TFRI-ONTARIO NODE RESEARCH SYMPOSIUM

THURSDAY DECEMBER 12, 2019

Novel Cancer Targets and Emerging Therapies

MaRS Collaboration Centre
(MaRS South Tower, Lower Level, 101 College Street, Toronto, ON M5G 1L7)

Program & Abstracts

THE TERRY FOX RESEARCH INSTITUTE
L'INSTITUT DE RECHERCHE TERRY FOX

Welcome

Symposium Hosts and Committee Members:

Robert Rottapel, MD, FRCPC
Ontario Node Leader, University Health Network

Donna De Francesco
Princess Margaret Cancer Centre

Marianne Koritzinsky, PhD
Princess Margaret Cancer Centre

Cheryl Arrowsmith, PhD
CSO, Structural Genomics Consortium
University of Toronto

Trevor G. Shepherd, PhD
Western University

Kristin Hope, PhD
McMaster University

Peter Greer, PhD
Queen's Cancer Research Institute

Carolina Ilkow, PhD
Ottawa Hospital Research Institute

Volunteers:

Zainab Azhar, Lora Cantarutti, Sally DeSilva, Charlyn Krahn, Arjun Naipaul, Julie Owen, Rosa Pileggi, Mary White
Princess Margaret Cancer Centre

Mela Maharaj
Princess Margaret Hospital

Statement on Respect for Confidentiality of Unpublished Material:

The Institute has invited everyone attending this meeting because of their contribution, or potential for contribution, to the work of our research community. In building our community, we are committed to respecting the confidentiality of ideas and data that are unpublished at this meeting.

We request and require that all registrants refrain from recording such confidential information, and do not discuss such information with colleagues outside of this meeting. It is only in this way that we will collectively build the trust and respect that is necessary for effective collaborations.

We appreciate your respect for and compliance with this important request.

The Terry Fox Research Institute Ontario Node Research Symposium

Novel Cancer Targets and Emerging Therapies

Opening Remarks:

Dr. Robert Rottapel, University Health Network

Mr. Darrell Fox, Terry Fox Research Institute

Keynote Speakers:

Professor Tim Willson, Structural Genomics Consortium, UNC

Professor Craig Crews, Yale University

Speakers:

Sheela Abraham, Queen's Cancer Research Institute
Rima Al-awar, Ontario Institute for Cancer Research
Liliana Attisano, University of Toronto
Fred Dick, Western University
Roman Melnyk, Hospital for Sick Children
Anton Neschadim, Immunobiochem Corporation
Jim Petrik, University of Guelph
Lisa Porter, Windsor Health Institute

Closing Remarks

Agenda

7:00am – 9:00am	**Early Registration**
7:00am – 9:00am	**Poster Set Up** (The Heritage Atrium, located on the 1st floor of the MaRS Centre)
7:30am – 8:45am	**Breakfast**
8:45am – 9:00am	**Welcome & Opening Remarks – Dr. Robert Rottapel, MD, FRCPC**, TFRI Ontario Node, Toronto & **Mr. Darrell Fox**, Board of Directors, TFRI

SESSION I:

9:00am – 9:45am	**KEYNOTE SPEAKER – Professor Tim Willson,** Structural Genomics Consortium, University of North Carolina Title: *Identification of Small Molecules to Shine Light on Dark Kinases*
9:45am – 10:10am	**Speaker – Jim Petrik, PhD**, Professor, University of Guelph Title: *Vascular normalization to improve therapeutic efficacy in advanced stage ovarian cancer*
10:10am – 10:40am	**Speaker – Liliana Attisano, PhD**, Scientist, Donnelly Centre, University of Toronto Title: *Targeting the Hippo pathway for Therapeutic Intervention*
10:40am – 11:05am	BREAK
11:05am – 11:35am	**Speaker – Sheela Abraham, PhD**, Scientist, Queen's Cancer Research Institute Title: *Investigating the Role of Extracellular Vesicles in Hematopoietic Stem Cell Regulation*
11:35am – 1:00pm	**LUNCH & Poster Session** (*Judges will review the Posters at this time) (The Heritage Atrium, located on the 1st floor of the MaRS Centre)

SESSION II:

1:00pm – 1:20pm	**Speaker – Anton Neschadim, PhD**, President & CEO, Immunobiochem Corporation, JLabs @ Toronto Title: *Targeting the Cancer Secretome of Solid Tumors with Potentiated Biologics*
1:20pm – 1:45pm	**Speaker – Rima Al-awar, PhD**, Director & Senior Scientist, Drug Discovery, Ontario Institute for Cancer Research Title: *Wdr5: The Translational Path of a First-in-Class Therapy for Leukemia*
1:45pm – 2:15pm	**Speaker – Fred Dick, PhD,** Professor of Pathology and Oncology, Western University Title: *A CRISPR-Cas9 Based Screen Implicates Netrin Signalling in Cell Survival During Ovarian Cancer Metastasis*
2:15pm – 2:45pm	**Rapid Fire Postdoc Talks –** 10 X 3 min
2:45pm –3:05pm	**BREAK**
3:05pm – 3:35pm	**Speaker – Roman Melnyk, PhD,** Senior Scientist, Sickkids Title: *Development of a Potent Ras-Targeted Enzyme for Precision Cancer Therapy*
3:35pm – 4:05pm	**Speaker – Lisa Porter**, Director, Windsor Health Institute, University of Windsor Title: *Reinstalling Cell Cycle Checkpoints as a Therapeutic Strategy*
4:05pm – 4:50pm	**KEYNOTE SPEAKER – Professor Craig Crews,** Yale University Title: *PROTACs: A New Therapeutic Modality*
4:50pm – 5:00pm	**Closing Remarks**
5:00pm – 6:30pm	**RECEPTION & Poster Session** (The Heritage Atrium, located on the 1st floor of the MaRS Centre)

Rapid Fire Talk Presenters

DOGAN-ARTUN, NERGIZ
Princess Margaret Cancer Centre
Supervisor: Mathieu Lupien

Integrating transcriptional regulatory network with chromatin accessibility as marker of drug response in triple-negative breast cancer

KRISHNAN, RAMYA
McMaster University
Supervisor: Peter Greer

The impact of tumour burden on oncolytic virus vaccine-induced anti-tumour immune responses

KUHLMANN, LAURA
Princess Margaret Cancer Centre
Supervisor: Thomas Kislinger

Identification of Cell Surface Proteins in Triple Negative Breast Cancer for the Development of Novel Targeted Therapies

MAIR, BARBARA
University of Toronto
Supervisors: Brenda Andrews and Jason Moffat

High-throughput genome-wide phenotypic screening via immunomagnetic cell sorting identifies QPCTL as modulator of CD47

MCGARRY, DAVID JOHN
Ryerson University
Supervisor: Michael Olson

MICAL1-mediated protein oxidation in tumour cell motility

NOURI, KAZEM
Queen's University
Supervisor: Xiaolong Yang

Identification of a Novel YAP-TEAD Inhibitor for Cancer Therapy by High Throughput Screening with Ultrasensitive YAP/TAZ–TEAD Biosensors

PRZEDBORSKI, MICHELLE
University of Waterloo
Supervisor: Mohammad Kohandel

Integrating Systems Biology and Machine Learning to Partially Overcome Limitations of Anti-PD-1 Immunotherapy

RAPIC, SARA
Princess Margaret Cancer Centre
Supervisor: Ralph DaCosta

Investigating radiation response of pancreatic tumors and their microenvironment using in vivo optical imaging to identify new treatment strategies

REHMAN, SUMAIYAH
Princess Margaret Cancer Centre
Supervisor: Catherine O'Brien

Tumor cell heterogeneity and dynamics in colorectal cancer development

SPARKES, AMANDA
Sunnybrook Research Institute
Supervisor: Jean Gariépy

ICOSL.COMP: A Powerful Enhancer of Anti-Tumoral Responses Restored by Immune Checkpoint Inhibition

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* *TFRI/TFF Supported*
† *Previous Rapid Fire Talk Presenter*
‡ *Previous Poster Winner*
🔥 *Rapid Fire Talk Presenter*

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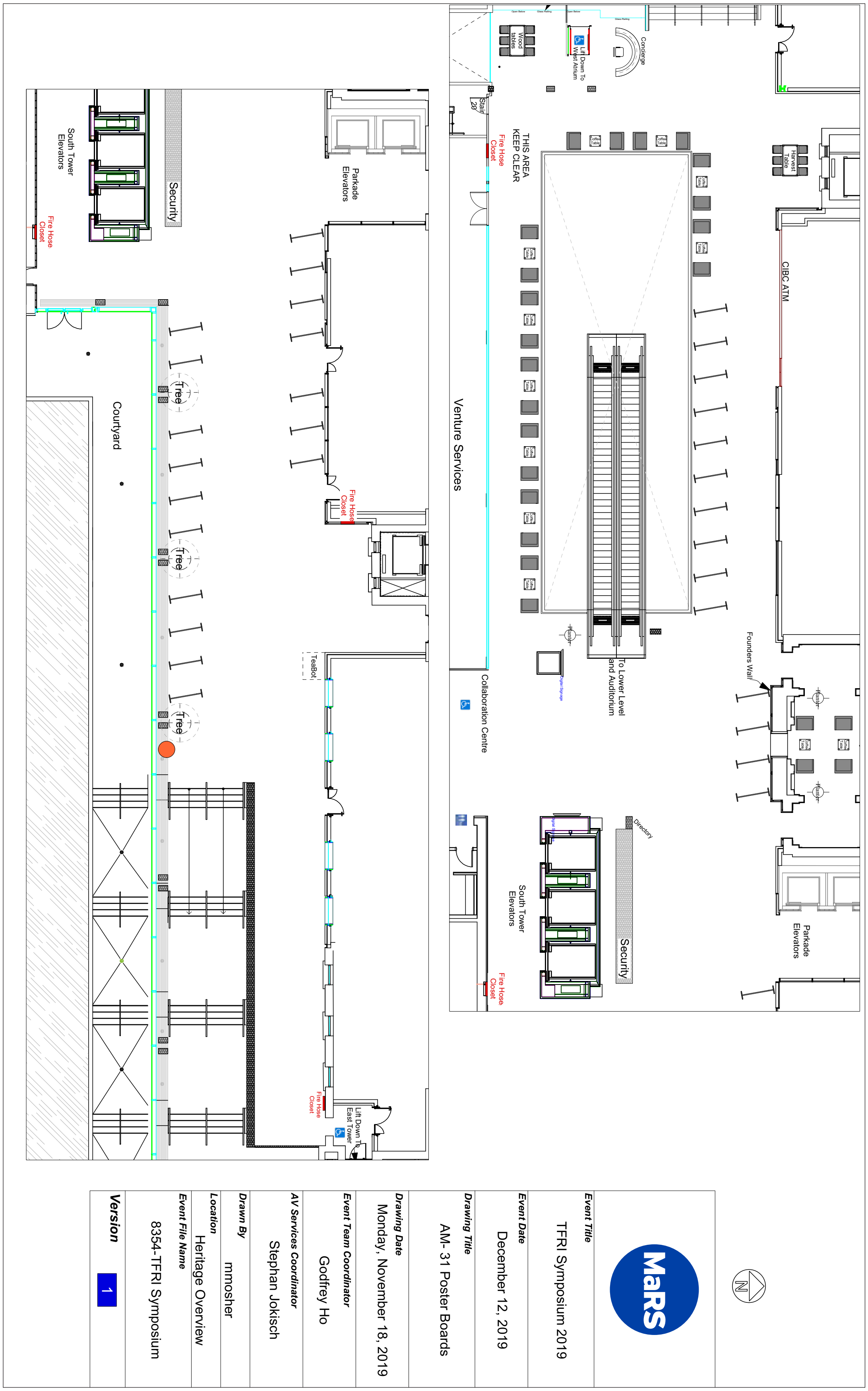

