associated with lung cancer in former smokers

Liam Brockley

Former tobacco smokers remain at high risk for lung cancer long after they quit smoking. Smoking induces a field of injury in airways, which can be detected as persistent genetic and epigenetic changes in airway epithelial cells. While most of these changes revert to normal levels after smoking cessation, a subset of 'irreversible' changes persists in former smokers. If some irreversible changes are shown to cause lung cancer, they could be intercepted before lesions form, used to stratify former smokers by cancer risk for screening programs, or detected in a non-invasive manner. While several studies have investigated irreversible expression changes in airways, they have not completely described the mechanisms underlying the differential expression, or substantiated links to lung cancer. For my project, I will incorporate data from multiple studies to identify irreversible genes in the airways of former smokers. I will further evaluate the degree of reversibility by considering pack-years, time since quitting, and other patient characteristics. I will identify which of these expression changes could be linked to lung cancer by performing comparisons with lung cancer 'omics datasets. To determine common mechanisms contributing to alterations in both airways and tumors, I will perform comparisons with epigenetic airway datasets and an expression dataset from tumor-adjacent lung tissue. I will also carry out cell signaling pathway and survival curve analysis to assess biological relevance. As a future direction for the project, pathways and genes of interest could be altered in vitro to evaluate the phenotypic effects of the irreversible changes.

84.

Defining the origins and metabolic pathways of osteoclasts in multiple myeloma

Melika Bakharzi

Osteolytic bone lesions are a characteristic feature of multiple myeloma (MM). Despite their prevalence in approximately 90% of MM patients, the therapeutic options available to manage bone absorption by inhibiting osteoclast (OC) activity are very limited and often accompanied by complex side effects. In healthy adults, OCs originate from osteoclast precursor cells derived from hematopoietic stem cells. However, in pathological conditions, such as in inflammatory diseases, OCs can also arise from other cells, such as physiological monocytes and dendritic cells, indicating the adaptable nature of OC genesis. Despite the emergence of single cell technologies, the exact origin of OCs in MM is not understood. This poses a critical challenge for the development of novel bone lesion targeting agents particularly suppress OCs genesis in MM and inhibit OC interactions with MM cells (MM-OCs). MM-OCs are thought to be characterized by elevated metabolic activity which could be a potential therapeutic weakness. Despite the emergence of drugs targeting the metabolism of cancer cells, the influence of MM cells on MM-OC metabolism and vice versa remains poorly understood. In this research proposal, we seek to investigate the origins of MM-OCs, along with their interactions with MM cells, and thus identify pathological pathways that can be targeted to prevent MM-OC differentiation and inhibit MM-OC energy metabolism to prevent osteolytic bone lesion formation in MM patients.

85.

Enhancing Early Relapse Detection in Testicular Cancer through Rolling Circle Amplification of microRNA Biomarkers

Andy Jia

This project aims to improve the accuracy of early-stage testicular germ cell tumor (GCT) relapse detection through rolling circle amplification (RCA) of plasma microRNA miR371 biomarker in patients with clinical stage I (CSI) on surveillance. GCTs pose a significant challenge in clinical management due to the lack of sensitive and specific biomarkers for early relapse detection. Patients are at risk of over-treatment and longterm chemotherapy toxicity that negatively impacts life expectancy and quality of life. Commonly used Polymerase Chain Reaction (PCR)-based methods for miRNA quantification have limitations, including timeconsuming setup, precise thermal cycling control requirements and false positive results due to contaminationprone samples. Isothermal amplification techniques lack thermal cycling which has greater sensitivity and ease of use. RCA is one such method that is effective in treatment and research applications. The clinical validity of microRNA miR371a-3p has been shown to be a potential biomarker for non-teratoma GCTs. miR371a-3p exhibits superior operating characteristics compared to CT scans and classic tumor markers. However, the sensitivity of this biomarker in early-stage GCT detection using current PCR methods is low, and more accurate methods to detect smaller amounts of circulating miR371 are needed. Patient samples from the provincial genitourinary biobank will be used, and RCA result sensitivity will be evaluated against PCR results. The successful implementation of RCA will validate a more sensitive method for miR371 analysis. This will enhance clinical decision-making, reduce treatment toxicity and extend the reach and applicability of early GCT relapse detection to resource-limited settings.

86.

Elucidating the immunomodulatory role of miR-210 in Acute Myeloid Leukemia

Leo Escano

Patients with Cytogenetically Normal Acute Myeloid Leukemia (CN-AML) comprise the largest AML patient group, with mutations in the nuclear trafficking protein Nucleophosmin1 (NPM1mut) being the most common genetic abnormality. Depending on co-mutations, patients with NPM1mut CN-AMLs have relapses rate between 20-70%. Interactions between AML and immune cells can create a tumor permissive immune microenvironment that facilitates AML survival, a process called immune escape. In contrast, enhanced anti-cancer immune responses are associated with higher overall survival. However, the extent to which NPM1mut CN-AMLs utilize and regulate immune escape remains poorly understood. I have found that microRNA-210 (miRNA-210) is significantly enriched in NPM1mut AML patients and that high levels of miR-210 associate with dismal outcome. I have found that overexpression of miR-210 in human AML cell lines results in 1) decreased expression of MHC class II expression through downregulation of the HLA master regulator CIITA and 2) increased surface expression of the immune checkpoint PD-L1. Reduced HLA expression and increased immune checkpoint expression are hallmarks of immune escape. These findings underline the unexplored role of miR-210 in modulating the immune microenvironment in AML.

87.

Development of PDX humanized mice model for HGSOC

Tsz Yin Lam

High grade serous ovarian cancer (HGSOC) accounts for 70% of all ovarian cases with the worst prognosis. First line treatment includes debulking surgery and chemotherapy. Majority of the patients suffered from recurrence with a 5-year survival rate under 50%. Various treatment approaches are employed to reduce recurrence, promote treatment outcomes, and prevent resistance. However, the design of a successful treatment approach is challenged by overall low response rates. This study aims to develop and characterize humanized patient-derived xenograft (PDX) mice model to decode the interaction of HGSOC tumor and the immune system, and to examine immunotherapy effects. We generated four humanized mice from female ENW mice (NRG-W41-3GS) (aged 7-8 weeks). 5x104 to 7x104 hematopoietic CD34+ cells were intravenously injected to five mice via tail vein. Bone marrow (BM) aspiration were performed at week 5 post injection and peripheral blood (PB) were drawn from saphenous vein at week 8, 9, 11, 14 to evaluate engraftment. BM samples were stained with two human CD45+ antibodies and PB samples were stained with an extra mouse CD45+ antibody. Samples were analyzed with flow cytometry. BM results showed robust engraftment, with 34%-46% huCD45+ cells in four mice. PB results showed 14%-37% at week 8 and maintained stable with slight deviation at week 9, 11, and 14. To conclude, hematopoietic CD34+ enriched cells transplantations in ENW mice can lead to the production of CD45+ human immune cells. We believe that our humanized mice models have the potential to develop HGSOC PDX model and evaluate various treatments, in particular immunotherapy. In the upcoming work, we will proceed to inject HGSOC patient tumor sample subcutaneously into humanized ENW mice once we confirm the robust engraftment in BM and PB samples.

88.

[68Ga]Ga-ProBOMB5 - a novel 68Ga-labeled [Leu13ψPro14]bombesin analog for imaging gastrin-releasing peptide receptor expression with positron emission tomography

Lei Wang

Gastrin-releasing peptide receptor (GRPR) is a promising target for cancer imaging and therapy. High pancreas uptake is the primary drawback of most published GRPR-targeted radioligands. Minimizing pancreas uptake is highly desirable for the development of effective GRPR-targeted radioligands, particularly for therapeutic application. Our group recently reported a potent GRPR antagonist, [68Ga]Ga-TacsBOMB5 (Wang L, et al. Molecules.2022) based on a modified bombesin(7-14) sequence with a reduced peptide bond (CH2-N) between residues 13-14 (Leu13ψThz14) and an NMe-Gly11 substitution. This tracer showed a high tumor uptake and a minimal pancreas uptake at 1 h post-injection (pi). However, its 4-thiazolidinecarboxylic acid residue (Thz14) is prone to oxidation in the final product formulation, leading to a short shelf-life. In this study, we replaced Thz14 in [68Ga]Ga-TacsBOMB5 with Pro14 to prolong the shelf-life and evaluated the potential of the resulting [68Ga]Ga-ProBOMB5 for imaging GRPR expression with positron emission tomography (PET). ProBOMB5(DOTA-Pip-[D-Phe6,NMe-Gly11,Leu13ψPro14]Bombesin(6-14)) was synthesized in 37% yields. Ga-ProBOMB5 binds to GRPR with a good binding affinity (Ki (GRPR) = 12.2±1.89 nM (n=3)). [68Ga]Ga-ProBOMB5 was obtained with >95% radiochemical purity. [68Ga]Ga-ProBOMB5 enabled clear visualization of PC-3 tumor xenografts in PET image and showed a very low uptake in all normal organs/tissues at 1 h pi. [68Ga]Ga-ProBOMB5 showed a good tumor uptake (12.4±1.35 %ID/g) at 1 h pi. The pancreas uptake value of [68Ga]Ga-ProBOMB5 was lower than [68Ga]Ga-TacsBOMB5 (1.37±0.40 vs 1.98±0.10 %ID/g). Compared with [68Ga]Ga-TacsBOMB5, despite a slightly lower uptake in PC-3 tumor xenografts, [68Ga]Ga-ProBOMB5 has superior tumor-to-background contrast ratios due to its extremely low background uptake. These features demonstrate that [68Ga]Ga-ProBOMB5 is a promising tracer for clinical translation for detecting GRPR-expressing tumor lesions with PET.

89.

Albumin binders to improve tumor uptake of CXCR4-targeted radiopharmaceuticals in advanced prostate cancer

Itzel Astiazarán-Rascón

Neuroendocrine prostate cancer (NEPC) is an aggressive subtype of prostate cancer necessitating novel targeted therapeutic approaches. Recently, we have confirmed CXCR4 expression in different NEPC patient-derived-xenografts (NEPC PDX) models suggesting its utility for radiopharmaceutical targeted therapy (RPT). Lutetium-177 is a beta-emitting radioisotope commonly used for RPT due to its potential to kill cancer cells. In vivo evaluation of [177Lu]Lu-BL34, a CXCR4-targeting peptide labelled with lutetium-177, showed specific tumor uptake and significant tumor growth delay at a high dose in a CXCR4 expressing NEPC PDX murine model. However, the therapeutic effect is limited due to the fast clearance of the drug. Novel CXCR4 targeting peptides conjugated to phenyl-derived albumin binder moieties were characterized using a NEPC PDX model. [natLu]Lu-BL34T1, a p-chloro-phenyl albumin binder derivative, showed high in vitro binding affinity to human CXCR4 (IC50= 2.1±1.6 nM). Moreover, increasing the albumin concentration in an in vitro cell uptake assay did not influence the total cell binding reaching >80% cell uptake after 3h. In vivo, [177Lu]Lu-BL34T1 resulted in rapid tumor uptake at 1h post-injection (16.6 ± 3.1 %IA/g) and maximum tumor uptake was reached after 24h (34.9 ± 2.6 % IA/g) in NEPC PDX-tumor bearing mice. The area under the curve (AUC) values were calculated from the biodistribution data. The AUC(0-168h) values for [177Lu]Lu-BL34T1 resulted in a 4.4-fold 2.6-fold and 15.5-fold increase in blood, kidney and tumor retention compared to [177Lu]Lu-BL34 ($p<0.05$). The slow blood pool clearance of [177Lu]Lu-BL34T1 led to a higher tumor uptake in a NEPC PDX model. Also, higher tumor AUC values in comparison to [177Lu]Lu-BL34 resulted in a higher tumor absorbed dose optimal for RLT.

90.