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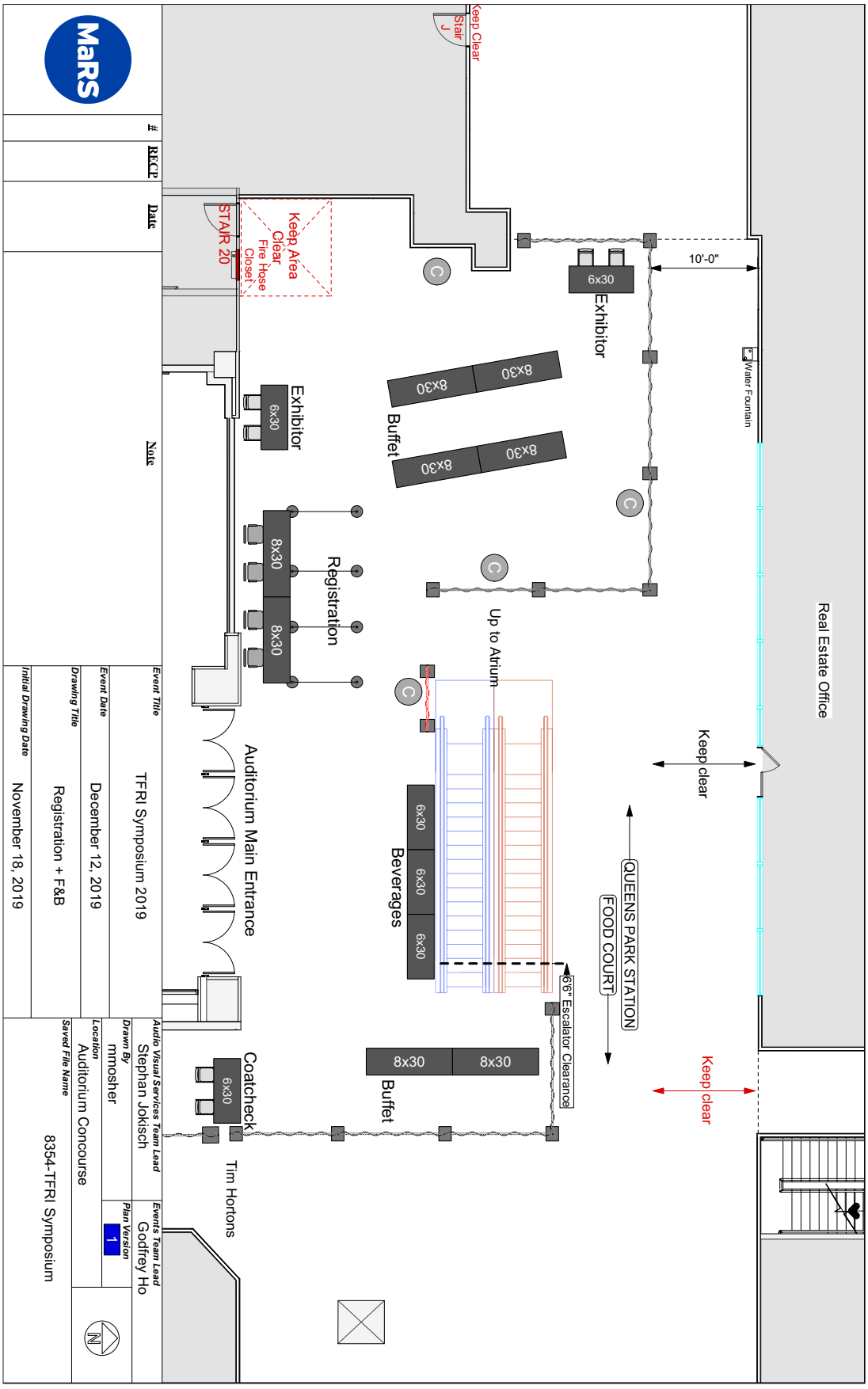
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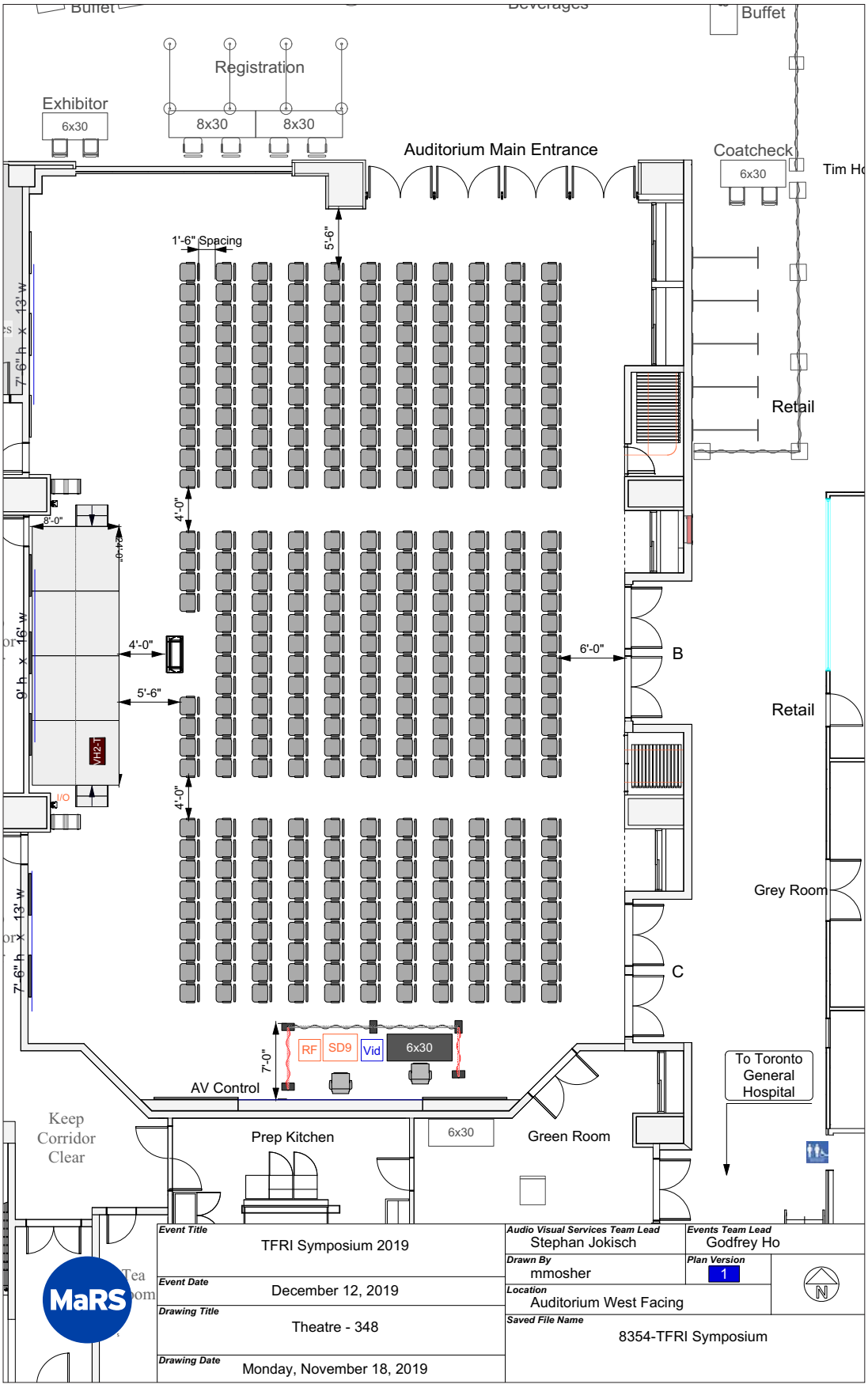
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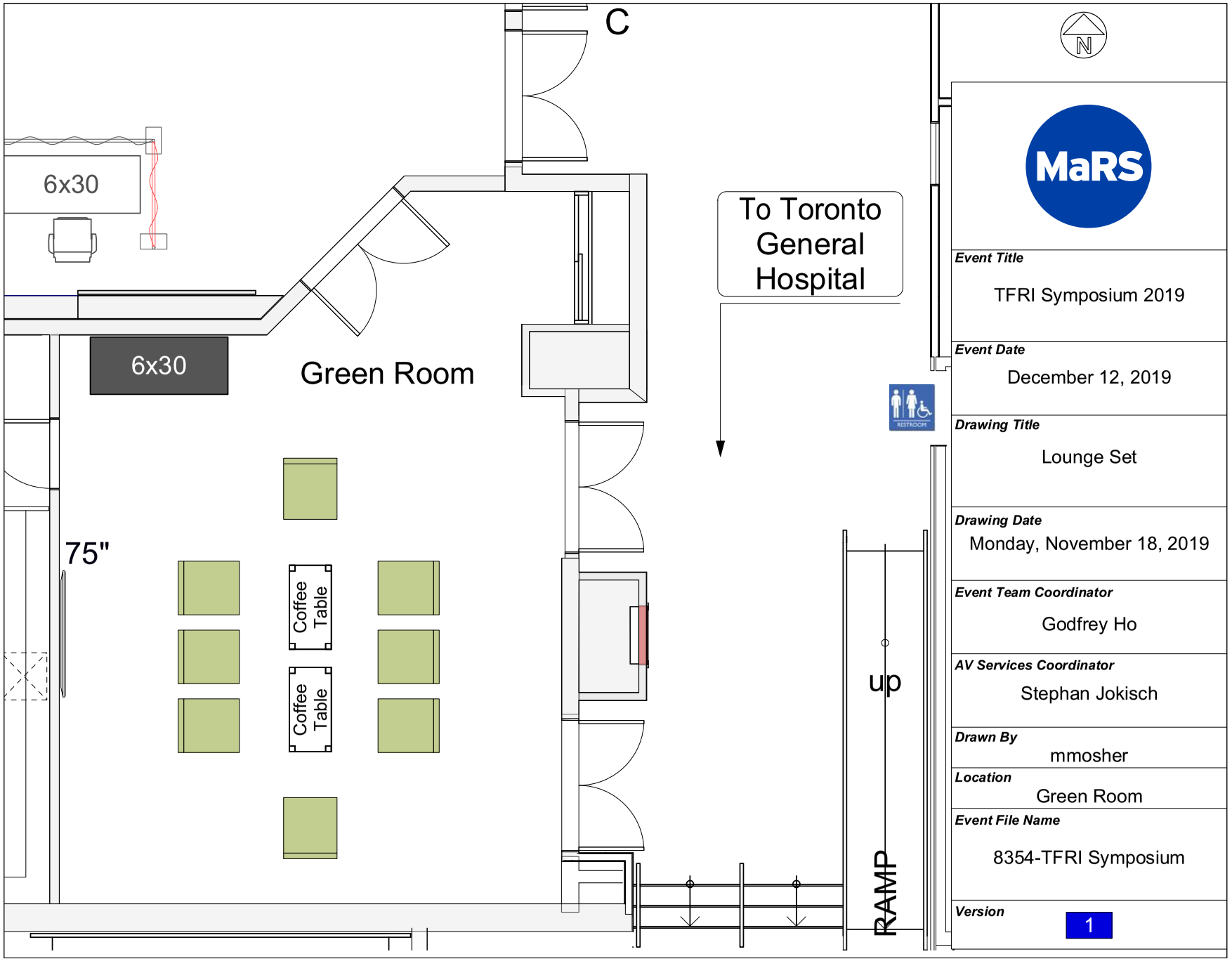