Elucidating the role of IL-33 in prostate cancer following androgen deprivation therapy

Claire Dourieu

Background: Prostate cancer (PCa) is the most common cancer in Canadian men. As androgen receptor (AR) is a driver of PCa, androgen deprivation therapy (ADT) is the standard of care for advanced PCa, however many patients go on to experience relapse, highlighting the need for earlier intervention strategies. As such, it would be optimal to target cancer cells in the earlier disease state when cancer is more easily treated. Interleukin 33 (IL-33) is a pluripotent cytokine and nuclear factor with conflicting roles in cancer. Our focus is to understand the role of IL-33 in PCa progression including tumor immune microenvironment (TME) remodelling. IL-33 has been shown to induce immunosuppressive properties in cancer, and as prostate cancer is considered an immunologically “cold” tumor, IL-33 may contribute to this phenotype. We hypothesize that IL-33 is upregulated following ADT in PCa cells, which acts as a mechanism of immune evasion allowing cancer cells to survive through TME remodelling. Methodology: Identification of IL33 upregulation was carried out in both patient derived xenograft (PDX) models and immunocompetent mouse models at chronological timepoints post-castration. Such discovery was validated in ADT-treated clinical PCa samples. Functional analysis will be carried out using PCa cells lines and shRNA knockdowns subject to androgen deprivation and immune cell co-culture assays. Our results indicate IL-33 upregulation across multiple PDX models, immunocompetent mouse models, and clinical cohorts following androgen deprivation. In vitro, androgen responsive cell lines such as Myc-CaP and LNCaP indicate upregulation in preliminary assays following androgen deprivation. In conclusion, we have established that upregulation of IL-33 is clinically relevant following ADT, and IL-33 upregulation is observed across many models in vivo and in vitro under androgen deprived conditions. Next steps will be to assess the functional role of this gene in PCa progression.

91.

Plasmablastic lymphoma (PBL) does not depend on B-cell receptor signaling and the NF-κB pathway

Jasper Wong

Plasmablastic lymphoma (PBL) is an aggressive type of non-Hodgkin lymphoma that predominantly occurs in patients with HIV or other causes of immunodeficiency, and are associated with Epstein-Barr virus (EBV) and MYC translocations. Current standard of care immunochemotherapy infrequently cures patients with PBL and conclusions on pathogenesis from previous studies, beyond the above factors, have been limited by the low number of samples used for genomic analyses. Here, we provide a comprehensive analysis of the genomic and transcriptomic landscape of PBL using a collection of 198 PBL exomes and genomes and 64 transcriptomes from archival diagnostic formalin-fixed paraffin-embedded tissue biopsies. Somatic mutations (SNVs/Indels) were identified using an ensemble approach of four variant callers (Strelka2, Lofreq, Mutect2, SAGE). Salmon and DESeq2 were used to assess differential gene expression. To compare PBL to other known B-cell malignancies, we compared PBL to genomes and transcriptomes of 92 multiple myelomas (MM), 238 Burkitt lymphomas (BL), and 208 diffuse large B-cell lymphomas (DLBCL). On a genomic level, we observed mutations affecting the JAK/STAT pathway (STAT3, SOCS1) and MAPK pathway (NRAS), as opposed to mutations that commonly affect the NF-κB signaling pathway observed in the activated B-cell-like (ABC) subtype of DLBCL – the entity thought to be the most closely related lymphoma to PBL. Through comparisons with transcriptomic data of other aggressive B-cell neoplasms (DLBCL, BL, MM), we identified a unique PBL expression signature that involves the down-regulation of genes involved in the canonical B-cell receptor signaling pathway. In vitro data showed lack of NF-κB signaling and resistance to therapeutics that inhibit B-cell receptor signaling pathways. Taken together, this may explain why current chemotherapeutic drugs targeting the NFκB pathway have limited efficacy in PBL.

92.

Repurposing disulfiram for cancer: a drug delivery and population-based approach

Devon Heroux

Disulfiram was approved by the FDA under the brand name Antabuse for treatment of alcohol-abuse in 1951, although for over 40 years there has been evidence supporting disulfiram's use as an anti-cancer drug. Upon administration, disulfiram is hydrolyzed to form two molecules of DDC, which upon binding Cu forms Cu(DDC)2, the proposed active form against cancer. It was our goal to formulate the water-insoluble Cu(DDC)2 in liposomes to facilitate its repurposing for breast and colon cancers. In addition, we also initiated an ongoing population-based study assessing the protective and treatment effects of disulfiram with cancer. Cu(DDC)2 liposomes were generated by the thin-film hydration method followed by extrusion, using DSPC and cholesterol (55:45 molar ratio). DDC and CuSO4 were added to the liposomes forming Cu(DDC)2, which was transported inside the liposomes. Anti-tumour activity was evaluated in tumour-bearing mice with or without competent immune systems, and immune system activation was assessed via a cancer vaccination model and the expression of markers of immunogenic cell death (ICD). A study was also initiated for cancer cases and controls in British Columbia (BC Cancer) with at least one prescription of disulfiram from 1996-2022 (PharmaNET), patients treated with immune-modulating therapies (BC Cancer), and outcome of death (Vital Statistics). Liposomes encapsulating Cu(DDC)2 caused a 40% reduction in tumour burden in a MDA-MB-231 xenograft model, and the results of a cancer vaccination study along with the presence of ATP, HMBG1 and surface-exposed calreticulin indicated the presence of ICD. The ongoing case-control study is expected to provide data to support the clinical potential of disulfiram, as a protectant and as an adjuvant with immune-activating therapies such as checkpoint inhibitors and anthracyclines to potentially improve efficacy through induction of ICD.

Poster Abstracts

93.

Large-scale DNA Organization Identifies Aggressive Prostate Cancer in Low and Intermediate Risk Patients

Fumi Inaba

Prostate cancer (PCa) is the most common cancer in men, with a lifetime risk of 1 in 8. PCa is clinically heterogenous, where patients my present indolent to aggressive cancers with a 5-year survival rate of over 99% or 41% respectively. Currently, patient risk stratification is done largely using the D'Amico classification system where patients are classified as low, intermediate, or high risk according to their serum PSA, Gleason score and clinical stage. However, recent literature suggests the inadequacy of this system where post-radical prostatectomy (RP) 5-year biochemical recurrence (BCR)-free survival was found to vary significantly, even within the same risk group. To work towards a refined, personalized risk assessment method, we propose a framework analyzing Feulgen-thionin stained needle biopsies on a nuclei-by-nuclei basis. Nuclear morphology, stain intensity and chromatin texture are quantified in ~140 large-scale DNA organization (LDO) features, previously demonstrated to be an effective prognostic marker for breast cancer survival. In this framework, LDO features are used as a predictive biomarker for post-RP BCR. Individually nuclei segmented by a sequential U-Net system are clustered into 10 groups based on unsupervised analysis of LDO features, and binary random forest classifiers are trained for the two clusters most predictive of BCR. Linear discriminant analysis on the proportion of nuclei suggesting BCR determines if a biopsy suggests BCR. Patients with more than half of their biopsies predicting BCR are predicted to experience BCR. On a cohort of 115 patients and 295 needle biopsies from Slovenia, the framework correctly classified 98.12% of biopsies, and 98.2% of patients. Analysis of the predictive models still needs to be completed to verify the framework is not overfit. The framework will be validated on additional prostate cancer cohorts to further elucidate the biological relevance of LDO features.

94.

Characterizing and targeting the interplay between the SWI/SNF chromatin remodeling complex and ASCL1 in prostate cancer lineage plasticity

Cassandra Cui

Prostate cancer (PCa) relies on the androgen receptor (AR) for growth and survival. Despite the success of androgen pathway inhibitors (ARPIs) in alleviating tumor growth, ~20% of patients can relapse with tumors independent of AR signaling. These tumors, known as treatment-induced neuroendocrine PCa (tNEPC), can trans-differentiate to acquire an alternative lineage with activated stem and neuronal programs via a mechanism known as lineage plasticity. Chromatin accessibility data from our lab in tNEPC and in ARPI-treated adenocarcinoma revealed widespread chromatin rearrangements during the progression towards tNEPC, pointing to an epigenetic basis of this disease. However, the mechanism underlying this chromatin remodeling remains unknown. Interestingly, increasing evidence has pointed to the deregulation of the SWI/SNF chromatin remodeling complex in NEPC, with a higher expression of its ATPase subunit SMARCA4 correlating with more aggressive forms of PCa. Using a PROTAC degrader of SWI/SNF ATPases, we observed that SWI/SNF inhibition in tNEPC cell lines led to potent inhibition of the neuronal lineage-determining transcription factor ASCL1 in a time-dependent manner. GIGGLE analysis, BioID, as well as proximity ligation assays suggested a potential co-operation between SWI/SNF and ASCL1 to reprogram the chromatin landscape and, in turn, activate a neurogenesis transcriptional program to induce tNEPC. As a next step, we aim to elucidate the molecular mechanism by which the SWI/SNF complex and ASCL1 co-regulate lineage plasticity.

95.

THE IMPACT OF EXTRACELLULAR VESICLES DERIVED FROM LUNG ADENOCARCINOMA CELLS ON CAF ACTIVATION

Jessica Trejo

Lung cancer is the leading cause of cancer deaths worldwide, largely due to metastasis. Its main subtype is lung adenocarcinoma (LUAD). Communication between cancer cells and cells within the tumor microenvironment, such as fibroblasts, can promote metastasis. This interaction can be mediated by extracellular vesicles (EVs) transferring bioactive cargo. Thus, this study aimed to identify the impact of EVs derived from LAC cells on lung fibroblasts.

For EV isolation, conditioned media derived from H2073 and H1437 LAC cell lines were ultracentrifuged multiple times after debris removal. Nanoparticle Tracking Analysis was used for quantification and size characterization and EV-associated markers were assessed by western blot (WB). For lung fibroblast activation, H6013 primary adult cells were treated with 1xPBS, TGFβ (10 ng/µL) or EVs (7.13x1011) every 24 hours over 3 days. Protein (for WB) and RNA (for gene expression profiling) were harvested 48 hours after treatment. RNA was extracted using the Qiagen miRNeasy kit and sequenced by Illumina NextSeq2000.

Most vesicles produced by both cell lines were between 50 and 250 nm in diameter. Fibroblasts treated with TGFβ or EVs had an increase in CAF markers (mainly α-SMA), especially in the EV group compared to the PBS treatment group. The activated fibroblasts shared differentially expressed genes in the TGFβ and EV treatments; however, there was an increase in some of the genes associated with epithelial-mesenchymal transition and cell cycle only in the EV-treated group, indicating unique features of CAF activation and the same was seen for the pathway analysis. LUAD EVs have a role in CAF activation by using both similar and unique pathways to TGFβ activation. Further understanding of the genes distinct to the EV-treated fibroblasts could lead to the identification of novel genes involved in CAF activation, and thus aid in the development of novel therapeutics that could improve patient outcomes.

96.

Sunflower Trypsin Inhibitor as Novel Scaffold For Development of Hepsin Inhibitor

Sabrina Skyba-Lewin

Hepsin is a type II transmembrane protein (TTSP) frequently overexpressed in a variety of cancers, such as ovarian, breast, and prostate cancer. Its upregulation activates proteolytic pathways and negatively interferes with cell-cell adhesion, impacting epithelial integrity. Due to its significant expression on many cancer-cell surfaces, and its pro-metastatic mechanism, hepsin has been viewed as a potential biomarker for tumor progression and a novel target for radiopharmaceutical therapy. Hepsin inhibitors have been explored as linear peptides; however, due to the hydrolytic activity of peptidases, may not be stable in vivo. An alternative scaffold is a sunflower trypsin inhibitor (SFTI), which has previously been used to engineer potent inhibitors against a variety of serine proteases, but not yet hepsin. I aim to explore the feasibility of targeting hepsin for radiopharmaceutical therapy, through the synthesis of hepsin inhibitors following an SFTI scaffold as a template. Select amino acids within the hepsin binding sequence will be altered, guided by molecular docking, to generate a small library of candidate peptides. Protein-peptide interactions will be evaluated through a BRET assay and enzyme inhibition assay, to determine which peptides would be strong candidates for radiopharmaceutical development.

97.