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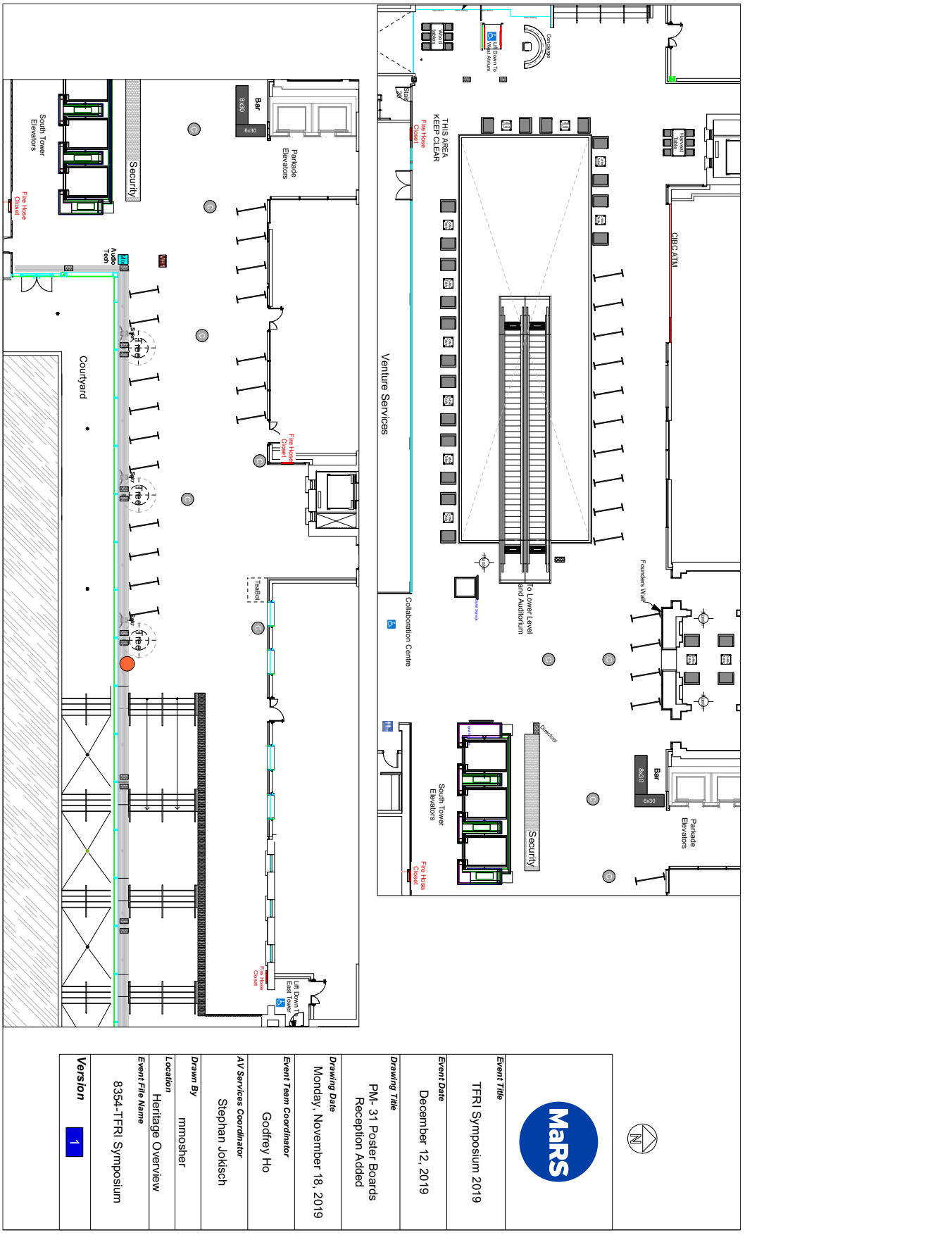
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Coatcheck
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6x30
Parkade Elevators
South Tower Elevators
Security
Courtyard
Audio Tech
Lift Down To East Tower

1. Chromosome 16q haploinsufficiency as a driver in IDC and ILC breast cancer

Yeji An (graduate student)[1,6], Jessica R. Adams[1], Amanda J. Loch[1], Lauryl Nutter[3], Wei Wang[1], Eldad Zacksenhaus[4,5], Charles M. Perou[2], SEAN E. EGAN[1,6].

[1]Peter Gilgan Center for Research and Learning, Hospital for Sick Children, [2]Lineberger Comprehensive Cancer Center, Departments of Genetics and Pathology, University of North Carolina, Chapel Hill, NC 27599, USA, [3]Science & Technology Development Team, The Centre for Phenogenomics, [4]Division of Cell and Molecular Biology, Toronto General Research Institute, University Health Network, [5]Department of Medical Biophysics, University of Toronto, [6]Department of Molecular Genetics, University of Toronto.

One of the most common events in human breast cancer involves hemizygous loss of chromosome 16q, which occurs in most hormone receptor positive tumors, including invasive ductal carcinomas (IDC) as well as invasive lobular carcinomas (ILC). To test for oncogenic effects of this event, we have created a Cre-conditional model with loxP sites flanking a 55 Mbp region on mouse chromosome 8 which is syntenic to human 16q. Deletion of this region in mammary epithelium by MMTV-Cre induced mammary tumor formation. Chromosome 16q includes candidate haploinsufficient tumor suppressor genes (TSGs), Cadherin 1, (CDH1) and Core-binding factor B (*CBFB*). Previously, we have shown that *Cdh1* is not a haploinsufficient or recessive TSG on its own. In addition, *Cdh1* cooperates with *Pik3ca*^{H1047R} as a recessive TSG and results in tumors that resemble a molecular subtype of ILC known as immune-related ILC. In contrast, we found that *CbfB* is a haploinsufficient TSG on its own. *CbfB* also cooperates with *Pik3ca*^{H1047R} as a recessive TSG to drive tumorigenesis. This study will define roles for several common events, including 16q hemizygous deletion, and will investigate cooperation between such events. Overall, these experiments will reveal novel pathways that contribute to initiation and progression of hormone receptor positive breast cancer.

1. Elucidating the role of CD44-Ezrin interaction in breast cancer cell invasion and drug resistance *in vitro*

RAYANNA BIRTCH, Yan Gao, Victoria Hoskin and Peter A. Greer
Division of Cancer Biology and Genetics, Queen's Cancer Research Institute
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Cancer is a debilitating disease that will affect 1 in 2 Canadians in their lifetime. While advances in cancer care and treatment have greatly improved survival for patients, the vast majority of cancer-related deaths are due to the spread of cancer cells to distant sites within the body, known as metastasis. Furthermore, resistance to anti-cancer therapies such as chemotherapy is a major problem limiting the effectiveness of these treatments. Therefore, understanding the molecular mechanisms regulating cancer progression and drug resistance are important for the development of novel therapeutic strategies to improve patient outcome. The cytoskeleton adaptor protein ezrin regulates a variety of cellular functions such as cell migration and survival. Ezrin's clinical relevance as a prognostic biomarker and its potential as a therapeutic target are supported by its association with poor outcomes in breast cancer patients, and preclinical model studies showing that genetic disruption this protein attenuates the metastatic potential of breast cancer cells and increases their sensitivity to chemotherapeutic challenges. As an adaptor protein, ezrin binds to a variety of membrane-associated proteins, including the cell adhesion molecule CD44. CD44 is a transmembrane glycoprotein that mediates communication between cells and the extracellular matrix. It is a marker of tumor-initiating/cancer stem cells in many human cancers and is well documented to promote cancer cell growth, survival and invasiveness. While both ezrin and CD44 are implicated in cancer metastasis and drug resistance, little is known about the how the CD44-ezrin interaction regulates these phenotypes. To address this, we utilize CRISPR-Cas9 gene editing technology and mutational approaches to modify the expression of, and disrupt the interaction between ezrin and CD44. Using the MDA-MB-231 human breast cancer cell line, we aim to conduct cell viability and clonogenic assays to assess changes in drug sensitivity to commonly used chemotherapy drugs. As well, migration and invasion assays will be used to evaluate the involvement of CD44-ezrin interaction on metastatic potential. By interrogating the role of CD44-ezrin interactions in breast cancer progression and drug resistance, we aim to have a better understanding of the mechanisms underlying cancer cell metastasis and treatment resistance, and to ultimately uncover potential novel therapeutic targets.

3. Assessing *TP53* Mutation Status' Influence On Response To Anti-Pd-1/Anti-Pd-L1 Immunotherapy In Non-Small Cell Lung Cancer

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Background: Recent studies with anti-PD-1/anti-PD-L1 immune checkpoint inhibitors have demonstrated remarkably durable responses in patients with non-small cell lung cancer (NSCLC). Although recent trials have proposed tumor mutational burden and intratumoral PD-L1 expression as unique biomarkers of response to anti-PD-1/anti-PD-L1 therapies, only a small subset of patients respond to current immunotherapy, and the factors that influence which patients will respond to drug treatment have not been fully elucidated (Dong et al., 2016). Thus, there is an urgent need to identify more effective biomarkers for immunotherapy response.

Objective: To investigate the potential predictive capacity of *TP53* mutation status on anti-PD-1/anti-PD-L1 response in NSCLC

Hypothesis: NSCLC patients harbouring *TP53* mutations would have increased PD-L1 expression and tumor mutational burden, leading to improved progression-free survival time (PFS) on anti-PD-1/anti-PD-L1 immunotherapy when compared to patients with wild-type (WT) *TP53*.

Methods: Clinical data were collected on 201 patients with advanced NSCLC who received anti-PD-1/anti-PD-L1 monotherapy utilizing data publicly available online through the cBioPortal platform *(J Clin Oncol, 2018)*. Loss of transcriptional activity for each *TP53* mutation was measured by functional assays described by Kato et al. *(PNAS, 2003)*. Statistical analyses were conducted using GraphPad Prism 8 (Version 8.2.0) and RStudio (Version 1.2.1355).

Results: Individuals with *TP53* mutations had a greater PFS when compared to individuals with *WT TP53* (P = 0.0059). As well, NSCLC patients harbouring *TP53* mutations had greater PD-L1 expression and mutation count than WT (*P* = 0.0099 and *P* = 9.4x10-9, respectively). Subsequent mutation stratification revealed that only patients with transcriptionally active (TA) *TP53* missense mutations had greater PFS than WT (*P* = 0.0019), whereas transcriptionally inactive (TI) missense mutations had similar PFS as WT (*P* = 0.99). Patients with TA, and not TI, *TP53* missense mutations had greater PD-L1 expression than WT (*P* = 0.0111 and *P* = 0.1328, respectively), while patients with both TA and TI *TP53* missense mutations had greater mutation count than WT (*P* = 0.00013 and *P* = 0.0039, respectively).

Conclusions: NSCLC patients with TA missense *TP53* mutations have increased PD-L1 expression and mutation count, contributing to improved response to anti-PD-1/anti-PD-L1 immunotherapy compared to patients with WT *TP53*. In the future, it would be beneficial to assess what specific *TP53* mutation a patient with NSCLC has before starting immunotherapy to potentially serve as a biomarker of drug response.

4. Targeting CXCL12/CXCR4 and Myeloid Cells to Improve the Therapeutic Ratio in Patient-Derived Cervical Cancer Models Treated with Radio-Chemotherapy

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Background: The CXCL12/CXCR4 chemokine pathway is involved in cervical cancer pathogenesis and radiation treatment (RT) response. We previously reported that radiochemotherapy (RTCT) and concurrent administration of the CXCR4 inhibitor plerixafor, an inhibitor of this pathway, improved primary tumor response. The aims of this study were to: **1.** To explore different ways of sequencing RTCT and plerixafor (small molecule CXCR4 inhibitor) to maximize efficacy; and its translation into a phase I/II clinical trial. **2.** Evaluate the role of hypoxia in RT-induced CXCL12 upregulation. **3.** Explore the effects of plerixafor with RTCT on intestinal toxicity, an important dose-limiting consequence of treatment in cervix cancer patients.

Methods: Primary cervical cancer xenografts (model OCICx 20), generated from cervix cancer biopsies from patients in clinical studies at Princess Margaret Cancer Centre were implanted in the cervixes of mice. Orthotopic cervical cancer xenografts were treated with RTCT (30Gy in 2Gy fractions and cisplatin) with or without concurrent, adjuvant or continuous plerixafor. This RTCT protocol was designed to mirror the clinic as closely possible. Mice were followed after treatment with serial CT imaging to monitor tumor regrowth. Endpoints were growth delay and molecular and immune cell changes including both CXCL12 circulating and expression levels at the end of treatment. Late intestinal toxicity was assessed by histologic examination of the rectum 90 days after a single 20Gy fraction.