Characterizing the role of NNMT in modulating metabolism and epigenetic reprogramming in prostate cancer lineage plasticity

Jalal Choupani

Prostate cancer (PCa) is the most common form of cancer and ranks the third leading cause of cancer-related death among men in Canada. Treatment-induced neuroendocrine prostate cancer (tNEPC) is an aggressive variant of PCa that emerges in the context of advanced castration-resistant prostate cancer (CRPC), by the transformation of prostate adenocarcinoma after prolonged treatment with androgen receptor pathway inhibitors (ARPIs). The transition from CRPC to tNEPC entails significant alterations in transcriptional patterns, indicating that its development is likely influenced by epigenetic factors. Recently evidence has shown that there is an increased level of DNA and histone methylation in NEPC compared to CRPC. Also, our group has discovered that metabolites that increase the methylation potential of the cell are elevated in NEPC cell models. Particularly, our preliminary data hint at the involvement of nicotinamide N-methyl transferase (NNMT) in the regulation of cellular metabolism which in turn influences the epigenetic landscape. Multiple functional assays showed that NNMT overexpression in NEPC-like cell models decreases the expression of specific neuroendocrine markers along with decreasing stemness features, proliferation, and aggressiveness of these cells. Suggesting that NNMT might be required for maintaining an adenocarcinoma phenotype and its loss creates an epigenetic landscape permissive to neuroendocrine differentiation. Interestingly, increasing evidence has pointed to the involvement of NNMT in the regulation of immune infiltration in tumor microenvironment (TME) via affecting the production of chemokines and cytokines. Based on the transcriptomic data we observed that NNMT overexpression can contribute to an induction of immune response, leukocyte migration, and chemokine production. As a next step, we aim to investigate the mechanistic role of NNMT in regulating immune response in TME which may affect treatment resistance and lineage plasticity.

98.

Immune Biomarkers on Tissue Microarray Cores Support the Presence of Adjacent Tertiary Lymphoid Structures in Soft Tissue Sarcoma

Elahe Shenasa

Immunotherapy has emerged as a new treatment modality in some soft tissue sarcomas, particularly for tumors associated with tertiary lymphoid structures (TLS). TLS are functional lymphoid aggregates, and their presence is indicative of an active anticancer immune response in the tumor microenvironment The assessment of TLS as a predictive biomarker at scale on patient specimens remains challenging. While tissue microarrays could facilitate this assessment, it is not clear if small microarray cores can represent associated TLS responses. We used multiplex immunohistochemistry to identify key components of TLS: B cells, T cells, and dendritic cells. The multiplex panels (CD20 or CD79a, CD3, CD208 and/or PNAd) were applied to 80 cases both on tissue microarrays and on their cognate available full-faced sections from epithelioid sarcoma and dedifferentiated/well-differentiated liposarcoma case series. Tissue microarrays were digitally scored for the number of immune cells using the HALO image analysis platform, and cognate full-faced sections were visually evaluated for the presence of TLS. We observed that a Combined Immune Marker (defined as the presence of more than 0.51% CD20 (B cell), or more than 24% CD3 (T cell), or more than 0.14% CD208 (mature dendritic cell) on a tissue microarray cores) is highly specific (100%) and moderately sensitive (61%) to predict the existence of TLS on full-faced sections. The Combined Immune Marker was validated on an independent soft tissue sarcoma series and showed a sensitivity of 25% and a specificity of 79%. The Combined Immune Marker assessed on tissue microarrays is a highly specific marker to infer the presence of tertiary lymphoid structures on full-faced sections. Therefore, despite the small area sampled, tissue microarrays may be utilized to assess the clinical value of TLS on datasets where specificity is critical and large sample size can mitigate moderate to low sensitivity.

99.

Optimizing Culture Conditions of Patient-Derived Multiple Myeloma Cells

Ariene Cabantog

Multiple myeloma (MM) is a prevalent and incurable hematological cancer with poor prognosis and a high rate of relapse. It presents a dire unmet need in its understanding as a disease and an urgency to improve outcomes through the pursuit of new treatment avenues. Studying MM with patient-derived cells is far superior and more biologically relevant compared to immortalized cell lines. However, this remains a challenge as current primary MM culturing techniques are incapable of maintaining cell viability and MM characteristics for a duration long enough to conduct meaningful experiments. Therefore, it is crucial to urgently establish new protocols for keeping primary myeloma cells alive while retaining their MM characteristics. I tested various culture conditions with different environmental factors. These conditions include determining which commercially available growth medium is best to support cell viability, oxygen pressure and 2D vs. 3D models. In addition, I identified cytokine candidates for MM growth and survival in vitro by bioinformatic-guided screens complemented with pathway and protein network analyses to supplement the growth medium. The current protocol has successfully cultured 3 patient samples for 11 days however, manual assaying proves to be challenging. Immediate future directions are to conduct high-throughput cytokine and compound screens by automated liquid handling. In addition to cytokine signalling, investigations are underway to elucidate the role of metabolism, particularly autophagy and senescence, in MM cell culture. Cell culture results will be measured by growth, survival and retention of MM characteristics such as immunophenotype and MM drug response. These protocols must be simple, controllable, reproducible and compatible with high-throughput experiments. Creation and establishment of such a protocol will aid the treatment of MM and provide possibilities for rapid development of novel and more specific therapies.

100.

Telomerase Activity Corresponds with T cell expansion

Charu Sankaran

with impressive results in the treatment of hematologic malignancies. However, patient to patient variability can greatly dictate therapy success, and no single marker has been able to predict this prior to CAR-T manufacturing or early in treatment. It is known that longer persistence of the CAR-T is associated with improved progression free survival (PFS) and given that CAR-T repetitively encounter their target antigen, we hypothesized that cellular senescence may play a role. Senescence is characterized by a loss of proliferative capacity due to shortening of the telomeres, which are maintained by the enzyme telomerase. To mimic the CAR manufacturing process and repetitive antigen encounter in vitro, we developed a repetitive stimulation assay, in combination with a molecular toolbox of techniques, to determine if senescence may play a role. This included flow-based T cell phenotyping, telomerase activity, expansion and senescence-associated (SA) -gal activity. In this system, we observed significant donor-to-donor variability, but during the initial stimulation, Day 3 telomerase activity is predictive of overall T cell fold expansion, whereas memory phenotypes were not. Additionally, SA -gal activity increased after the first round of stimulation and decreased on subsequent rounds. We hypothesize these cells are primed for apoptosis and are performing further work to characterize this. Lastly, we are investigating whether these patterns hold true for patient samples, including B-ALL and lymphoma samples from the CLIC-01 trial, and whether this is predictive of CAR manufacturing success or patient outcome.

Poster Abstracts

101.

Investigating Intra-tumor Heterogeneity in Non-small Cell Lung Cancer Using Multiplexed Immunohistochemistry and Deep Learning

Kouther Noureddine

Non-Small Cell Lung Cancer(NSCLC) is the most common cause of cancer death in Canada and worldwide. As the immune component of the NSCLC tumor microenvironment(TME) is highly prognostic of patient outcome, further understanding of TME and the spatial organization of the immune cells within the TME is needed for better patient prognosis and treatment planning. Current immunohistochemistry techniques quantify immune cell counts and density, but generally cannot asses the spatial relationship between tumour and immune cells. We have developed a multiplexed Immunohistochemistry(mIHC) procedure combining multiple labels per round with several rounds, enabling analysis of immune cell populations on a slide through consecutive cycles of staining, destaining & hyperspectral imaging. By integrating serial imaging, sequential labeling & image registration, we are able to spatially map the TME. Robust, accurate, segmentation of cell nuclei for overlapping nuclei is one of the most significant unsolved issues in digital pathology. We have trained a deep learning segmentation method to accurately segment individual cell nuclei within overlapping clusters of nuclei. By combining a mIHC technique which enables the detection of multiple markers with deep learning segmentation methods to segment every individual cell nuclei in tissue sections with an accuracy comparable to human annotation, we can analyze the cell-cell interactions between immune and tumour cells, enhancing our ability to perform molecularly based single cell analysis of multiple cell types simultaneously within the tissue. These two techniques joined can be scaled up to the entire tissue section level, improving our understanding of the biological aggressiveness of NSCLC's.

102.

Elucidating the Role of the IR-A:IR-B Ratio in Pancreatic Ductal Adenocarcinoma

Lan Valerie Tao

Pancreatic ductal adenocarcinoma (PDAC) is a deadly disease characterized by the asymptomatic presentation at early stage, high metastatic potential, intra- and inter- tumour heterogeneity, and limited treatment options. As PDAC arises from the exocrine cells of the pancreas, they are uniquely exposed to a high concentration of insulin due to the portal structure that transports insulin from the pancreatic beta cells to the liver. We aim to investigate the role of the insulin receptor and its isoforms, IR-A and IR-B, in contribution to PDAC. By leveraging whole genome and transcriptome data of 63 metastatic PDAC patient tumours from both PanGen (NCT01855477) and Personalized Oncogenomics (NCT02155621) trials, we identified a higher INSR expression ($p = 0.00069$) in classical versus basal-like tumours of the Moffitt transcriptomic subtypes. Furthermore, IR-A and IR-B levels quantified in biopsies and organoids by RNAseq, and organoids and cell lines by qPCR, demonstrated that most samples had higher IR-B than IR-A levels. Additionally, a correlative trend in the IR-A:IR-B ratio quantification was revealed between organoid RNAseq versus biopsy RNAseq and organoid qPCR. Transcriptomic analysis unveiled subtype-specific differences in the gene expression of insulin receptor signaling and pentose phosphate pathway. Specifically, Spearman's correlation analysis demonstrated a significant positive correlation of IR-A:IR-B ratio and the upregulation of genes in the MAPK pathway and downregulation of genes in the PI3K-AKT pathway in basal-like tumours. Moreover, the IR-A:IR-B ratio was associated with higher gene expression in the pentose phosphate pathway (PPP) compared to the TCA cycle in basal-like tumours. Meanwhile, a small subset of top IR-A:IR-B ratio tumours in our cohort displayed higher gene expression related to carbohydrate metabolism compared to PPP. Overall, IR-A:IR-B ratio may contribute to subtype-specific differences in INSR signaling pathway and metabolism.

103.

DNMT3A Limits Myeloid Signaling Responses In Committed T Cells During Normal And Leukemic Development

Marissa Foo

T-cell development and lineage commitment are temporally protracted processes in which transcription factors and epigenetic modulators coordinate the sequential exclusion of alternative fates and acquisition of specialized T-cell functions. Alterations during this process can lead to diseases such as T-cell acute lymphoblastic leukemia (T-ALL). While many efforts have focused on the transcription factors which drive normal development and leukemogenesis, the underlying epigenetic constraints remain poorly understood. DNA methyltransferase 3A (DNMT3A) is recurrently mutated in human T-ALL with identified variants thought to result in protein loss-of-function (LOF). The role of DNMT3A in early stages of T cell lineage commitment was explored using an in vitro cord blood (CB) differentiation system in combination with lentiviral shRNA-mediated knockdown (KD) and CRISPR/Cas9-mediated knockout (KO) to mimic DNMT3A LOF mutations. To assess durability of commitment to the T lineage, we transferred cells at the post-commitment CD44- stage from culture conditions supporting T cell differentiation to conditions favoring myeloid differentiation. T cells with DNMT3A KD/KO were able to grow in myeloid media and expressed an aberrant T/myeloid phenotype at significantly higher frequency as compared to negative controls. These results support a working model in which DNMT3A loss in T-cell progenitors restores their ability to respond positively to myeloid growth factor stimulation both in terms of cell growth and myeloid marker expression. Thus, DNMT3A LOF mutation may afford T-ALL cells the ability to exploit alternate myeloid factor replete niches in the body. Better understanding of the developmental framework in which specific genetic alterations operate in T-ALL, as well as the functional implications of these alterations will hopefully accelerate the advancement of targeted therapies for this disease.

104.