Results: Combination of RTCT and plerixafor produced substantial tumor growth delay compared to RTCT alone regardless of sequencing. The continuous and adjuvant plerixafor arms were associated with significantly longer growth delay than the concurrent only arm. Increased duration of adjuvant plerixafor (3 vs 6 weeks) did not show additional benefit. RTCT increased CXCL12/CXCR4 signalling and the intratumoral accumulation of myeloid cells; the addition of plerixafor mitigated these effects. All of the RTCT and plerixafor arms showed prolonged tumor growth delay compared to RTCT alone, with the adjuvant arm showing the greatest improvement. Dose dependent increase in CXCL12 gene expression and circulating levels by qRT-PCR and ELISA respectively were observed with post-RT treatment. End of treatment CXCL12 (qRT-PCR), pCXCR4, pERK, PD-L1 expression and intratumoral accumulation of Ly6G and F4/80 levels were abrogated with RTCT+plerixafor compared to RTCT. Similar expression levels were observed for adjuvant treatment. Plerixafor also reduced late intestinal toxicity.

Conclusion: Adding plerixafor to RTCT blunts treatment-induced increases in CXCL12/CXCR4 signalling, improves primary tumor response and reduces intestinal side effects. These benefits may apply to other tumors where RT plays a curative role. Plerixafor protects normal tissue from RT injury. Few if any drugs have been identified previously that both improve the effectiveness of RT and prevent side effects. This combination warrants testing in future clinical trials.

5. Identifying Epigenetic and Metabolic Targets in Drug-Resistant Triple-Negative Breast Cancer

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Breast cancer is the most common cancer and has the second highest cancer mortality rate in Canadian women[1]. It is stratified into molecular subtypes by immunohistochemical analysis of estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2). Triple-negative breast cancer (TNBC) is an aggressive subtype characterized by earlier age of onset, poor prognosis, and lower 5-year survival rate compared to other subtypes. While targeted therapies against ER or HER2 have proven effective in other subtypes, TNBC lacks these molecular features and as such, treatment defaults to chemotherapy. However, despite initial response to chemotherapy, TNBC has a high rate of relapse[2,3]. Thus, resistance to therapy remains an obstacle to complete remission and there is a demand for novel approaches to treat TNBC. There has been growing interest in the role of metabolism in tumour progression as cancer cells acquire metabolic adaptations to survive and proliferate in response to environmental challenges. In addition, metabolic processes directly affect substrate availability for chromatin-modifying enzymes, thereby modulating the epigenetic landscape[4]. We hypothesize that exploring the metabolome and epigenome will highlight potential new targets to treat chemoresistant TNBC.

We have established gemcitabine-resistant TNBC cell lines and identified perturbations in metabolic gene expression upon chemotherapy drug treatment. We also showed that interferon response gene expression is upregulated upon acute chemotherapy in drug-sensitive cells but not in resistant models, indicating an underlying adaptation. Global histone methylation and acetylation were accessed by Western blotting and we followed up on our observations with ATAC-seq, probing chromatin accessibility to determine alterations in the epigenomic landscape associated with resistance. Leveraging an extensive library of chemical probes to metabolic and epigenetic targets from the Structural Genomics Consortium (Toronto, Canada), we screen for drugs effective against our resistant TNBC models. Overall, our goal is to understand the epigenetic changes contributing to drug resistance to identify new vulnerabilities and find new therapeutic opportunities.

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6. Stable J-aggregation of an aza-BODIPY-lipid in a liposome for optical cancer imaging

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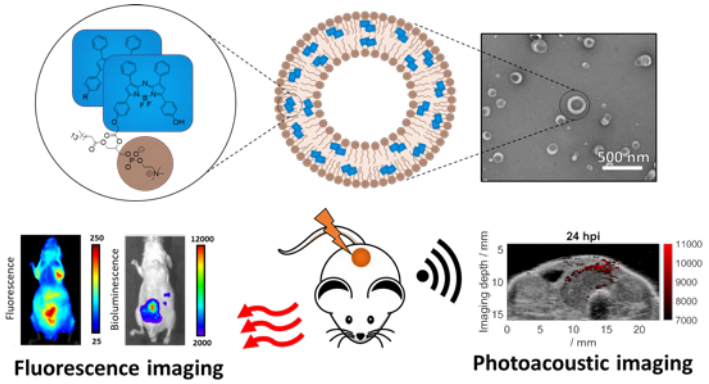
Organic building blocks are the centerpieces of "one-for-all" nanoparticle development, wherein their self-assembly into a nanostructure can induce novel nanoscale properties and advance design of multimodal nanoparticles for cancer imaging and therapy.1 These advantages have led to the development of liposomes with the phospholipid itself serving as a multifunctional building block.2-4 However, new dye subunits that are capable of self-assembling into liposomes are difficult to synthesize. Herein, we report the synthesis of a novel aza-BODIPY-lipid building block and its self-assembly into a liposomal nanoparticle (BODIPYsome). We observed NIR J-aggregation within the BODIPYsome that is optically stable under various serum and temperature conditions, which is likely attributed to J-dimerization. BODIPYsomes exhibit a high extinction coefficient (128 mM-1 cm-1) and high fluorescence quenching (99.70 ± 0.09%), which enables photoacoustic (PA) properties from its intact structure and recovered NIR fluorescence properties when it is disrupted in cancer cells. Finally, the BODIPYsome was proven as a highly biocompatible nanocarrier and its multimodal imaging (PA/fluorescence) capabilities were demonstrated in an in vivo orthotopic prostate tumor mouse model 24 h after intravenous administration. Overall, the BODIPYsome opens the door for engineering new building blocks in the design of optically stable biophotonic imaging agents.



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7. Identifying mechanisms that determine sensitivity to p38 MAPK inhibition in castration-resistant prostate cancer

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Androgen receptor (AR) signaling is the major driver of castration-resistant prostate cancer (CRPC). Tumor hypoxia increases AR signaling and is associated with treatment resistance.
p38 MAP kinase is involved in AR signaling by activating heat shock protein 27, a chaperone for AR translocation. Additionally, the activation of p38 has been found to be an early response to hypoxia. However, the role of p38 in AR signaling under hypoxia in CRPC has not been explored. In this study, we evaluated the role of p38 on AR signaling under hypoxia in CRPC cells. Our results demonstrate that p38 activation is an early response to hypoxia. Hypoxia increased ligand-dependent AR binding to androgen-responsive element and expression of AR target genes. Pharmacological p38 inhibition decreased the hypoxia-induced increase in AR activity. Additionally, pharmacological inhibition and siRNA knockdown of p38 decreased cell proliferation and survival in prostate cancer cells dependent on AR signaling for survival. These results suggest further investigation of p38 inhibition as a therapeutic strategy to disrupt AR signaling in CPRC.

8. Identifying mechanisms that determine sensitivity to p38 MAPK inhibition in castration-resistant prostate cancer

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Ovarian carcinoma (OC) has the highest disease mortality amongst gynecologic cancers. Recent clinical trials using immune checkpoint inhibitory nivolumab (anti-PD1) have shown promising therapeutic results in patients with high-grade serous ovarian carcinoma (HGSOC), warranting further investigations on immune microenvironment in OCs. Clear cell carcinoma (CCC) and endometrioid carcinoma (EC) account for approximately 20% of diagnosed OC and are often platinum-chemotherapy resistant. The majority of studies have focused on HGSOC; few have investigated the immune composition within the tumour microenvironment of CCC and EC. Non-serous non-mucinous OC were surgically resected and processed for multi-parametric flow cytometry to determine the proportions of lymphocyte subsets ($CD4^+$ T cells, $CD8^+$ T cells, yd T cells, $CD19^+$ B cells). From the 17 non-serous non-mucinous ovarian samples processed, 7 were identified as CCCs and 11 were ECs. There was no significant effect of ovarian subtype on proportion of tumour-infiltrating $CD8^+$ T cells and $CD19^+$ B cells. There was a trend towards increased CD4+ T cells and decreased yd T cells in EC compared to CCC. Furthermore, $CD45^+$ lineage- innate lymphoid cells (ILCs) – further subdivided into NK cells, ILC1s, ILC2s, and ILC3s – were also present in some ovarian tumours. To our knowledge, we are the first to characterize intratumoral ILC subsets in OC. Further investigations on immune microenvironment are warranted to determine whether TILs can be used as a prognostic tool for CCC and EC. Furthermore, presence of CD8+ TILs may provide justification for clinical trial development for nivolumab in patients with CCC and EC.

9. Gene expression profiles that influence lymphocytic infiltration in sarcomas of the extremities

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Objective: Sarcoma is arguably the most heterogeneous group of cancers with over 50 subtypes and as a result, survival rates have remained relatively stagnant in recent decades. Sarcoma treatment strategies rely on surgical resection, meaning immunotherapy interventions applied to other malignancies are largely underutilized. Adoptive cell transfer therapy (ACT) is a relatively non-invasive immunotherapy approach wherein a patient's immune cells are externally isolated, expanded and then re-administered. While some cases show positive results, it is unknown why certain patients do not have long-lasting results and some do not respond at all. A recent advance in the sarcoma field by Wunder *et al* (2019) demonstrated that a subset of Undifferentiated Pleiomorphic Sarcoma (UPS), Osteosarcoma (OSA) and Myxofibrosarcoma (MFS) cases harboured tumour-infiltrating lymphocytes (TILs) and express Programmed Cell Death Ligand 1 (PD-L1). Further, OSA and UPS cases with high PD-L1 expression were associated with a better clinical outcome. Through RNA-sequencing, a list of differentially-expressed genes between UPS and OSA cases with and without a positive prognosis was determined. With this discovery, we hypothesize that there are tumour-related gene expression profiles that influence immune infiltration and potentially clinical outcome. Our aims are to create a reliable system to investigate the gene expression profiles of patient cases with respect to their clinical features. This system will also be used to determine if an association lies between a specific gene expression profile and autologous tumour-immune reactivity.

Methods: 230 Sarcoma tumor specimens from patients undergoing open biopsy or surgical resection without pre-operative adjuvant treatment have been collected for the isolation of both tumour cells and the respective tumour-infiltrating lymphocytes (TILs). Various dissociation and tumour culturing methods were optimized for each case, with UPS and MFS cases being the most viable subtype (Table 1). Cases were analyzed for tumour specific mutations before being co-cultured with autologous TILs. Indicators of cell activity and toxicity were evaluated with an ELISA. Expression levels of discriminatory genes determined by Wunder *et al* has been investigated in cultured tumour cells by qPCR.

Results: Due to the intra and inter-subtype heterogeneity of sarcoma, optimal culturing conditions were variable. It was determined that culturing method and materials influence cell morphology, therefore techniques were optimized on a case-specific basis. Currently, we have been investigating the relative expression levels of discriminatory genes in cultured tumour cells by qPCR.

Conclusion: A subset of resected patient samples are capable of forming successful primary cell cultures. Genetic analysis of successfully cultured cases will be validated and correlated with the patient's clinical features. Autologous TIL-tumour interactions will be examined and it will be determined if a tumour-specific expression profile is associated with higher reactivity.

Successful Culture: Case shows persistent growth over 5 passages and can produce enough cells for functional assays.

Success By Subtype		
	Successful	**Total Attempted**
UPS	8	10
MFS	7	10
LMS	3	5
OSA	2	4
Lipo.	1	2
Misc.	3	7
Total	24	38

Table 1: A summary of Sarcoma cases that have attempted to be cultured by subtype.

10. Establishing Patient and PDX-Derived Organoids to Model Therapeutic Response

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Organoids provide a unique 3D-culture method of growing patient-derived tumours in vitro which better reflect their respective tumours of origin than do 2D culture systems. We have successfully established organoids from both patient and PDX-derived breast cancers and have used these to model tumour response to chemotherapeutic drugs. These organoids have been characterized by immunohistochemistry and represent the heterogeneity observed in the tumour from which they were derived. Ongoing work involves comparing the therapeutic response between organoids and PDX models derived from the same breast tumours with the long-term goal of using the organoids to assist in predicting and informing patient response to treatment. We are also establishing methods to perform targeted genetic screens on patient organoids to identify novel therapeutic vulnerabilities.

11. Dual Functionality of Smart Delivery Biomaterials in the Treatment of Cancer

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Alexandria DeCarlo – Master's Student in Dr. Szewczuk's Laboratory

Current cancer therapies broadly target rapidly proliferating malignant cells while also affecting healthy cells. Currently, an area of intense research focus is the development of a highly specific delivery method of cancer therapeutics. Functionalized and extended-release drug-delivery systems that actively target tumor-specific receptors have been investigated as possible cancer treatments. We have previously reported on a pH-responsive folic acid (FA) conjugated nanopolymer that has the requisite features for efficient drug delivery. This biocompatible copolymer (FA-DABA-SMA), self-assembles in a pH dependent manner and provides active targeting and gradual release of hydrophobic chemotherapeutics simultaneously. We have demonstrated that this nanopolymer can effectively penetrate the inner core of 3-dimensional cancer cell spheroids and deliver anti-cancer therapies. Surprisingly, nanopolymers not loaded with therapies also decreased spheroid volume and revealed a previously unknown intracellular mechanism of action of the nanopolymer. In the present study, we investigated the possibility of a secondary therapeutic mechanism of action post-internalization. We characterized a size and shape-dependent mechanism of FA-DABA-SMA that actively targets folic acid receptors (FR) to initialize internationalization. This was shown to lead to intracellular disruptions of essential oncogenic proteins including p53, the product of the highly mutated *TP53* gene, and candidate proliferative proteins STAT-3 and c-Myc. Furthermore, FA-DABA-SMA activates early and late apoptotic events in MDA-MB-231 breast cancer cells. These findings indicate that the large size and nanostructure of the FA-DABA-SMA polymer has multiple tumor-targeting mechanisms with the ability to internalize hydrophobic chemotherapeutics and disable critical oncogenic proteins controlling cell division and induce apoptosis.

12. Label-free detection of nerves and lymphatic vessels *in-vivo* with optical coherence tomography

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Analysis of semi-transparent low scattering biological structures in optical coherence tomography (OCT) has been actively pursued in the context of lymphatic imaging, with most approaches relying on the relative absence of signal as a means of detection. Here we present an alternate methodology based on spatial speckle statistics, utilizing the similarity of a distribution of given voxel intensities to the power distribution function of pure noise, to visualize the low-scattering biological structures of interest. In a human tumor xenograft murine model, we show that these correspond to lymphatic vessels and nerves; extensive histopathologic validation studies are reported to unequivocally establish this correspondence (Fig. 1). The emerging possibility of OCT lymphangiography and neurography is novel and potentially impactful (especially the latter), although further methodology refinement is needed to distinguish between the visualized lymphatics and nerves.

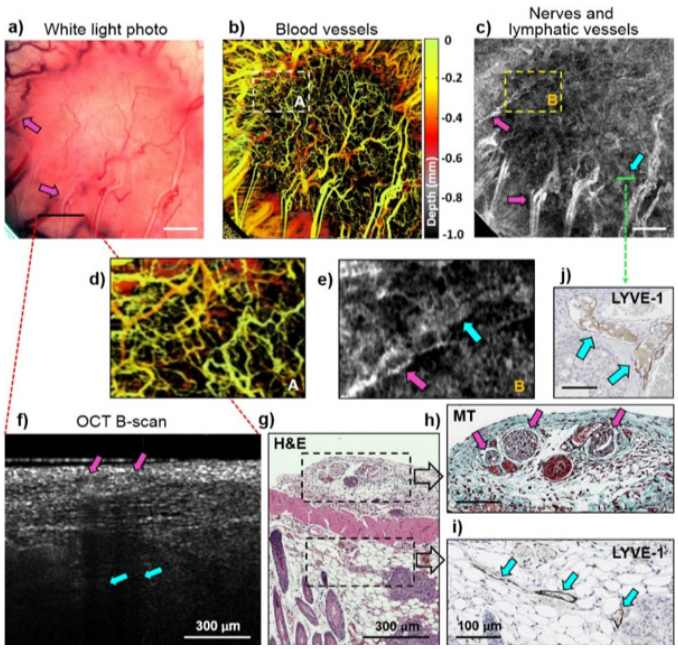

Fig. 1. Angiography, neurography / lymphangiography, and histology of pancreatic adenocarcinoma grown in a mouse dorsal skin window chamber. **(a)** White-light photo of dorsal skin in window chamber, field of view – 6 x 6 mm^2; **(b)** Depth-encoded blood microvasculature map of **(a)**; **(c)** Grey-scale average R^2– thresholded projection for low-scattering regions in **(a)**. Dashed-line white **(A)** and yellow **(B)** rectangular areas are expanded in **(d)** for blood microvasculature, and (e) for lymphatic vessels and nerves, some of which are labeled with arrows, the former as cyan and the latter as purple. Scale bars in (a), (b) and (c) are 1 mm; **(f)** OCT B-scan with corresponding Hematoxylin and Eosin (H&E) staining in **(g)** from approximately same location in tissue. Black dash-lined rectangular areas are enlarged in (g) and (h); **(h)** Nerves are distinguished from surrounding connective tissues with Masson's Trichrome (MT) staining; **(i)** Peri-tumoral lymphatic vessels are visualized with lymphatic vessel endothelial hyaluronan receptor 1 (LYVE-1). Nerves are labeled with purple arrows, lymphatic vessels – with cyan arrows. **(j)** LYVE-1 staining of the tumor core tissue (location labeled with green line in (c), identifying two newly formed lymphatic vessels with thinner walls. Scale bars in (h), (i) and (j) are 0.1mm.

13. Exploring the mechanism of the acoustic conversion of microbubbles to nanostructures for use as an EPR-independent nanomedicine delivery platform†

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INTRODUCTION – Nanomedicine represents the culmination of research across the fields of chemistry, physics, and biomedical engineering to generate innovations in healthcare, with a strong commitment to the enhancement of cancer diagnosis and treatment. However, it has seen limited clinical translation despite preclinical success, a problem largely attributed to its reliance on passive tumor accumulation via the enhanced permeability and retention (EPR) effect[1–3]. As growing evidence suggests that the EPR effect has a heterogeneous clinical profile[4,5], the field has been driven to consider more active strategies to improve delivery. One such platform that may bypass the EPR effect is the in situ conversion of microbubbles to nanostructures using low frequency focused ultrasound – a micro-to-nano conversion – which was first explored using porphyrin-containing microbubbles for the localized delivery of photosensitive nanostructures to tumors[6]. In order to facilitate an intentional design process for the development of this technique as a versatile means of nanoparticle delivery, a thorough characterization and optimization of the micro-to-nano conversion is necessary.

OBJECTIVE – To elucidate the mechanism and dependencies that enable the microbubble-to-nanostructure conversion under clinically-relevant ultrasonic conditions.

METHODOLOGY & FINDINGS – A library of microbubbles was formulated to assess the impact of lipid chain length, composition, and agent concentration on the micro-to-nano conversion efficiency. These microbubbles were characterized optically and morphologically using fluorescence, microscopy, and particle sizing. Ultrasound irradiation using a variety of pulse lengths was conducted in a channel phantom to optimize conversion criteria, and the irradiated products were characterized via acoustic detection and particle sizing. Insight into the mechanism of the conversion was gained via FRET using microbubbles functionalized with fluorescent lipid probes.

IMPACT & INNOVATIONS – The microbubble-to-nanostructure acoustic conversion represents a novel platform for the stimulated delivery of nanoparticles to tumors that operates beyond the EPR effect. Through a deeper understanding of the material properties and acoustic conditions which influence this phenomenon, its use as a generalized strategy for EPR-independent delivery of nanoparticles can be utilized in a more predictive and consistent manner. In addressing this obstacle to clinical translation, this approach will allow for improved nanomedicine delivery to a more diverse clinical population, broadening its impacts for cancer therapy and diagnostics.

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14. Inhibiting PWWP Reader Domains

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Protein methyltransferases are an emerging target class in oncology. The modification of chromatin by histone methyltransferases (HMTs) is critical for both the maintenance and plasticity of gene expression. In cancer, aberrant methylation patterns enforce oncogenic transcription programs. In addition to their methyltransferase activity, many HMTs contain ancillary 'reader' domains that recognize distinct modifications to integrate epigenetic signaling events. Inhibition of reader activity may provide additional opportunities for therapeutic intervention, in particular, where the identification of active site inhibitors has failed. The nuclear SET domain-containing proteins (NSD), NSD2 and NSD3, both read and write H3K36 dimethylation via PWWP and SET domains, respectively. NSDs have been shown to drive oncogenic gene expression in both hematological malignancies and solid tumors. Despite many valiant efforts, selective and potent inhibitors of their methyltransferase activities have not been achieved. Here we describe the cellular characterization of two first-in-class NSD2/3 PWWP1 chemical probes (inhibitors), discovered by scientists at SGC and Boehringer Ingelheim, respectively. These molecules are highly-characterized, potent, and selective tool compounds that will enable novel biological discovery and target validation. We demonstrate activity at 1 μM in live cell assays and demonstrate superb selectivity for their respective targets. We further evaluate the therapeutic potential of NSD PWWP1 antagonism in disease-relevant cell models. The NSD2 and NSD3 PWWP chemical probes will provide the scientific community with valuable research tools to interrogate the role of PWWP reader domains in cancer biology and their potential as targets in the treatment of disease.

15. Investigating the Effects of Metformin and Radiation on Pancreatic Tumour Growth and Spread *In Vivo*

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Note: The presenting author is a research trainee, and the project is supported by the TFRI New Investigator's Grant.

Background

Ionizing radiation is heavily dependent on the presence of intracellular oxygen to produce cytotoxic reactive oxygen species (ROS) and cause cell death. However, a high dose of radiation, such as the one delivered by stereotactic body radiation therapy (SBRT), can damage the tumour's vasculature as well. A lack of blood flow creates a low oxygen tumour microenvironment (hypoxia) and consequent upregulation of hypoxia inducible factors (HIFs), which can cause treatment resistance and promote tumour cell migration. Metformin, an FDA-approved treatment for diabetes mellitus patients, reduces cellular oxygen consumption by inhibiting oxidative phosphorylation and accordingly increases intracellular oxygen levels. Thus, the resulting oxygen increase can potentially enhance cancer cell SBRT response through mitigating radiation resistance associated with hypoxia. We hypothesize that metformin treatment could have radiosensitizing effects on pancreatic cancer. Therefore, our study aims to determine the effects of metformin and SBRT on tumour hypoxia, burden, and spread in a mouse model of orthotopic pancreatic cancer.

Materials & Methods

BxPC-3 human pancreatic cancer cells transfected with luciferase were injected into the pancreas of radiosensitive NRG mice (n=20). 4 weeks post-inoculation, mice were randomized into 4 treatment groups: control, metformin (5mg/ml in drinking water for 7 days), SBRT (daily 8Gy for 5 consecutive days) and a combination of the two treatments (metformin treatment was started 2 days prior to SBRT). Following treatment, mice underwent weekly bioluminescent imaging (BLI) and lateral saphenous vein blood collection. When endpoints were reached, mice were sacrificed and BLI was performed of the exposed thorax and abdomen, before and after primary tumour removal to analyze tumour burden and spread. The excised primary tumour was measured using a digital caliper and processed for *ex vivo* analysis.

Results

While SBRT treatment decreased the primary tumour volume, mice exhibited notable tumour spread, similar to untreated mice. Metformin-treated mice showed inhibition of tumour spread without changing primary tumour volume. By combing the two forms of treatment, both limitations were addressed, with the primary tumour decreasing in volume and complimenting the decreasing tumour spread.

Conclusions

In conclusion, SBRT inhibited tumour growth, while metformin reduced tumour spread. The combination of the two treatments resulted in the smallest total tumour volume as well as the lowest tumour spread. Therefore, radiotherapy used in combination with metformin treatment has the potential to supress tumour growth rate of pancreatic cancer as well as tumour metastasis, therefore providing a promising therapeutic avenue for improving patient prognosis.