A subset of development-associated PIWI-interacting RNAs show prognostic potential in lung cancer

Michelle Pewarchuk

The reactivation of development-associated regulatory networks can play an important role in tumour development and progression. PIWI-interacting RNAs (piRNAs) are a class of small non-coding RNAs that modulate gene expression in both adult and fetal tissues. Aberrant expression of piRNAs has been associated with cancers, such as lung cancer - the leading cause of cancer deaths worldwide. Therefore, we sought to identify fetal piRNA transcripts which were not expressed in normal adult lung tissue but showed expression in lung cancer tissues. PiRNA expression profiles of fetal lung (FL) tissues (BCWH; n=25) were compared to paired adult non-neoplastic lung (ANL) and lung adenocarcinoma (LUAD) tissue (TCGA; n= 92). Sequencing data was processed using the online tool miRMaster2. PiRNAs which had ≥ 1 reads per million (RPM) in ≥ 10% of samples within each tissue type were “expressed”. A list of 504 piRNA transcripts was considered expressed between all three tissue types. Upon differential expression analysis, a subset of 43 piRNAs was expressed in LUAD and FL, but not in ANL and thus was considered oncofetal (p-adjusted < 0.05; fold change > 2). A univariable Cox analysis identified 8 oncofetal piRNAs which were associated with survival ($p < 0.05$). A combined expression signature of these 8 piRNAs improved stratification of patients into high and low risk categories. As these piRNAs are less abundant or not present in normal adult tissue, they may prove useful as biomarkers or therapeutic targets with little toxicity to normal tissue in the treatment of lung cancer.

Poster Abstracts

105.

Bloody Sweet: The Role of Chondroitin Sulfate Glycocalyx in Prostate Cancer Vascularization

Zakir Tahiry

Prostate cancer (PC) progression depends on its ability to form new blood vessels through angiogenesis, enabling tumors to receive oxygen and nutrients and transition from a dormant state to rapid growth. PC tumor vessels differ from normal blood vessels and are characterized by features such as abnormal leakiness, chaotic arrangement, and a lack of organized structure. Within the tumor microenvironment (TME), the glycocalyx, composed of glycoproteins and glycosaminoglycans (GAGs), regulate PC vascularization. Our research reveals the role of Chondroitin sulfate (CS) glycocalyx, a sulfated subtype of GAGs, in PC vascularization. By testing in the PC LNCaP xenograft model, we observed that the degradation of CS led to alterations in the tumor vasculature and reversed PC tumor erythrocyte leakiness. The removal of CS not only modified the morphology of stabilizing pericellular cells, pericytes, and enhanced their coverage on PC tumor vessels but also resulted in a more normalized endothelial architecture surrounding the tumor. CS degradation brought about a structural resemblance to normal prostate tissue architecture, characterized by more organized and diffuse vascular patterns. This was further supported by increased infiltration of mouse stroma and elevated expression of fibroblast markers in CS-depleted tumors. Through RNA sequencing and mass spectrometry, we identified distinct stromal-related pathways changes associated with CS glycocalyx degradation. These findings highlight the role of CS in the tumor vasculature and its potential as a therapeutic target for normalizing tumor blood vessels, which could be exploited for therapeutic benefits in PC.

106.

Overcoming aggressive EMT-driven phenotypes in t(9;11) acute myeloid leukemia through the modulation of microRNA-204

Liam MacPhee

Improved genetic sequencing and tracking of clonal evolution in t(9;11) acute myeloid leukemia (AML) have led to the advent of novel targeted therapies, such as MENIN inhibitors. However, vast intratumoral heterogeneity and the inability to drug established oncogenes such as HOXA9 and MEIS1 underlie a 5-year survival under 30%, highlighting the need to characterize mechanisms driving refractory disease and relapse, and to establish novel treatment strategies. The expression of microRNA-204 (miR-204) has been predicted to regulate MEIS1 in AML and its increased expression has been shown to correlate with better clinical outcomes after chemotherapy, suggesting a potential anti-leukemic role in t(9;11) AML. We show that low-dose hypomethylation increases endogenous expression of miR-204, supporting evidence in other cancers that the hypermethylation of the host gene TRPM3 may be driving the repression of miR-204 in AML. Upon enforced lentiviral overexpression of miR-204, both human CD34+ cord blood and murine MLL-AF9 cells showed a significantly reduced colony forming capacity. Overexpression in transplantable murine models driven by MLL-AF9 or the co-overexpression of its downstream effectors Hoxa9/Meis1 significantly prolonged survival and promoted a cell morphology and immunophenotype consistent with increased myeloid differentiation. RNA-Seq analysis on bone marrow from MLL-AF9 mice post-mortem revealed the repression of genes and predicted targets of miR-204 that include regulators of cell motility and chemotaxis with well-established clinical significance in t(9;11) AML, including downstream targets of HOXA9/MEIS1. Further, miR-204 overexpression also increased sensitivity to cytarabine in vitro. Combined, our results define an anti-leukemic role of miR-204 in MLL-AF9 AML, where its increased expression represses regulators of chemotaxis downstream of the HOXA9/MEIS1 complex, antagonizing disease progression and rendering cells more susceptible to cytarabine.

Poster Abstracts

107.

Pyruvate supplementation alters metabolism and improves effector molecule expression in CD8+ T cells

Michael Hall

Adoptive T cell therapy has emerged as a successful treatment for hematologic malignancies such as lymphoma; however, there seems to be a lack of therapeutic efficacy in solid tumours. One of the main reasons is the competition for nutrients which leads to weaker T cell activation and killing of tumour cells. Metabolism is critical to the function of CD8 T cells. It is well established that once CD8 T cells are activated, they become highly glycolytic which promotes proliferation and tumour killing. The purpose of glycolysis is to: 1) produce a number of glycolytic intermediates that are used for anabolic processes 2) make ATP rapidly and 3) To maintain NAD+/NADH pools through lactate dehydrogenase (LDHA) and glyceraldehyde-6-phosphate dehydrogenase (GAPDH). We hypothesize that activated CD8 T cells supplemented with pyruvate can more efficiently recycle NADH to NAD+ so glycolysis can run more efficiently, ultimately leading to improved CD8 T cell effector function. So far, we have discovered that supplementing CD8+ T cells with sodium pyruvate (NaPyr) improves the killing of tumour cells in co-culture assays, as well as increases the expression and secretion of inflammatory cytokines: IFNγ and TNFα. Furthermore, NaPyr supplementation significantly increases pools of TCA cycle intermediates which may promote epigenetic alterations leading to increased transcription of CD8 T cell functional genes. Additionally, the supplemented pyruvate appears to be preferentially used in producing lactate (and recycle NAD+) compared to lactate that was derived from glucose. In summary, we have demonstrated that NaPyr alters metabolism and improves effector molecule expression in CD8+ T cells. Understanding how pyruvate supplementation improves CD8 T cell function will provide new insight into CD8 T cell biology while also providing a new way to prepare CAR T cells for better therapeutic efficacy.

108.

Increased Glut1 Expression Improves Adoptive T Cell Therapy

Betty Yao

Adoptive cellular therapy (ACT) is an immunotherapeutic strategy that often results in complete remission in blood cancers. A major challenge with ACT effectiveness in solid tumours is the hostile tumour microenvironment (TME) due to metabolic competition between immune and tumour cells, lack of tumour infiltration, and poor survival of infiltrated cells. We have previously shown that transiently restricting glucose in fully activated CD8+ effector T cells in vitro reprograms cellular metabolism and dramatically upregulates protein expression of the glucose transporter Slc2a1/Glut1 without altering Glut1 mRNA expression. Glut1hi CD8+ T cells showed improved tumour clearance upon infusion in tumour bearing mice, however, how this high Glut1 expression is induced and its downstream effects on T cell function remain unclear. A better understanding of the non-transcriptional upregulation of Glut1 72 hours after initial CD8+ T cell activation will be crucial for us to utilize this key glucose transporter to improve or recover donor CD8+ T cell persistence and tumour infiltration. We found that genetic deletion of Glut1 or transiently blocking with a Glut1-specific inhibitor CD8+ T cells 72 hours post-activation reduces cytokine production in vitro. Transient Glut1-inhibition also blunted in vivo anti-tumour function of Glut1hi CD8+ T cells. We found decreased donor-derived CD8+ T cells in blood, tumour, and spleen, and decreased cytokine production in tumour infiltrating lymphocytes. Taken together, increased Glut1 expression in fully activated CD8+ T cells can augment tumour clearance in pre-clinical models. These results suggest that Glut1 plays a vital role in CD8+ T cell ACT, and that generating Glut1hi effector T cells that have a metabolic reprogramming advantage may provide a strategy to select cells with improved in vivo function.

109.

Single-cell multi-omics profiling of Chronic Myeloid Leukemia stem and progenitor cells across disease stages

Yingying Liu

Chronic myeloid leukemia (CML) is a clonal myeloproliferative disease driven by the oncogenic BCR-ABL1 gene fusion within a hematopoietic stem cell. ABL-targeting tyrosine kinase inhibitors (TKIs) have proven effective in treating early-stage chronic phase (CP) CML patients. However, about 15% of CP-CML patients experience treatment failure, relapse, and potential progression to late-stage accelerated phase (AP) and blast crisis (BC), which have a median survival of just a few months. Addressing drug resistance and enhancing the prognosis for AP/BC-CML patients remain major challenges in CML management. Prior studies have highlighted that CD34+ leukemic stem and progenitor cells are notably less responsive to TKIs than differentiated cells. In this study, we aim to characterize the heterogeneous CD34+ cells across disease stages at single-cell resolution to identify critical molecular drivers of disease progression and TKI resistance. We have performed single-cell multi-omics sequencing (scRNA-seq + scATAC-seq) on CD34+ cells from paired CP and BC samples, cultured in vitro with or without TKI for 16 hours, from three CML patients. We observed considerable heterogeneity in cell type composition between disease stages and among different patients. Using differential analysis, motif enrichment, and co-accessibility analysis, we identified dysregulated genes and transcription factors (TFs) along disease progression. Interestingly, we found a subpopulation of CP cells with gene expression and chromatin accessibility profiles similar to BC cells, allowing for potential discovery of biomarkers predictive of BC transformation. Future work will involve validating our candidate genes and TFs in a larger collection of patient samples, and performing functional studies to further characterize the mechanisms contributing to disease progression and TKI resistance.

110.

Center Detection of Overlapping Nuclei in Micrographs

Shunsuke Ishige

In histology tissue samples, multiple translucent objects can attenuate the illuminating light of the microscope that traverses the tissue as part of the image formation process, with the resultant projection showing object overlaps. Neural networks, especially convolutional neural networks (CNNs), are very effective at processing digital pathology images such as providing cell segmentation to render them amenable to quantitative analysis. Thus, pathological changes in cellular morphology or the interactions of cells in tissues can be quantified in images containing multitudes of cells of various types. In particular, boundary approximation and segmentation can leverage estimated nucleus centers. In this project, we explore image processing methods used to measure crowd sizes even with partially hidden people for possible translation to pathology. One of the challenges is the difference in opacity of target objects (people vs nuclei). A particular problem of interest for us is how CNNs can correctly process overlapping objects: when finding object centers, at least an implicit understanding of the overlap areas is required, making the recognition of the morphological characteristics of individual nuclei challenging, particularly with multiple objects (clumps) in the image plane.

Poster Abstracts

111.