16. Molecular Profiling of Glioma Subtypes using MS Proteomics for Personalized Drug Selections

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Diffuse gliomas, as a group, represent the most common brain tumors in adults with grade IV glioblastomas (GBMs) carrying a uniformly dismal prognosis of 15 months median survival. Genomic and transcriptomic analyses of large GBM cohorts defined few clinically-actionable markers of patient outcomes. Here, we utilize mass spectrometry to define protein landscapes of genomically-annotated primary gliomas (n=20), patient-matched recurrent GBMs (n=10) and tumor-derived GBM cell lines (GSCs, n=10). Our objective was to use mass spectrometry (MS) to detect (phospho)proteome abundance changes that distinguish glioma samples, predict GBM recurrence and establish proteomically-matched GSCs for chemical screens. Our hypothesis is that glioma subtypes can be defined according to (phospho)proteomic profiles which can be used in conjunction with chemical screens in GSC models of tumor development. Our current dataset contains a total of ~6,300 proteins, among which 486 are differentially abundant across glioma subtypes with 297 proteins (FDR<0.1) defining GBM recurrence-related molecular pathways. GO term enrichment analysis indicates that epithelial to mesenchymal transition-related proteins define higher grade gliomas while extracellular matrix or nuclear proteins are enriched in primary and recurrent GBMs, respectively. Using our integrative approach, calcium signaling, proteins of the endoplasmic reticulum and extracellular integrin proteins are most conserved proteomic markers that distinguish IDH-wildtype from -mutated GBMs in primary tissues as well as in our GSC lines. Treatments for diffuse gliomas tumors are largely non-specific and overlap between genomic subtypes. We are now initiating a large-scale chemical screen to determine differential chemosensitivities of cell lines with unique proteomic profiles. Given the central position proteins occupy in driving biology and phenotype, deeper characterization of the substantial proteomic diversity that exists between molecular subtypes of gliomas may help define more personalized prognostic and predictive biomarkers for precision care.

17. Integrating transcriptional regulatory network with chromatin accessibility as marker of drug response in triple-negative breast cancer

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Breast cancer is the most common and second leading cause of cancer death in women worldwide. 15-20% of breast cancer patients are diagnosed with triple-negative breast cancer (TNBC) which is negative for receptors estrogen and progesterone, and for overexpression of *HER2* gene. Importantly, TNBC is the most heterogeneous and aggressive subtype of breast cancer, and no targeted therapy is available against it. To identify transcriptional regulators in TNBC, we analyzed 1,607 transcription factors (TFs) and their regulons (expression of targets of these TFs) based on transcriptional (RNA-seq) profiling of TNBC primary tumors (TCGA-BRCA[1]), in-house patient-derived xenografts (PDXs), and cell lines. We first assembled transcriptional interaction network of breast cancer tumors using the algorithm for the reconstruction of accurate cellular networks, then measured the regulatory TF activity in TNBC vs Non-TNBC tumors (n=1,099), in-house PDXs (n=54), and cell lines (n=83) using VIPER[2] algorithm. As a result, we identified TNBC-specific TF regulatory network with 126 differentially active TNBC-specific TFs and their activated and repressed targets. In addition, we integrated chromatin accessibility data (ATAC-seq) with these network-based signatures to detect active TFs whose binding site motifs enriched in chromatin accessible regions in TNBC samples. Furthermore, we examined the activities of these TFs as markers of response to 90, 250 and 544 compounds tested in different TNBC cell lines from GRAY, GDSC1000 and CTRPv2 datasets[3], respectively. We determined significantly correlated drug(drug target)-TF pairs, and are currently validating the findings in PDXs.

Together, our study develops an understanding of regulatory networks driving the unique identity across TNBC and identifying biomarkers for patient stratification.

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18. Metabolic reprogramming in high-grade sarcomas, repurposing anti-cholesterol agents as a novel therapeutic strategy

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Objective: Soft tissue sarcomas (STS) are a diverse group of mesenchymal tumors with over 50 histologically distinct subtypes. Undifferentiated pleomorphic sarcoma (UPS), leiomyosarcoma (LMS) and liposarcoma (LPS), are three common types and comprise >50% of adult sarcoma. Current treatment includes chemotherapy, radiation and/or surgery and survival rates remain largely unchanged in the last decade. Since the main patterns of failure are metastatic disease and multifocal local recurrence, development of more efficacious systemic therapy is essential to improve disease outcomes. To identify novel therapies for sarcoma that are effective and potentially less toxic, we screened patient derived cell lines with >3,300 compounds and identified that UPS cells were exquisitely sensitive to statins (simvastatin and pitavastatin). Statins inhibit the rate limiting enzyme in the mevalonate (MVA) pathway, HMGCR – critical for the production of cholesterol. Cancer cells can exploit this pathway through PI3K/mTOR signaling to enhance proliferation and survival. The use of statins as an anticancer agent has been tested in other solid tumors, however to date it has not been investigated in STS. Thus, goals of our study are **to define the mechanism(s) responsible for statin sensitivity** in UPS and determine if LMS and/or LPS are also statin sensitive.

Results: We observe that simvastatin reduces viability of patient-derived LMS cell lines, but not LPS. To determine if dysregulation of the PI3K/mTOR pathway renders sarcoma cells statin sensitive, we assayed the activity of downstream effectors of PI3K/mTOR. In UPS, we found a decrease in phosphorylation of AKT and 4EBP1 indicating that simvastatin disrupts PI3K/mTOR signaling. We are currently investigating if PI3K/mTOR signaling is similarly dysregulated in LMS cell lines to determine if this is a candidate mechanism of simvastatin sensitivity. To analyze if simvastatin could be successfully combined with doxorubicin, the current standard of care, as a future therapeutic strategy, we performed a BLISS analysis, which demonstrated that these two agents are synergistic in vitro. Finally, as our laboratory has a panel of UPS and LMS xenografts, we are currently investigating if statin inhibition is an effective therapy in vivo and mechanistically dissecting the basis for tumor growth inhibition. During the pharmacokinetic study we treated mice for 5 days via oral gavage (n=5) or intraperitoneal injections (IP) (n=5), and found that simvastatin is present in the tumors treated by IP injections but not gavage. Thus, in our pre-clinical murine studies, we are administered simvastatin by IP injections alone and in combination with doxorubicin to assess efficacy and toxicity. These results are currently being assessed.

Summary: Taken together, we report the promising findings that primary UPS and LMS cells are highly sensitive to simvastatin. This work suggests that two common high-grade sarcomas rely on metabolic reprogramming to support the increased energy demand and proliferation through the PI3K/AKT pathway. Simvastatin, a repurposed and well tolerated drug, is able to inhibit this process which may give rise to treating sarcomas with this novel therapy. Future studies will focus on elucidating connections between major metabolic pathways and preclinical drug studies of UPS, LMS and other sensitive sarcoma models.

19. Blocking the restorative feedback response by novel drugs potentiates the anti-cancer activity of approved cholesterol control agents (statins)

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Statins are FDA approved cholesterol lowering drugs that inhibit the mevalonate pathway. Our lab recently showed that statins have strong anti-cancer activity, however, they induce a feedback response which confers resistance by activating the SREBP transcriptional activators to restore expression of mevalonate pathway genes. We have recently identified dipyridamole (DP), an anti-platelet agent, as an inhibitor of SREBP-mediated feedback that potentiates the anti-cancer activity of statins. However, given its polypharmacology and anti-platelet activity, DP is not a specific SREBP inhibitor and may not be suitable for every cancer patient. To identify novel SREBP inhibitors, we have performed an unbiased image-based screen of the Selleck-Drug library (>1500 Drugs). We identified 34 novel drugs that potentiate the pro-apoptotic activity of statins more robustly than DP. We have shown that two drugs show a similar structure and inhibit the statin-induced feedback response in HCC-1143 breast cancer and LNCaP prostate cancer cell lines. We further validated the efficacy of these 2 compounds in 2 breast cell lines (HCC-1143 and SUM149PT) and 4 prostate cancer cell lines (LNCaP, 22rv1, DU-145 and PC-3) using an MTT assay. We have shown that these two drugs potentiate Fluvastatin anti-proliferative activities in all cell lines that have an intact feedback response, however they fail to potentiate Fluvastatin in a cell line that doesn't have an intact feedback response (PC-3). These findings show that these novel drugs potentiate statin proapoptotic activities at clinically relevant concentrations by blocking the statin-induced feedback response and suggest further *in vivo* investigation to develop new (Statin+Drug) combination therapies to impact patient outcome in a timely manner.

20. CLU-ing into intestinal regeneration and colorectal cancer: The role of revival stem cells

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Understanding the normal, or homeostatic, activity of a tissue is an essential aspect of defining that tissue in states of injury and disease. The intestinal epithelium, otherwise known as "the gut", faces continuous stress, requiring a remarkable rate of cellular turnover to maintain healthy tissue. This homeostatic turnover is a result of precise intestinal niche signaling that dictates the self-renewal and differentiation of Lgr5+ crypt base columnar (CBC) intestinal stem cells (ISCs). However, the mechanisms responsible for the post-injury regeneration of the gut have remained elusive, serving as a point of contention in the field of ISC biology. Recently, work from our lab (Ayyaz *et al.* 2019) identified the unique revival stem cell (revSC) through single cell RNA sequencing (scRNAseq). RevScs are marked by expression of a stress response gene, Clusterin (Clu), and represents a damage-induced cell type that restores the homeostatic Lgr5+ ISCs, ultimately reinstating a healthy, functioning gut. The discovery of this novel, damage-induced ISC signifies a huge advancement in our knowledge of non-homeostatic regenerative processes which have long since been linked to intestinal disease.

In particular, clinical observations have repeatedly shown association between colorectal cancer (CRC) and other regenerative and inflammatory responses in the gut, such as those tied to inflammatory bowel disease. CRC is a leading cause of cancer related death in Canada, with a majority of deaths attributed to tumor recurrence and metastasis. CRC arises through the step-wise accumulation of mutations in driver pathways, yet tissue regeneration and inflammation are highly implicated in cancer initiation. Mechanisms underlying this link have yet to be thoroughly established, but the Wrana lab has begun to bridge this gap through the discovery that Yap, a Hippo pathway co-transcriptional activator, mediates intestinal regeneration and plays a definitive role in the formation of tumorigenic legions. Additionally, the recently discovered revSC population has proven to be Yap-dependent, with complete Clu expression blocked upon Yap knock-out in murine models, further providing evidence for a link between tissue regeneration and tumor initiation.

This novel ISC redefines the framework of post-injury regeneration, yet questions remain regarding their regulation and contributions to disease. My initial studies show that Clu+ cells are abundant in an organoid model of intestinal adenoma formation driven by mutation in *Adenomatous Polyposis Coli* (Apc-/-). Additionally, high Clu is associated with poor prognosis in human CRC, further implicating revSCs in CRC progression and recurrence following therapeutic treatment. Here I will present findings from my studies focused on defining the signling networks that regulate revSC production, and their capacity to reconstitute the homeostatic Lgr5+ISC. These discoveries may provide insight into how regeneration may be tied to various stages of CRC progression. Therefore, understanding how revSCs are regulated and function in CRC will provide advanced understanding of human pathologies, shaping how we approach them therapeutically.

21. Investigating the Tumor-Immune Microenvironment in Li-Fraumeni Syndrome

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Objective: Li-Fraumeni syndrome (LFS) is a highly penetrant cancer predisposition disorder caused by germline mutations in the TP53 tumor suppressor gene. Affected individuals have a lifetime cancer risk of 93% to develop a wide spectrum of early-onset cancers and over 30% of LFS patients develop cancer before age 20. To date, no effective therapies to prevent or treat these tumors have been developed. Immunotherapy is a particularly attractive treatment option for LFS cancers because of the damaging long-term side effects of conventional treatments and the inherent risk of inducing secondary malignancies. The aim of this research is to investigate the mechanisms underlying tumor immunosurveillance in LFS.

Methods: We are using a mouse model of LFS that accurately mimick the human LFS genotype and cancer-prone phenotype. We have cultured tumor cell lines from spontaneous tumors developed in these 'LFS mice' that are used for tumor challenges in a syngeneic tumor model. We are characterizing the tumor-infiltrating immune cell populations using flow cytometry in LFS mice versus healthy littermates carrying wild-type p53.