Integrative Analysis of Germline and Somatic DNA Repair Gene Variants in Prostate Cancer Metastasis: Identification and Functionalization of Lead Candidates

Hamideh Sharifi Noghabi

Prostate cancer (PCa) is a significant public health concern in Canada, affecting 23,300 men annually. Metastasis is a major contributor to PCa-related mortality, necessitating innovative therapeutic strategies. This study addresses the need for biomarker-driven tumor stratification based on metastatic potential and predictive markers for emerging therapies. Utilizing an extreme phenotype approach, we analyzed 26 localized high-grade tumors with bone metastasis and 26 non-metastatic counterparts. Whole exome sequencing of formalin-fixed paraffin-embedded specimens revealed germline nonsynonymous rare variants (nsRVs) exclusive to metastatic tumors. A non-biased assessment identified 61 genes with nsRVs recurrent in metastatic tumors. These nsRVs, predicted or known to be pathogenic, exhibited higher frequencies despite their rarity. The significance lies in their potential pathogenicity and positive selection during tumor progression. We explored the functionalization of one lead candidate, KDM6B, selected based on criteria related to their association with aggressive cancers, metastasis, and potential druggability. KDM6B, a histone demethylase, was identified as highly expressed in metastatic PCa, playing a crucial role in super-enhancer dynamics. NsRVs in KDM6B were recurrent and pathogenic, suggesting a potential link to metastasis. We employed CRISPR prime editing, to introduce RVs into the PCa cell line LNCaP. Initial characterization involved assessing viability, proliferation, migration, and invasion, with further examination of sensitivity to KDM6B inhibitors. Expected outcomes include demonstrating that nsRV candidates modify metastatic potential and may serve as potential targets for inhibitors. The study's confidence is supported by the robust association of KDM6B with aggressive PCa. This research sheds light on potential biomarkers for personalized therapeutic interventions in metastatic prostate cancer.

112.

Interplay between miR-146a downregulation and high TP53 activity is associated with DNMT3A CHIP

Debajeet Ghosh

Clonal Hematopoiesis of Indeterminate Potential (CHIP) is a condition characterized by the presence of a dominant clonal expansion of mutant hematopoietic cells, predisposing patients to increased risk of hematological malignancies, cardiovascular and inflammatory disorders. DNMT3A variants are the most common CHIP mutation seen, and these tend to be specific alterations at arginine 882 (R882). However, it is unclear what molecular factors drive the acquisition or expansion of these mutant clones. Our previous work has elucidated that downregulation of miRNA, miR-146a, is associated with inflammation, ageing and poor prognosis in acute myeloid leukemia (AML) patients. GSEA analyses across de-novo AML patient cohorts (TCGA, AML PMP, n = 275) show low miR-146a to be associated with high TP53 pathway activity. Further investigation showed that miR-146a inhibits TP53 S-15 phosphorylation by targeting the TP53 kinase, PRKCD, and that TP53 downregulates miR-146a expression by inhibiting the expression of the miR-146a transcription factor, TCF3. This positive feedback loop between low miR-146a expression and high TP53 activity was further validated in mouse models and patient expression data. DNMT3A mutated cells tend to exhibit a clonal advantage in inflammatory conditions. Our data shows that AML patients with DNMT3A mutations are enriched for inflammatory pathways and express significantly lower TCF3 and miR-146a while having a higher expression of PRKCD and TP53 activation. Only DNMT3A R882 variant bearing patients exhibit these expression trends, in contrast to patients with other DNMT3A mutations. This leads us to hypothesize that high TP53 activity-associated inflammation (acting via miR-146a inhibition) confers DNMT3A mutant clones a competitive advantage, promoting CHIP. Classically TP53 associated cancers have been attributed to loss of function. Here we propose an aging-associated oncogenesis model secondary to TP53-induced senescence.

Poster Abstracts

113.

Non Invasive trtDNA early detection screening in Pancreatic Ductal Adenocarcinoma

Sara Singh

Pancreatic ductal adenocarcinoma (PDAC) represents a significant oncological challenge, ranking as the 10th most prevalent cancer in Canada and the 4th leading cause of cancer mortality. The majority of PDAC patients are diagnosed with advanced, unresectable disease, and survival beyond one year is uncommon. Current screening protocols are economically viable only for high-risk populations, underscoring the urgent need for cost-effective, non-invasive screening modalities suitable for broader application. Trans-renal tumor DNA (trtDNA) analysis emerges as a promising technique for early PDAC detection, offering both sensitivity and quantifiability. Canadian research initiatives are focused on developing Circulating Tumor DNA-based (ctDNA) screening tests, utilizing plasma samples for early cancer detection. However, the requirement for blood draws poses a significant barrier to participation. This project proposes a pilot study to evaluate urine as an alternative biospecimen for trtDNA screening in patients with active PDAC or those at elevated risk. The goal is to extend this screening to individuals with a lower risk profile. The success of this research program would be game-changing for a disease with a dismal 5-year relative survival rate of only 8%. The study aims to determine if patient-led home urine trtDNA collection is feasible, and if ddPCR analysis for mutated KRAS in urine-derived trtDNA has a comparable sensitivity to plasma-derived ctDNA. Use of urine trtDNA would make sample acquisitions amenable to patient-led home urine collections, that are simple, non-invasive, accessible to patients in remote regions and less expensive. Long-term surveillance for KRAS-HM in individuals at risk of PDAC by urine monitoring promises a high-benefit, low-risk method for improving patient outcomes at low cost.

114.

STEAP1 facilitates iron transport in Ewing sarcoma to support mitochondrial activity

Taras Shyp

Ewing sarcoma (EwS) is a highly aggressive bone and soft tissue tumor in children and young adults with the presence of major disease-driving gene fusions, in 85-90% of cases - EWSR1-FLI1. Six-transmembrane epithelial antigen of the prostate 1 (STEAP1), a member of the STEAP membrane-bound protein family of transient metal reductases, was shown to be overexpressed in EwS and other malignancies. However, a precise functional role of STEAP1 in EwS remains elusive. Genetic silencing of STEAP1 and STEAP2, another STEAP protein that was discovered to be overexpressed in EwS, led to lower intracellular iron and copper levels, implying STEAP1's metal reductase function in the presence of STEAP2. Furthermore, we discovered that cells that lack STEAPs have a significantly reduced mitochondrial labile iron pool with altered rates of oxygen consumption and impaired assembly of supercomplexes of the electron transport chain. Targeted metabolomics identified a depletion of numerous central carbon metabolism metabolites in STEAP1KO cells, including the intermediates of glycolysis and the pentose phosphate pathway. Moreover, using the ex vivo pulmonary metastasis assay and intramuscular paratibial EwS tumor model, we showed that the loss of STEAPs decreases primary tumor volume and metastatic capabilities of EwS. In conclusion, this study, for the first time, demonstrated in vitro interaction between two members of the STEAP that is required for sufficient transition metals homeostasis and cancer metabolism. Hence, loss of STEAPs significantly decreases tumor growth at primary and distant sites. Moreover, STEAP2 as well as STEAP1, are membrane-bound proteins with abundant expression on the cell surface of cancer cells and low levels of expression among normal tissues. Therefore, they can be utilized as promising candidates for developing targeted therapies for EwS patients, including ADCs and mono/bispecific antibodies.

115.

Telmisartan-mediated myofibroblast inhibition in the tumour

Che-Min Lee

Cancer associated fibroblasts (CAFs) have been strongly implicated in skewing patient outcomes and have shown mostly anti-tumorigenic activity. Efforts to target CAFs to improve therapeutic outcomes have been underway, however, repurposing existing therapies has not been fully explored. Recently, we found that a common blood pressure drug, telmisartan, inhibits collagen accumulation in a murine tumour model. High collagen density is known to be correlated with worse outcome in certain tumours, including head and neck tumours. Interestingly, telmisartan did not eliminate specific cell types, but decreased expression of a myofibroblast marker, αSMA within a CAF subset (CD29-hi, Ly6C-low). This indicated that telmisartan was likely inhibiting myofibroblast-like CAFs (myCAFs) in particular. To understand the CAF heterogeneity in this model and their responses to telmisartan, we applied scRNA-seq on the same murine tumour model with telmisartan treatment. Preliminary scRNA-seq analysis has revealed changes in signalling pathways related to the canonical signalling telmisartan affects including angiotensin II-related signalling and PPAR-gamma signalling. However, changes in other signalling pathways such as the Wnt-beta catenin cascade were also found to be altered. To validate our scRNA-seq findings, we created a model for CAF cells from murine dermal fibroblasts. These fibroblasts were induced into an inflammatory CAF (iCAF) or myCAF phenotype using cytokines. We validated the iCAF and myCAF phenotypes by expression of Ly6C-hi, aSMA-low for iCAFs and the reverse for myCAFs. We found in this model, iCAFs were not sensitive to telmisartan but that myCAFs were, which corroborated with what we saw in the in vivo model. We are currently using this model and the scRNA-seq results to uncover the specific mechanism through which telmisartan inhibits myCAFs, but not iCAFs. We hope this work will be able to uncover a new group of neoadjuvant cancer therapy that targets CAFs.

116.

GABARAPL2: Grim Reaper and GATEkeeper of viability in pancreatic ductal adenocarcinoma

Jennifer Chan

Pancreatic ductal adenocarcinoma (PDAC) accounts for more than 90% of all pancreatic cancer diagnoses. Fast progression, late-stage diagnosis, and drug resistance are among factors that make PDAC an aggressive and difficult cancer to treat. One proposed mechanism of PDAC survival and treatment resistance is through an increased dependence on autophagy, an intracellular recycling process that is fundamental to cellular health and homeostasis. Previous findings from our group suggest that high levels of the autophagy-related protein, GABARAPL2, otherwise known as GATE-16, are associated with improved survival outcomes in PDAC patients treated with adjuvant chemotherapy, specifically gemcitabine. The overall objectives of this project are to first determine if GABARAPL2 levels are similarly associated with treatment response in experimental models of PDAC and, second, to investigate if modulating GABARAPL2 levels have a direct functional consequence on treatment response and viability. To date, our results indicate that levels of endogenous GABARAPL2 do not associate with treatment response to gemcitabine in PDAC cell lines (r = -0.007). However, PDAC cells with a loss of GABARAPL2 demonstrate increased cell death and reduced cell proliferation, which is coupled with elevated p21 expression. Conversely, ectopic expression of GABARAPL2 has no effect on proliferation but appears to reduce death. We further show that there is a modest additive benefit with gemcitabine plus GABARAPL2 loss on PDAC cell growth inhibition. Our data suggest that there may be a threshold to GABARAPL2 levels that are important in PDAC growth, such that a loss of GABARAPL2 leads to a significant impairment in their proliferative capacity, but that ectopic expression has no effect on proliferation. In summary, we have identified GABARAPL2 as a potential biomarker and modulator of PDAC viability.

117.

Leveraging mutational screening to uncover dominant Rev3 alleles as a novel synthetic lethal therapeutic strategy

Cathy Cozma

Synthetic lethal (SL) therapies can exploit genome instability present in rapidly dividing cancer cells by targeting key DNA damage repair pathways. Clinically successful SL therapies, such as PARP inhibitors, act by 'trapping' the PARP protein at DNA damage sites, creating persistent cytotoxic protein-DNA lesions and limiting accessibility by redundant repair mechanisms. While PARP trapping is an effective SL therapeutic approach, it is difficult to identify inhibitors de novo with trapping properties. Cancer cells come to rely on error-prone mechanisms like translesion synthesis (TLS) for chemoresistance, making TLS polymerases a high-value cancer therapeutic target. We have leveraged high-throughput mutagenesis techniques to screen for missense variants in DNA polymerase Z (REV3) that cause dominant negative growth phenotypes. Dominant Rev3 variants in the Rev7 and Pol31 interaction interfaces have been identified and will be characterized for altered DNA binding kinetics and protein-protein interaction strength. Additional analysis of this Rev3 variant library and its effects on fitness, genetic interactions and mutation signatures are ongoing. The identification of Rev3 domains mutable to dominant forms may help to guide rational inhibitor development to selectively kill cancer cells dependent on TLS.

118.

Investigating dominant negative mutations in DNA2 as a model for targeted cancer therapy.