Results: In our analysis of tumors developed in LFS mice versus healthy littermates, we found that LFS mice have a strikingly lower total number of tumor-infiltrating immune cells. Immunophenotyping of these populations reveals lower numbers of immune cell types of both the lymphoid and myeloid compartments in the tumors of LFS mice.

Conclusion: These results suggest that the LFS immune system, harboring non-functional mutant p53 proteins, has an impaired response to tumor formation. Further characterization of the mechanisms underlying this immune deficiency are underway, with the exciting potential to uncover novel treatment strategies through immunological means.

22. Targeting SMG1 to induce proteotoxicity in high-grade serous ovarian cancer

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Background: High-grade serous ovarian cancer (HGSOC) is characterized by widespread genomic aberrations and *TP53* mutations. SMG1 and its substrate UPF1, mediate nonsense-mediated mRNA decay (NMD) to degrade pre-termination codon containing mRNA to mitigate against proteotoxic stress associated with misfolded and truncated proteins. Due to the high levels of recurrence after chemotherapy and the ability to evade the immune system, there is an urgent unmet need to find effective therapies that induce immune detection through the presentation of cancer-specific antigens.

Methods and Results: Here, we perform a genome-wide shRNA screen and identify SMG1 as an essential kinase in 14 of 28 HGSOC cell lines. HGSOC cells expressing shSMG1 were injected into immunodeficient mice and reduced tumour growth suggesting SMG1 is essential for tumor progression *in vivo*. Knockdown of SMG1 or UPF1 inhibited cell proliferation highlighting the dependency on NMD in a subset of HGSOC cell lines. Inhibition of SMG1 using the small molecule inhibitor 11j demonstrated a variable range of sensitivity amongst a panel of HGSOC cell lines whereas the immortalized fallopian tube secretory cells were insensitive to SMG1 inhibition. 11j treatment induced apoptosis, autophagy and an unfolded protein response through the activation of ATF6 and IRE1a in HGSOC cells. We identified physiological and cancer-specific NMD target transcripts with increased abundance and translation during 11j treatment of HGSOC cells. Using proteogenomics, we identified 133 MHC class I-associated peptides (MAP) from coding and noncoding transcripts in HGSOC cells during SMG1 inhibition. 11 MAPs are derived from transcripts undetected in normal tissues suggesting these peptides are candidate tumour-associated antigens.

Conclusion: SMG1 is an acquired vulnerability in HGSOC where inhibition induces an unfolded protein response, apoptosis, and presents a considerable set of MHC class I-associated peptides for tumour immune detection.

23. Gastrointestinal transcription factors drive lineage-specific developmental programs in organ specification and cancer

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Transcription factors (TFs) are spatially and temporally regulated during gut organ specification. Although accumulating evidence shows aberrant reactivation of developmental programs in cancer, little is known about how TFs drive lineage specification in development and cancer. We first defined gastrointestinal tissue-specific chromatin accessibility and gene expression during development, identifying the dynamic epigenetic regulation of SOX family of TFs. We revealed that *Sox2* is not only essential for gastric specification, by maintaining chromatin accessibility at forestomach lineage loci, but also sufficient to promote gastric/squamous transformation upon *Cdx2* deletion. We also exhibited, another SOX TF, *Sox9*, maintains gastric specification in the absence of *Sox2*, suggesting functional redundancy.

By comparing our gastrointestinal lineage-specific transcriptome to human gastrointestinal cancer data, we found that stomach and intestinal lineage-specific programs are reactivated in $Sox2^{high}/Sox9^{high}$ and $Cdx2^{high}$ cancers, respectively. By analyzing mice deleted for both *Sox2* and *Sox9*, we revealed their potentially redundant roles in both gastric development and cancer, highlighting the significance of developmental lineage programs reactivated by gastrointestinal TFs in cancer.

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24. Investigating FGL2 as a Therapeutic Target in Ovarian Cancer

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Background: The tumour microenvironment (TME) in ovarian cancer is infiltrated by populations of highly suppressive immune cells, such as regulatory T cells (Tregs), which allows the tumour to escape the immune system. Fibrinogen-like protein 2 (FGL2) is a regulatory T cell (Treg) effector protein that promotes immunosuppressive activity. High levels of FGL2 are associated with poor survival in several malignancies. In ascites fluid from ovarian cancer patients, FGL2 levels by ELISA ranged from 14-222 ng/ml, in patients with all subtypes of ovarian cancer, indicating a widespread potential for targeting FGL2. Analyzing the TCGA database of 374 ovarian cancers, FGL2 expression correlated with expression of markers for immunosuppressive immune cells (FOXP3, PD1, CTLA-4).

Objective: Given that increased numbers of Tregs in ovarian cancers predict poor patient survival, we hypothesized that blocking FGL2 activity would alleviate the immunosuppressive environment and prolong survival.

Methods: Two syngeneic mouse models of ovarian cancer were studied; ID8 (C57BL/6) and STOSE (FVB/n) cells derived from spontaneously transformed ovarian surface epithelium. Mice 7-9 weeks of age were injected intraperitoneally and assessed for survival until humane endpoint.

Results: In the syngeneic ID8 model of ovarian cancer, the expression vs. absence of FGL2, either in the tumour microenvironment (*Fgl2*$^{-/-}$mice) or in the ID8 cancer cells, did not affect survival. However, mice bearing syngeneic ovarian STOSE tumours treated for seven days with an anti-FGL2 blocking antibody (150µg/day) survived longer (median: 69 days) than mice treated with IgG control antibody (60.5 days; P= 0.0280 Log-Rank test). Decreased frequencies of Tregs and decreased frequencies of antigen presenting cells (CD11b+) were observed by flow cytometric analysis in spleens of mice treated with anti-FGL2.

Conclusions: Further investigations showed that the ID8 model is poorly immunogenic and does not respond to immunotherapy (oncolytic viruses), while STOSE tumours do. Therefore, targeting FGL2 has therapeutic potential in more immunogenic models of ovarian cancer and prolongs survival by modulating several immune cell populations.

25. Paradoxical protective effects of hepatic BRCA-1 deletion in hepatocellular carcinoma

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Hepatocellular carcinoma (HCC) is one of the most lethal cancers with only 19% 5-year survival post-diagnosis and its mortality rate continues to rise with limited treatment options. An emerging leading cause of HCC is non-alcoholic fatty liver disease (NAFLD) which is predicted to continue increasing due to the obesity and diabetes epidemic. Breast cancer susceptibility gene 1 (BRCA1) was initially discovered as mutations linked to breast and ovarian cancers. This tumour suppressor has fundamental cellular functions including maintaining genomic stability through DNA damage repair, cell cycle arrest and apoptosis. Recently, BRCA1 has also been implicated in metabolism. Yet, its role in liver metabolism and cancer is unknown and we therefore sought to investigate the role of BRCA1 in NAFLD and HCC.

Liver-specific BRCA1-KO (L-BRCA1-KO) and wildtype (WT) littermate control mice were generated using the Cre-LoxP system under the control of albumin promoter. These mice were fed a high fat diet (HFD) over 16 weeks from 6 weeks of age to induce fatty liver disease. L-BRCA1-KO were more glucose tolerant than WT mice and exhibited less fatty liver as assessed by Hematoxylin and Eosin and Oil-Red-O staining of liver sections.

To investigate whether their protection against fatty liver also extended to HCC, another cohort of mice were injected with a carcinogen, Diethylnitrosamine (DEN; 25mg/kg) at 14 days of age and sacrificed after 10 months. Liver tumours were counted and measured to quantify HCC parameters which revealed that while 100% of the WT litter control mice (16/16) developed HCC, only 66.67% of L-BRCA1-KO mice (10/15) showed tumours. Moreover, L-BRCA1-KO mice had less than 5% of tumours larger than 1 mm compared to nearly 15% in WT mice. qPCR and western blots performed on liver tissues showed that in non-tumour hepatocytes from L-BRCA1-KO mice had increased cyclin E mRNA expression and yH2AX foci compared to WT mice.

To examine for acute and subacute effects of DEN, cohorts was examined at 48 hrs and at 8-10 weeks post-injection. qPCR was performed on liver tissues to assess for markers of inflammation, apoptosis, and cell proliferation, which showed L-BRCA1-KO livers to express higher p21, Cyclin D1 and Cyclin E mRNA expression and yH2AX foci formation. TUNEL assay was performed revealing more apoptosis in L-BRCA1-KO livers compared to WT.

To assess for cellular mechanisms, we assessed for polyploidy which is associated with tumour protection. We found that L-BRCA1-KO livers had increased polyploidy. Since p21 is associated with promoting polyploidy, we measured p21 in HepaRG and HepG2 hepatocyte cell lines which also showed increased p21 after BRCA1 knockdown, along with increased polyploidy.

Overall, our data show that BRCA1 deficiency protects against hepatic steatosis and HCC. BRCA-1 deficient hepatocytes exhibit more DNA damage but also increased apoptosis, which, along with increased polyploidy, may contribute to a paradoxical protection against HCC.

26. Role Of Tumor-Specific CD4 T Cells In Cancer

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CD4 T cells are critical for orchestrating robust primary immune responses by promoting CD8 T cell cytolytic function and B cell secretion of high-affinity antibodies. Interestingly, mounting evidence suggest that CD4 T cells are also important in controlling cancer growth and their abundance is often associated with better outcomes to immunotherapies. Yet, the mechanisms of CD4 T cell help and the type of help required remain unclear. In this project, we are interested in investigating the mechanisms of CD4 T cell help in different murine tumor models. We show, using multiple murine tumor models (PyMT [breast cancer] and MC38 [colon adenocarcinoma]) that mice lacking CD4 T cells (CD4KO mice) have accelerated tumor growth compared to WT mice. Using time-of-flight mass cytometry (CyTOF), we demonstrate a significant change in the phenotype of tumor-infiltrating CD8 T cells between CD4KO and WT mice. In contrast, minimum changes are found on other immune cells (e.g., DCs and macrophages). To further profile the tumor-immune landscape, we have developed two new murine tumor models, MC38GP and PyMG15, which express a truncated glycoprotein (GP) of lymphocytic choriomeningitis virus (LCMV). The expression of LCMV-GP mimics the emergence of neoantigens in cancer, and enables investigation of tumor-specific T cells via tetramers and TCR transgenic CD4 and CD8 T cells (SMARTA cells and P14s). We are currently elucidating the impact of different T helper cells on tumor microenvironment with a specific focus on antitumor CD8 T cell response.

27. Identifying Novel Regulators of MYC Stability

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As a regulator of gene transcription, c-MYC (MYC) modulates cell proliferation, growth, and metabolism. Experiments in animal models of cancer provide proof-of-concept that inhibiting MYC drives the eradication of oncogenic tissue while sparing normal tissue. Unfortunately, traditional approaches to develop an effective small molecule inhibitor (SMI) that binds and targets MYC have been unsuccessful. To fill this gap, we have used a novel tool to identify SMIs that regulate MYC at the level of protein stability. In non-transformed cells, MYC is highly regulated, with a half-life of 30 min. Only one pathway (GSK-3/SCFF^{bxw7}) has been well characterized as a regulator of MYC degradation, however loss of this pathway only extends MYC half-life to 60 min. We hypothesize that additional pathways regulating MYC stability exist, and can be exploited to develop novel therapeutics against cancer.

The standard techniques for measuring MYC turnover are cumbersome and labour-intensive, which has limited research in this field. To overcome this barrier, we fused MYC and the Venus fluorescent protein (MYC-Venus), allowing us to measure MYC levels in non-transformed MCF10A cells. We applied this stability beacon to screen a panel of 320 kinase inhibitors across a panel of cell lines. Following three replicate screens at 0.1 and 1 μM of library compounds over 16 hours of treatment, we identified a panel of 22 inhibitors that increased the percentage of MYC-Venus fluorescent cells. We also used unsupervised data analysis to identify compounds with similar phenotypes to our top priority compounds. All identified compounds were then validated using 8-point dose response curves to determine the maximum effective dose that yielded minimum cell death. Compounds were then assayed at the selected dose to determine whether they regulated endogenous or ectopic MYC RNA. Finally, six compounds were assayed for regulation of ectopic MYC-Venus or endogenous MYC half-life, and two of those compounds increased the stability of both ectopic and endogenous MYC.