Katie Baillie

DNA2 is a combined nuclease-helicase with important roles in DNA repair and restart of stalled replication forks. As cancer cells rely on these mechanisms to tolerate uncontrolled cell division, DNA2 is a promising therapeutic target. However, targeting DNA repair is challenged by redundant and rescue pathways. To address this, we are studying dominant-negative mutations. Understanding how these mutants are detrimental even to unperturbed cancer cells is a useful model for future therapeutics. To identify dominant-negative alleles of DNA2, known disease and catalytic mutants were overexpressed in U2OS cells. Cells were assessed for markers of DNA double strand breaks, single stranded DNA (ssDNA), and signalling by DNA Protein Kinase and ATR. Overexpression of the nuclease inactivating mutations increased double strand breaks compared to unmutated DNA2. Inactivating the helicase domain in the nuclease dead mutants rescued this phenotype. Nuclease mutants also increased ssDNA, and activation of DNAPK and ATR signalling. These effects were rescued in the double mutant. Finally, expression of nuclease dead mutants induced G2/M cell cycle arrest and decreased cell fitness. Mechanistically, DNA2 interacts with 5'-3' ssDNA through a central tunnel, entering at the nuclease domain and passing through to the helicase domain. The helicase is thought to secure protein-DNA interactions. These results support a trapping mechanism whereby the nuclease dead mutant engages DNA and forms a strong interaction with the help of the helicase domain. However, without cleavage activity, the bound protein is unable to release itself. Future experiments will focus on characterizing the mechanism of dominant DNA damage, as well as identifying genetic backgrounds and drug combinations that increase sensitivity to the mutant. These results will guide efforts to develop effective DNA2 inhibitors for cancer treatment.

Poster Abstracts

119.

O-GlcNAc Transferase (OGT) is a novel therapeutic approach for EVI1+ AML through increased mitochondrial priming.

Junbum Im

Acute myeloid leukemia (AML) that expresses ecotropic viral integration site 1 (EVI1), a stem cell regulator that enhances survival, is associated with dismal treatment response and survival. Although resistance triggered by EVI1 is not yet elucidated, recent research indicates that adverse risk AML is frequently characterized by diminished mitochondrial priming. Mitochondrial priming dictates the execution of intrinsic apoptosis through the mitochondrial release of Cytochrome C (CYC), which requires the proteins BIM and BID to bind and facilitate the oligomerization of BAK and BAX into channels. When BAK and BAX are suppressed, the cell enters a mitochondrial unprimed state where treatments do not elicit CYC release and apoptosis; therefore, restoring the mitochondrial primed state shows therapeutic potential for EVI1+ AML. Using the BEAT AML patient RNAseq dataset, we found that EVI1 is positively correlated with the post-translational modifier O-GlcNAc transferase (OGT). OGT adds a sugar called O-GlcNAc to target proteins inside the cell, altering their function and stability and regulating various cellular processes downstream such as survival. In EVI1+ AML samples, we found abnormally high OGT expression and activity compared to EVI1- AML. Using the OGT inhibitor OSMI-4b, inhibiting OGT in EVI1+ AML followed by proteomics reveals that expression of the apoptotic proteins BAK and BAX increases, indicating increased mitochondrial priming. To confirm this, we treated EVI1+ AML samples with synthetic peptides of BID and BIM that mimic binding to BAK and BAX and found that OGT inhibition significantly increased CYC release upon treatment with both BID and BIM peptides. Subsequently, combining OSMI-4b with the AML treatment Venetoclax significantly increased apoptosis in these samples. Here, we show that mitochondrial priming in EVI1+ AML can be modified through OGT inhibition and aim to establish a preclinical rationale for OGT inhibition as a treatment approach.

120.

Identifying Modifiers of EGFR Induced Tumourigenesis to Develop New Therapeutic Strategies for Lung Cancer

Jana Jajarmi

Lung cancer is the deadliest form of cancer in Canada, accounting for 24% of all cancer related deaths. Lung adenocarcinoma (LUAD) is the most common subtype and is frequently driven by activating mutations in epidermal growth factor receptor (EGFR). While the development of targeted therapies such as tyrosine kinase inhibitors has significantly improved patient outcomes, acquired resistance continues to be a major challenge. It is therefore essential to identify other targetable genes that drive LUAD tumourigenesis to improve treatment outcomes. Our lab previously used a sophisticated in vivo Sleeping Beauty (SB) screen to model EGFR driven LUAD in mice and identify functionally relevant genes that cooperate with mutant EGFR in LUAD tumourigenesis. A thorough computational analysis of the SB screen results along with TCGA data was performed to determine the genes that were frequently mutated in both SB mice and human LUAD patients. This produced a list of 385 genes, several of which have never been implicated in LUAD. To further investigate these candidate genes, we are using a targeted in vitro CRISPR screen with mouse fibroblasts (3T3s) overexpressing mutant EGFR. Candidate genes identified after culture under numerous conditions will be further studied to determine their role(s) in essential cellular pathways, drug resistance, and LUAD progression. The results of this study will greatly expand our understanding of LUAD progression and lay the groundwork for future therapies against this devastating disease.

Poster Abstracts

121.

Investigating the clinical relevance of pre- and post-treatment serum biomarkers in oropharyngeal cancer

Jamie Kwon

Oropharyngeal cancer (OPC) presents a persistent challenge with a five-year survival rate of approximately 50%, which is largely attributed to occult and recurrent cases. Recent demographic changes, including a notable rise in younger, HPV+ patients, demonstrate the urgency for new approaches to detect and manage this disease. The conventional method of "watchful waiting" for disease monitoring is hindered by post-treatment scar tissue, leading to late-stage diagnoses in nearly 90% of cases. Hence, there is a pressing need for more effective early detection methods. Liquid biopsies have emerged as a non-invasive and promising avenue for detecting various cancers. They offer a minimally invasive alternative to traditional tissue biopsies and can detect microRNAs (miRNAs), which are small non-coding RNAs that play a crucial role in regulating gene expression. miRNAs are released through extracellular vesicles, allowing for quantification and detection in blood. We previously identified a circulating miRNA signature capable of distinguishing individuals with oral premalignant lesions, oral squamous cell carcinoma, and early detection of recurrent disease. We aim to assess the adaptability of this miRNA classifier in patients with OPC. To this end, serum samples were collected from OPC patients pre-treatment and during follow-up appointments. RNA was extracted from serum and profiled using RT-qPCR. A biomarker score was calculated using our two-miRNA classifier. The preliminary findings demonstrate distinct patterns in the miRNA classifier over the course of treatment for recurrent and non-recurrent patients. The classifier shows promise as a straightforward, non-invasive monitoring tool for OPC.

122.

The effect of chronic allergic stimulation on group 2 and group 3 innate lymphoid cells

Davit Khijakadze

Group 2 Innate lymphoid cells (ILC2s) are tissue resident cells that can be found in the lung, liver, spleen, intestine, lymph nodes and fat-associated lymphoid clusters of mice. ILC2s in the lung, upon activation by epithelium-derived cytokines IL-33, IL-25 and thymic stromal lymphopoietin (TSLP), produce T helper 2-type (type 2) cytokines IL-5 and IL-13, which induce eosinophilia and mucus hyperproduction, respectively, leading to allergic inflammation. We established a mouse model of chronic allergic lung inflammation using fungal and/or protease allergens to investigate the behavior of ILC2s in prolonged inflammatory conditions. Following initial treatment, we observed a significant increase in ILC2 populations in the lung and liver. However, repeated exposure to allergens did not lead to further expansion; instead, ILC2 numbers declined after 5 weeks of chronic treatment. Flow cytometry analysis revealed functional impairment and an exhausted phenotype in small subset of lung ILC2s, characterized by reduced IL-5 and IL-13 production. ILC2s retained memory capabilities and exhibited enhanced responsiveness upon re-stimulation with IL-33 compared to naïve mice. Unexpectedly, we also observed expansion of Group 3 Innate Lymphoid Cells (ILC3s) and identified a subset of cells producing both IL-5 and IL-17, suggesting a potential transition state between ILC2s and ILC3s. Surprisingly, ILC3 number stayed steady after initial expansion compared to ILC2s. Histological examination demonstrated COPD-like lung tissue alterations and emphysematous changes, indicating a link between chronic inflammation and structural lung damage. These findings underscore the complexity of ILC2 behavior in prolonged allergic conditions, revealing functional and phenotypical changes, including exhaustion and memory subset emergence. Furthermore, our model implicates both ILC2s and ILC3s in the pathogenesis of emphysema and COPD, likely mediated by IL-5 and IL-17 production.

123.

Identification of misclassified multiple myeloma patient risk subgroups with a novel biological disease stratifier

Panahi, A

Recent insights into the pathogenesis of multiple myeloma (MM) have highlighted inflammation and APOBEC enzyme-induced genome editing, as major drivers of disease onset and progression. Therefore, we assumed that inclusion of molecular features reflecting these mechanisms can improve the accuracy of MM risk classification and can be utilized to define novel MM risk groups at initial diagnosis. Using two independent patient cohorts (MMRF and IFM/DFCI 2009), we developed and validated our novel risk-score based on mRNA expression levels of APOBEC2 and APOBEC3B, as well as inflammatory cytokines (IL11, TGFB1 and TGFB3) and serum levels of ß2-microglobulin and LDH. Performance of the Editor- and Inflammation-based score (EI-score) was superior to current cytogenetics-based risk classifiers. The EI-score identifies patient subsets who classified in ISS/ R-ISS stage II/III with good prognosis and patients classified in ISS/ R-ISS stage I/II with poor prognosis. The EI-score also identified subgroups of MM patients with adverse risk cytogenetics [carried either del(17p)/ gain(1q)/ t(4;14)] but with favorable outcomes. Furthermore, we found that patients that carried del(17p) and high EI-score, display an enrichment of APOBEC-induced genomic mutations compared to intermediate and low EI-score patients supporting the hypothesis that del(17p) along with high APOBEC expression levels activate the APOBEC mutation program and thus create an optimal environment for tumor progression. These findings support the necessity of a prognostic score that more accurately reflects MM disease biology. Through accurate risk stratification we can identify patients who are currently over-or undertreated. The EI-score is a contemporary and superior prognostic score, calculated based on transcript levels at diagnosis, allowing the identification of unrecognized MM risk subgroups potentially leading to adjustment of clinical treatment and improvement of patient outcomes.

124.

Investigating the biological effects of outdoor air pollution on lung cancer in patients who have never smoked using an integrated genomics approach

Peipei Wang

Introduction: Lung cancer is the leading cause of cancer deaths worldwide, and the subset of patients with lung cancer who have never smoked (LCNS) is increasing in incidence despite the general decrease of overall lung cancer cases each year. Outdoor air pollution has been revealed as a key risk factor in LCNS, with many industrial sources generating fine particulate matter smaller than 2.5 micrometers (PM2.5), which accumulates in the small airways over time. This work aims to elucidate the impact of PM2.5 exposure on lung tumour biology.

Methods: Patients with LCNS will be assessed for their PM2.5 exposure through a detailed questionnaire. Fresh frozen tumour and adjacent normal lung tissue from these patients who qualify for having high (>10 µg/m3) or low (<5 ug/m3) PM2.5 exposure will be sent for RNA sequencing and whole genome sequencing to discover molecular alterations that result from outdoor air pollution. All dimensions of data will be integrated for functional analyses and relevant pathways related to PM2.5 will be determined.

In tandem, lung epithelial cells will be exposed to PM2.5 in vitro for both short- and long-term exposure periods to assess the genomic impact of PM2.5 in controlled conditions. This will further inform the genomic analyses of the patient tumour data and allow for a comprehensive exploration of the influence of PM2.5 in both pre-malignant and malignant settings.

Expected Results and Future Directions: PM2.5 may leave a distinct genomic imprint on lung epithelial cells and lung tumour tissue, constituting a mutational signature that has not previously been defined. Combined with transcriptomic analyses, this work will reveal pathways that are altered by outdoor air pollution and thus inform public health policies, therapeutic targets, and avenues for preventative care.

125.

The Role of SHPRH in Lung Adenocarcinoma Initiation and Development

Serena Chuang

Late-stage diagnosis of lung cancer (LC) is associated with poor prognosis and survival, highlighting a need for increased understanding of risk factors to support early screening and treatment strategies. While environmental factors play a significant role, genetic factors can greatly enhance LC risk in smokers and non-smokers. Using whole exome sequencing of never-smokers with lung adenocarcinoma (LUAD), our lab identified a candidate gene which could be linked to LC susceptibility: SHPRH. Previous work demonstrated that SHPRH acts as a tumour suppressor gene in the context of LUAD, but its mechanism has yet to be elucidated. This project aims to investigate the mechanism of SHPRH-mediated tumour suppression in LUAD cells and identify the key pathways involved with its phenotype. RNA-sequencing and immunoprecipitation-mass spectrometry (IP-MS) will be performed to identify transcriptomic and proteomic changes with SHPRH expression. This will help identify major biological processes and interactors associated with SHPRH, which can increase understanding of SHPRH's function and contribution to tumorigenesis. Genes found to be associated with SHPRH-mediated tumour suppression will be genetically manipulated via lentiviral transduction to be either conditionally expressed or knocked down. This will help validate the contribution of these genes to SHPRH's tumour suppressive phenotype. Increased understanding SHPRH's tumour suppressive function and contribution to LC initiation and development may help identify at-risk patients and increase opportunities for early intervention.

126.

Elongation control of mRNA translation drives Group 3 medulloblastoma adaptation to nutrient deprivation

Namya Sharma

Group 3 affiliation and MYC genetic amplification are associated with poor life expectancy and substantial in children suffering from medulloblastoma (MB). However, the high metabolic demand induced by MYC-driven transformation sensitizes MYC-overexpressing MB to cell death under conditions of nutrient deprivation (ND). MYC-driven transformation is known to promote mitochondrial oxidative phosphorylation (OXPHOS). We previously found that eukaryotic Elongation Factor Kinase 2 (eEF2K), the master regulator of mRNA translation elongation, promotes survival of MYC-overexpressing tumors under ND. Interestingly, eEF2K is overexpressed in MYC-driven MB and our preliminary proteomics data highlight large-scale alterations in OXPHOS components affecting eEF2K deficient MB cells. We therefore hypothesized that eEF2K activity is required for the selective translation of mRNAs needed for efficient OXPHOS, and for the progression of MYC-driven MB. We performed Multiplexed Protein Dynamic Mass Spectrometry in eEF2K knockdown MYC-overexpressing D425 MB cells to identify mRNAs selectively translated upon eEF2K activation. Messenger RNAs encoding multiple components of the mitochondrial OXPHOS pathway are selectively translated upon eEF2K activation. Inactivation of eEF2K by genetic KO leads to the disassembly of electron transport chain (ETC) complexes I-IV without affecting mRNA levels of their respective components. Consistently, eEF2K KO MB cells display decreased mitochondrial membrane potential and 20% increased proton leak thorough the mitochondrial membrane. In addition, eEF2K inactivation results in increased Group 3 MB cell death under ND and doubles survival of MB bearing mice fed with calorie restricted diets ($p < 0.05$). Control of mRNA translation elongation by eEF2K is critical for mitochondrial ETC complex assembly and efficient OXPHOS in MYC-overexpressing MB, likely representing an adaptive response by which MYC-driven MB cells cope with acute metabolic stress.

Poster Abstracts

127.

Expansion and characterization of immune suppressive CD56brightCD16- regulatory natural killer cells for chronic graft-versus-host disease (cGvHD)

Madeline Lauener

cGvHD is a major cause of morbidity after Hematopoietic Stem Cell Transplantation. Previously, in large patient cohorts we identified regulatory NK cells (NKreg) to associate with cGvHD suppression. We hypothesized that NKreg cells may be a candidate for cGvHD cell therapy and aimed to expand NKreg cells while maintaining regulatory phenotype and function. To meet our objective, NK cells were first expanded with ligands associated with immune tolerance: IL2/4/7/10/18/23, GPR183L, GMCSF. The optimal expansion cytokine (IL2) was then combined with ligands to prevent cell differentiation: rapamycin, TGFβ1, NECA, metformin, dexamethasone. The functional characteristics and phenotype were evaluated. The optimal expansion protocol was compared in terms of function and metabolism for 3 NK expansion medias, and cells from cord vs. peripheral blood. Further, expanded NKreg protein and gene expression was characterized using the Olink Proximity Extension Assay and bulk RNAseq, respectively. Finally, NKreg cells were differentiated from CD34+ HSPC's and compared in terms of proliferation and function. The expansion of total NK cells found IL2 to result in the greatest proliferation (up to 100-fold), and the combination of TGFβ1 and/or NECA with pulsing of rapamycin prevented NKreg differentiation (up to 200-fold). These expanded cells maintained similar phenotype, transcriptome, and T cell suppression to fresh NKreg cells. NKreg expansion was greatest in the Immunocult media (up to 300-fold). NKreg cells from peripheral blood demonstrated significantly greater proliferation than cells from cord blood (65-fold). The metabolic profile of NKreg cells and cytolytic NK cells was not significantly different, though rapamycin induced a lower OCR/ECAR. Additionally, expanded NKreg compared to CD56dim NK cells upregulate proteins associated with regulatory function, such as TGFβ1, TRAIL, and ADA ($p<0.01$). Further, suppressive NKreg cells may alternatively be expanded from CD34+ cells.

128.

CPSF1 drives cell mortality via alternative polyadenylation

Yue Li

The dysregulation of Alternative polyadenylation (APA) is a common characteristic across various types of cancer. While progress has been made in studying APA in tumor progression, our understanding about its impact on cancer metastasis, the leading cause of cancer mortality, remains limited. In this study, we report CPSF1, a frequently overexpressed cancer-related protein, as an alternative polyadenylation regulator contributing to epithelial-mesenchymal transition, tumor local invasion and metastasis. A pan-cancer analysis across 33 TCGA cancer types revealed frequent amplification in CPSF1 among 19 APA components. CPSF1-depleted cells exhibited a shift toward longer 3'UTR isoforms in TGFBR1 and SOX9 transcripts, resulting in decreased protein expressions. Mechanistically, CPSF1 induced 3'UTR shortening by preventing CPSF complex from ubiquitin-mediated protein degradation. Using sub-renal capsule xenograft models, we showed that ablation of CPSF1 decreased tumor local invasion and distant metastasis. Our observations provide insights into the molecular mechanisms of APA as a metastasis-promoting drive, representing a therapeutic target in the treatment of metastatic tumors.

129.

Investigating Autophagy-related Cysteine Protease Atg4a in Drosophila melanogaster Models of Cancer

Jessica Felix

Pancreatic ductal adenocarcinoma (PDAC) accounts for over 90% of all pancreatic cancer cases with a worldwide five-year survival rate of a mere 9%. There is an urgent need for further research into the molecular and cellular mechanisms that contribute to PDAC progression and treatment failure in PDAC. One advancing idea is that an upregulation of autophagy contributes to PDAC tumor survival and resistance to therapy, suggesting autophagy as a potential therapeutic target. Our recent work in human PDAC cell lines suggests that the autophagy related cysteine proteases ATG4A and ATG4B are key players in the progression of PDAC. Knocking out ATG4A and ATG4B in PDAC cells resulted in growth inhibition and a decrease in Atg8-family processing. Further research into the mechanisms of ATG4A/B and their interactions with cancer-related cell signaling pathways in an in vivo model may help provide insight into potential ATG4A/B-related treatments for cancer. Therefore, we will investigate ATG4A/B using Drosophila and its single ortholog for both ATG4A and ATG4B, Drosophila Atg4a. Drosophila is a well-studied and widely used in vivo model with conserved cell signaling pathways, making it a powerful study tool for the complex interactions that occur in cancer host-tumor interplay. Our preliminary data shows that a mutant line Atg4aMB03551 has a decrease in Atg8 processing, as well as an accumulation of p62 and ubiquitin. These phenotypes indicate that autophagy is compromised. This mutant line also shows a delay in an ovary degeneration phenotype, which is known to be mediated by autophagy and is typically observed upon starvation of wild-type flies. A ubiquitous RNAi knockdown of Atg4a also exhibits a decrease in Atg8 levels, as well as a decrease in motor function as measured by climbing assays. Characterization of potential genetic interactions between Atg4a and pancreatic cancer-related mutations in vivo is currently in progress.

130.

Decoding the Effects of Air Pollution on Older Adults with COPD: A Comprehensive Transcriptomics Study

Ho Jung Yoon

Chronic obstructive pulmonary disease (COPD) is a debilitating lung disease, affecting over 384 million individuals worldwide. Key features of COPD include chronic inflammation, airflow obstruction, chronic cough, and alveolar destruction. A significant risk factor contributing to COPD development includes exposure to particulate-rich air pollution. Air pollution exposure may trigger respiratory flare-ups, termed exacerbations, that require in-hospital treatment. To date, studies characterizing the impact of air pollution on COPD using omics approaches have been in cross-sectional population cohorts. To complement and strengthen these population cohorts, we conducted an integrative transcriptomic analysis in a controlled human exposure study. We employed a systems biology approach involving the integration of transcriptomic datasets across lung and blood to uncover novel biomarkers to better comprehend the interplay between COPD and air pollution. For our investigation, we recruited thirty research participants aged 40-80, with and without COPD. Each participant was exposed to diesel exhaust and filtered air (control) for 2 hours, during 2 visits randomized to order and separated by a washout period. Peripheral blood and endobronchial brushings were collected after each exposure for bulk-RNA sequencing. Differential gene expression was assessed by linear mixed-effects models, accounting for repeated measures. We performed integration for novel biomarker discovery using latent components utilizing the R package mixOmics. Differential gene expression analysis revealed that genes related to immune function were altered following exposure. These genes included HSPA1, MTND4P1, MICB, TRIM39, and GPX2. Blood-lung omics integration showed shared clusters of dysregulated genes in the blood that are reflective of the response of the airways to air pollution. This gene set signature in blood is a candidate biomarker for determining response to acute air pollution exposure.

131.

Copy Number Signatures identify therapeutic opportunities for p53 abnormal Endometrial Carcinomas

Juliana Sobral de Barros

Endometrial carcinoma (EC) is the most common gynecologic cancer in North America, with p53 abnormal (p53abn) having the worst outcomes, responsible for 50-70% of EC mortality. Therapeutic advances are urgently needed to improve outcomes for these patients. Shallow whole genome sequencing (sWGS) has been successfully used to derive copy number (CN) signatures in high grade serous ovarian cancer (HGSOC). p53abn ECs share genomic features with HGSOC, supporting applying sWGS to this EC molecular subtype. Identifying CN signatures may allow prognostic stratification and identification of therapeutic options. Tumor DNA was extracted from 187 formalin-fixed paraffin-embedded p53abn ECs. sWGS and targeted panel sequencing was performed. The raw data were aligned and treated to correct CG content and discard low-quality reads. The samples were exposed to the HGSOC signatures and our own custom generated signatures and then compared. Our p53abn EC signatures were composed of 5 distinct groups. Signature 5 was associated with homologous recombination deficiency (HRD) due to CN loss of BRCA1/2 and these were also associated with the HGSOC HRD signature. Both signatures 3 and 4 were associated with a high ploidy state, CCNE1amp, ERBB2amp and MYCamp. Signature 3 is differentiated from signature 4 by an enrichment for PIK3CA mutations. We found no molecular associations for signature 1 and 2, although signature 2 was associated with endometrioid histotype. In this p53abn EC cohort the most common mutations beyond TP53 were PIK3CA (32%), PPP2R1A (29%), PIK3R1 (18%), FBXW7 (16%) and PTEN (16%). sWGS is a relatively inexpensive tool that has been successfully used to derive CN signatures. In this study we identified opportunities for targeted therapy such as PARPi for HRD ECs, anti-HER2 therapy for ERBB2amp and targeting Wee1 inhibitors for CCNE1amp ECs. CN signatures derived from sWGS can provide a clear path to clinical implementation and open new avenues for clinical trials.

132.

Brain-Age Prediction: Systematic Evaluation of Site Effects, and Sample Age Range and Size

Jordan Yu

Structural neuroimaging data have been used to compute an estimate of the biological age of the brain (brain-age) which has been associated with other biologically and behaviorally meaningful measures of brain development and aging. The ongoing research interest in brain-age has highlighted the need for robust and publicly available brain-age models pre-trained on data from large samples of healthy individuals. To address this need we have previously released a developmental brain-age model. Here we expand this work to develop, empirically validate, and disseminate a pre-trained brain-age model to cover most of the human lifespan. To achieve this, we selected the best-performing model after systematically examining the impact of site harmonization, age range, and sample size on brain-age prediction in a discovery sample of brain morphometric measures from 35,683 healthy individuals (age range: 5-90 years; 53.59% female). The pre-trained models were tested for cross-dataset generalizability in an independent sample comprising 2,101 healthy individuals (age range: 8-80 years; 55.35% female) and for longitudinal consistency in a further sample comprising 377 healthy individuals (age range: 9-25 years; 49.87% female). This empirical examination yielded the following findings: (1) the accuracy of age prediction from morphometry data was higher when no site harmonization was applied; (2) dividing the discovery sample into two age-bins (5-40 years and 40-90 years) provided a better balance between model accuracy and explained age variance than other alternatives; (3) model accuracy for brain-age prediction plateaued at a sample size exceeding 1,600 participants. These findings have been incorporated into CentileBrain [https://centilebrain.org/#/brainAGE2], an open-science, web-based platform for individualized neuroimaging metrics.

133.

Detection of Mitochondrial 8oxoG using Nanopore Sequencing

Andrew Galbraith

Reactive oxygen species can result in oxidative stress, potentially leading to neurodegenerative disorders and tumorigenesis. Due to its low redox potential, guanine is particularly susceptible to oxidation resulting in 8-oxoguanine (8oxoG). The syn conformation of 8oxoG base pairs with adenine resulting in G > T transversion mutations. Further, 8oxoG can regulate gene transcription through a variety of epigenetic and posttranscriptional roles such as by interference with CpG island methylation. To detect 8oxoG, we sequenced 11 conserved mitochondrial oligonucleotide sequences across mice and humans, 8 with 8oxoG at a given sequence motif and 3 without. We then compared the signal of oligonucleotides with and without 8oxoG to develop neural network models for 8oxoG detection. Further, we finetuned a base caller to correctly call the nucleotide sequence even with 8oxoG present. These models were found to differentiate modified oligonucleotides with a greater than 98% accuracy and false positive rate of less than 0.5%. Additionally, the models could correctly predict a significantly higher rate of 8oxoG in PolG mutant mice in comparison to wildtype. These mice are more vulnerable to oxidative damage due to having impaired mitochondrial replication. One of the models also showed higher rates of 8oxoG in cancers with mutation signatures associated with oxidative damage such as SBS 18 and 36. This project presents the first methodology to detect and quantity 8oxoG at single base pair resolution without the need of chemical conjugation or enzymatic treatment. Levels of 8oxoG can be profiled alongside standard nanopore sequencing analysis to do in-depth genetic and epigenetic screening of samples. This will facilitate 8oxoG analysis and help illuminate its role in pathogenesis. Future development of models will be needed to detect 8oxoG in more contexts, improve specificity of models, and profile nuclear and telomeric DNA 8oxoG.

134.

Investigating the role of epigenetic regulator SUV420H2 in Neuroendocrine prostate cancer development and aggressiveness.

Ace Shi

Neuroendocrine prostate cancer (NEPC) is a highly lethal form of prostate cancer. While less than 1% of clinical cases arise de novo, escalating incidence of treatment-induced NEPC has been observed in patients with castration-resistant prostate cancer via neuroendocrine (NE) transdifferentiation as an AR-independent therapeutic resistance mechanism. NEPC cells exhibit low or absent AR activities and high expression of NE markers such as CHGA, SYP, CD56 ,and NSE. Dismal clinical outcomes can be attributed to a lack of potent therapeutics and limited understanding of the underlying biology related to this malignancy, which hindered the development of more effective treatments. To better understand this malignancy and identifying therapeutic targets, our lab has developed the first-in-field NEPC LTL-331/331R patient-derived xenograft (PDX) model that recapitulates the process of transdifferentiation from adenocarcinoma (LTL-331) to NEPC (LTL-331R) following castration. Despite drastic variation on the transcriptomic level between the two stages of model, LTL-331 and LTL-331R show a consistent genomic profile, indicating that epigenetic regulation may play a critical role in the transdifferentiation process. Using the model, we identified SUV420H2, a histone methyltransferase for repressive H4K20 trimethylation (H4K20me3) marker, as a key regulator of driving NEPC development and aggressiveness. We demonstrated an upregulation of SUV420H2 in multiple NEPC PDXs and clinical samples compared to adenocarcinoma PDXs and clinical samples and confirmed its steady upregulation after castration along the longitudinal timeline (LTL331, Castration 1wk, 3wk, 8wk and relapsed LTL331R). Functional study demonstrated SUV420H2 knockdown inhibited cell growth and led to expression change of NE markers. Ectopic expression of the gene in adenocarcinoma cell line induced NE markers expression and H4K20 trimethylation and enhanced resistance to cisplatin treatment.

Poster Abstracts

135.

Development and Characterization of a Novel Topotecan Liposomal Formulation

Sarthak Garg

Topotecan (Topo) is a poorly soluble camptothecin derivative. Due to its high toxicity and low therapeutic index, Topo is only used as second line treatment for metastatic lung and ovarian cancer. Decades of Topo delivery formulation are therapeutically interesting but to date none have been approved for clinical use. Our research team has found that although Topo can be encapsulated easily, it is not well retained in the liposomes after administration. A novel approach with a different water-soluble camptothecin increased the amount of liposome associated camptothecin while also improving drug retention inside the liposome after administration. The goal of this project was to apply the novel approach to Topo. Liposomes with internal copper (Cu) containing and external Cu lacking solution were prepared by extrusion methods. After changing the outside buffer to pH 7.5 Topo was added to the liposomes. At various time points after drug addition, the liposomes were separated from unassociated drug by column chromatography (Sephadex G-50 spin columns). The liposomes were characterized and the cytotoxicity was measured in vitro using A549 (human lung cancer) and CT26 (mouse colorectal cancer) cells. This innovative approach could generate liposomes with drug-to-lipid ratios (D/L) (mol:mol) that were equal to or greater than 0.3. The maximum D/L achieved previously was 0.2. The new formulation was stable as determined by an in vitro drug release assay (less than 5% of the encapsulated drug dissociated from the liposomes over 24 hours at 37oC). Liposomal Topo exhibited an IC50 (concentration of Topo that affected 50% of the cells) of 52 ± 0.5 nM and 104 ± 1.3 nM when incubated with the A549 cells and the CT26 cells, respectively. The IC50 of free Topo was 53 ± 0.2 nM and 124± 0.9 nM. The resulting liposomal formulation is suitable for further development, and studies assessing the formulation's pharmacokinetic characteristics and its therapeutic effects are ongoing.

136.

Modeling the genome and exposome contribution to newborn DNA methylome variability with the RAMEN package

Erick Navarro-Delgado

DNA methylation(DNAme) is an epigenetic mark that can regulate the genome, and its variability has been associated with potentially long-term phenotypic changes. Studies suggest that genetics(G) and environmental(E) factors jointly best explain DNAme variability in most of the newborn epigenome(75-89%). Limitations of previous works are that the prenatal environment's influence could be underestimated due to the small set of variables analyzed(<20) and that past methodologies do not address the genome-exposome variable-number imbalance. We aimed to address these gaps and 1)identify Variable Methylated Regions (VMRs) at birth, and 2)analyze the contribution of G and E to VMR's DNAme. Using cord blood samples of CHILD (n=699, 94 E variables across four dimensions of the prenatal environment: parental psychosocial, maternal health, built environment, and maternal nutrition), we identified 28,480 VMRs using variance as a measure of variability. We conducted an E and G variable selection procedure for each VMR using LASSO, and then fitted single-variable G, E, pairwise additive(G+E) and interaction(GxE) linear models. After selecting the winning model using AIC, and labelling VMRs with non-conclusive models identified through a permutation analysis(41.2%), we found G+E to be the predominant best explanatory model(27%), followed by G(18.4%), GxE(13.4%) and E(0.1%). Furthermore, we found that the SNP terms explain a higher proportion of variance across all winning models at birth (mean partial R2=0.22) compared to the environment (mean partial R2=0.008) and interaction terms (mean partial R2=0.007). Finally, we developed an R package: Regional Association of Methylome variability with the Exposome and geNome (RAMEN; github.com/ErickNavarroD/RAMEN), which provides the community an easy-to-use pipeline to conduct integrative genome-exposome analyses with DNAme data. Overall, our work highlights the role of G in DNAme and the importance of addressing it in epigenetic studies.

137.

Repurposing Telmisartan as an Immunotherapy Adjuvant: Modulating CD8+ T Cell Activation in the Tumour Microenvironment

Meredith Clark

Immune checkpoint blockade (ICB) has emerged as a ground-breaking therapy for many cancer types. While effective in immunogenic cancers such as triple-negative breast cancer, enhancing response rates remains a challenge. This project explores repurposing the blood pressure drug Telmisartan (Tel) as a potential adjuvant for ICB. We have previously shown that while Tel decreases collagen deposition by cancer-associated fibroblasts and improves radiotherapy response, it does not affect xenograft growth in an immunodeficient mouse model. However, Tel treatment significantly delayed tumour growth in an immunocompetent murine mammary carcinoma model; suggesting an immune-mediated mechanism of tumour suppression. We hypothesize that Tel improves CD8+ T cell activation and anti-tumour activity through its role as a partial PPARγ agonist, and that Tel treatment will improve ICB response. We have identified that Tel treatment promotes a more activated CD8+ T cell phenotype in E0771 tumour-bearing mice, demonstrated by the increased expression of T cell activation markers, CD44 and PD-1. Further, ex vivo CD8+ T cells treated with Tel exhibited increased expression of effector molecules such as TNFα and granzyme B, suggesting that Tel may increase CD8+ T cell anti-tumour activity. Currently, we are investigating the tumour killing capacity of Tel-treated CD8+ T cells ex vivo and further characterizing the mechanism of Tel-mediated T cell modulation. We are also working to combine Tel treatment with ICB in tumour-bearing mice, as well as investigating other immune cell populations that are modulated by Tel treatment in the tumour microenvironment. This research highlights Tel's potential as an ICB adjuvant, providing a rationale for further exploration of its impact on T cell function and its viability as a complementary approach to cancer immunotherapy. Ultimately, combining Telmisartan with current ICB therapies may lead to more durable clinical responses for cancer patients.

138.

Characterizing the Interactome of the MET Exon 14 Oncogene in Lung Adenocarcinoma

Sarah Anna Okun

Splice site mutations in the MET receptor tyrosine kinase leading to skipping of exon 14 (METex14) are established driver mutations in non-small cell lung cancer (NSCLC), of which lung adenocarcinoma (LUAD) is the most common subtype. Targeted therapies for METex14 have been developed, however patient response is poor, and development of better inhibitors or combination therapies is necessary. The oncogenic potential of METex14 has been attributed to its increased half life and subsequent hyperactivation of downstream signaling pathways. Recent evidence from our lab suggests that it also preferentially upregulates the RAS/MAPK pathway, indicating that METex14 may display altered receptor affinity for its binding partners relative to its wildtype (WT) counterpart. We therefore aim to characterize the METex14 interactome to allow for better understanding of the pathways and mechanisms contributing to its transforming potential, and consequently identify possible therapeutic targets. To this end, V5-tagged constructs will be developed for MET WT, METex14, and MET Y1003F, the latter of which should partially phenocopy METex14's transforming ability. V5-tagged MET will then be pulled down and tandem mass tag mass spectrometry will be performed on co-immunoprecipitated binding partners to identify those which preferentially interact with METex14. Functional impacts of interactions of interest will then be elucidated through a pooled CRISPR-Cas9 screen utilizing a targeted library; enrichment levels of sgRNA before and after several population doublings will elucidate the impact of knocking out target proteins before individual effectors are validated through knockdown cell lines and transformation assays. This information will ultimately help uncover mechanisms through which METex14 drives LUAD oncogenesis and identify potential therapeutic targets, contributing to the design of more effective treatment strategies for lung cancer patients harbouring METex14 mutations.