To elucidate the functional targets of inhibitors that increase MYC half-life, we will down-regulate each of the reported target kinases associated with those inhibitors using RNAi or CRISPR/Cas9. We will then prioritize kinases that modulate MYC stability independently of the classical pathway. To identify signaling pathways related to the identified kinases, we will conduct a network analysis using STRING and BIO-GRID, followed by elucidation of those components of the signaling pathways that, when targeted, will increase MYC turnover. It may also be possible to identify a MYC interactor within the pathway that could be directly targeted to modulate MYC stability. Identifying multiple kinases, and genes, that regulate c-MYC stability will reveal potential pathways regulating c- MYC turnover. Ultimately, developing an arsenal of therapeutic targets against MYC would enable the use of targeted cancer treatments based on pathway activity in a wide range of cancer types to help impact patient care.

28. Small molecule inhibition of ezrin increases T cell activation in tumour-draining lymph nodes

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Ezrin, part of the ERM (ezrin-radixin-moesin) family of membrane adaptor proteins, regulates many cellular processes including cytoskeleton remodelling and signal transduction. In cancer cells, over-expression of ezrin promotes an invasive and metastatic phenotype. Interestingly, ezrin is also expressed in lymphocytes, acting as an A-kinase anchoring protein (AKAP) in effector T cells to localize PKA to the immunological synapse, resulting in suppression of T cell activation through the PGE2/cAMP/PKA/Csk pathway. While many studies have looked at the tumour cell-specific function of ezrin, little is known about the role of ezrin in repressing T cell activation in cancer. Our group has developed an intravital model to monitor T cell trafficking in the tumour-draining lymph nodes (TDLNs). We demonstrate that systemic treatment with a small molecule ezrin inhibitor (NSC668394) leads to enhanced T-cell engagement with EO771 metastatic breast cancer cells in the TDLN. Flow cytometric analysis of the TDLN revealed an increase in the proportion of active ($CD8^+/CD69^+/CD62L^-$) cytotoxic T cells in mice treated with the ezrin inhibitor, compared to TDLNs of control tumour-bearing mice. We also demonstrate an increase the number of apoptotic cancer cells by immunohistochemistry within the TDLN of ezrin inhibitor-treated mice, consistent with our intravital and flow cytometry data, and that TDLN and lung metastatic burden in these mice are also markedly reduced. Our findings suggest that targeting ezrin may enhance T cell activation, leading to anti-tumour responses against metastatic breast cancer cells. (Supported by CCS, CIHR and OMPRN)

29. Validating the WD Repeat Protein CDC40 as a Potential Therapeutic Target for Lung Cancer

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Lung cancer is the most commonly diagnosed cancer in Canada and is the leading killer of Canadian cancer patients, with an overall 5-year survival rate as low as 17%. There is a pressing need to uncover novel targets for the development of improved therapeutics. Data mining across multiple large genetic screens suggests that lung cancer cell survival is strongly dependent on the presence of Cell Division Cycle 40 (CDC40). CDC40 is a WD repeat protein (WDR) family member involved in pre-mRNA splicing, serving as a scaffolding protein within spliceosome C complex. Splicing is a fundamental cellular process for gene expression and protein production, and could support carcinogenesis if hijacked. Therapeutic modulation of cancer-sustaining splicing patterns may thus be an effective strategy for lung cancer treatment, although the precise role of CDC40 in splicing regulation and the consequences of its inhibition remains unknown. We and other groups have demonstrated that several WDR protein-protein interactions are druggable by small molecule inhibitors, making CDC40 an interesting target for therapeutic evaluation.

Hypothesis: Inhibition of the WD40 repeat protein CDC40 will disrupt pre-mRNA splicing and cell cycle control mechanisms in lung cancer cells leading to cell cycle arrest and apoptosis.

The first objective was to validate CDC40 as an essential factor for lung cancer cell growth and survival. Using doxycycline (DOX)-inducible shRNAs, we verified CDC40 dependency in 3 lung cancer cell lines. Cell growth suppression, apoptosis induction and cell cycle arrest were the 3 main phenotypes observed in cell lines with CDC40 knockdown. Kinetically, we observed CDC40 mRNA and protein knockdown as early as 8 hours and 24 hours respectively after shCDC40 induction, followed by changes in cell cycle distribution after 48 hours. This led to Caspase 3/7 activation and an anti-proliferative phenotypes after 72 hours. As CDC40 is postulated to provide a crucial structural role within the spliceosome C complex, the second objective was to investigate whether a protein-protein interaction (PPI) inhibitor could be developed to disrupt CDC40 protein interactions. Preliminary in silico analysis of CDC40 interactions points to the top central pocket of the WD propeller domain as the most therapeutically tractable site, which interacts with another spliceosome complex C member CDC5. Further work in the development of pharmacologic (small molecule PPI inhibitors) and genetic (complex-disrupting mutations) interventions targeting the CDC40-CDC5 interaction will improve our understanding of the essential binding residues and their functional roles in lung cancer cells. In conclusion, our results indicate that CDC40 is important for lung cancer cell growth and survival, and points to the need for further investigation into how CDC40 participates in splicing activity (i.e. constitutive/alternative splicing) as well as cell cycle progression.

Note: Die Hu is a trainee (Ph.D.)

30. The potential role of cyclooxygenase-2 as a folding factor of vascular endothelial growth factor

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Supported by the Terry Fox Research Institute

Hypoxia promotes an aggressive and lethal tumour phenotype through changes in cellular biology largely mediated by cell-surface and secreted proteins. The majority of these proteins require disulfide bonds to ensure their stability in the extracellular space. However, proteins possess different abilities to form disulfide bonds and be secreted in anoxia, suggesting that there is a selective requirement for oxygen in the protein folding process. It remains unclear then how certain proteins have the ability to fold successfully when oxygen is scarce. Vascular endothelial growth factor (VEGF) is among the proteins that fold and function under hypoxic conditions. To investigate the folding mechanism of VEGF, Ascorbate Peroxidase 2 (APEX-2) proximity labelling assay was used to map the interactome of VEGF. Cyclooxygenase-2 (COX2) was identified as a significant potential interactor in the screen. Here, we propose that the ability of VEGF to fold and be secreted is coupled to specific protein-protein interaction during maturation. Canonically, COX2 is a cellular oxidoreductase enzyme involved in the production of prostaglandins, however, there has been literature to suggest a non-canonical role of COX2 through the reduction potential of its peroxidase active site. We set out to determine if COX2 is necessary for the folding and secretion of VEGF in both the presence and absence of oxygen. To this end, we created a HeLa COX2 knockout cell line using CRISPR/Cas9 for a radioactive pulse chase assay. Newly synthesized proteins were labeled with a short pulse of sulphur-35 labeled cysteine and methionine. VEGF was immunoprecipitated and were quantified from media and cell lysates after varying chase lengths. Preliminary data suggest that a fraction of VEGF is unable to dimerize by disulfide bonds without COX2 and therefore remains in monomeric form. This research aims to discover a novel protein folding pathway that will give insight into protein assembly in the ER and may be of particular importance in hypoxia that occurs during embryonic development and pathological conditions such as wound healing, stroke and cancer.

31. Comprehensive Genomic Profiling (CGP) of Metastatic Castrate Sensitive Prostate Cancer (mCSPC) Reveals Potential Biomarkers and Therapeutic Targets

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Background: Docetaxel and androgen receptor (AR) targeted agents have become standard options for mCSPC. CGP data is limited in mCSPC, however has the potential to guide treatment selection. We aimed to establish the feasibility of using CGP to characterize genomic alterations (GAs) in mCSPC.

Methods: Patients with mCSPC were prospectively recruited at the Princess Margaret Cancer Centre to the OCTANE trial (NCT02906943), which aims to establish a genomically-characterized and clinically-annotated patient base that can be enrolled into specific research initiatives. Archival tumour specimens (after clinical testing completed) were profiled using next-generation sequencing (NGS) with a custom hybridization capture DNA-based panel (555 genes) or a targeted DNA/RNA amplicon panel (Oncomine Comprehensive Assay v3, 161 driver gene panel). GAs were classified according to the 2017 AMP/ASCO/CAP consensus recommendation. Clinical data were extracted from electronic health records.

Results: Since 2016, 17/31 (55%) mCSPC patients who enrolled had sufficient tissue (15 from primary, 1 lymph node, 1 bone) for CGP, and were analyzed for feasibility. All had adenocarcinoma histology, most had high volume (76%) or *de novo* (71%) mCSPC. Median presenting PSA was 56 µg/L (range 8.4 – 1394). Patients received docetaxel (88%) or abiraterone (12%). Tier I/II GAs consistent with possible benefit from approved or investigational targeted therapies were identified in 14/17 patients (82%). AR pathway GAs (in 29%) were present regardless of prior androgen deprivation therapy or disease volume. DNA damage repair GAs (in 65%) included *BRCA2, ATM, PALB2, MLH1/3, CDK12, FANCA, ERCC4, and SPOP*. Other GAs involved the cell cycle, mTOR, *EGFR*, wnt, hedgehog, notch, and epigenetic pathways.

Conclusions: In this small cohort, CGP was feasible in the setting of mCSPC for half of the patients. Potentially actionable GAs was identified in over 80% of patients tested. Pending confirmation from larger cohorts, these data support CGP in mCSPC prior to systemic therapy initiation, and highlight its potential role in future biomarker development and trial design in mCSPC.

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32. Intravital Imaging of Acute Myeloid Leukemia within the Living Bone Marrow as a Preclinical Model for Evaluating Novel Therapies

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Introduction: Acute myeloid leukemia (AML) is a disease of the bone marrow (BM) microenvironment, in which AML-BM interactions play important protective and promotive functions for cancer development. In addition to important cell-cell interactions, leukemic cells are also bathed in various cytokines and extracellular vesicles while existing in one of the lowest oxygen regions of the body. Current experimental systems are not able to recapitulate these environmental conditions ex vivo, while *in vivo* methodologies lack the specificity to study diseases at the cellular level. For example, interleukin 12 (IL-12) is a key primer of the initial specific T-cell immune response for resisting cancer progression, but given the irreproducibility of a host's immune system *in vitro*, the potential of IL-12 therapy has been largely under-examined. Recently, our lab has developed a novel femur window chamber (FWC) intravital imaging mouse model capable of long-term spatiotemporal assessment of leukemias within the *in vivo* femoral BM at cellular resolution. This model allows for direct and quantitative observation of a therapeutic maneuver working *in situ* in real time, providing a rapid and cost-saving means for preliminary drug efficacy testing and dosing/scheduling for therapy development. In an ongoing IL-12 Princess Margaret Cancer Centre clinical trial (NCT02483312), patients are injected with their own blast cells engineered to express IL-12. To better understand the pharmacodynamics of this treatment and help inform the clinical trial, our parallel preclinical study will be determining i) how long post-AML engraftment IL-12 therapy continue to be effective, ii) optimal effect dosages, and iii) whether repeated infusions yield added benefit. The capability of the FWC to quantify changes in fluorescent cells *in vivo* makes it the ideal model to answer such questions.

Methods: The murine lymphoblastic cell line, 70Z/3, was transfected with either a constitutively active GFP marker (70Z/3-GFP) or a lentivirus expressing mCherry IL-12 (70Z/3-IL12). The FWC was installed by shaving the femoral corticalis to reveal the BM cavity and securing a cover glass on top. Mice were then intravenously injected with 1x106 70Z/3-GFP cells 30 min post-surgery and imaged immediately afterwards via confocal fluorescent microscopy every 24 h until cancer cells were seen; imaging was reduced to every 3 days thereafter.

Results and Discussion: 70Z/3 cells were first detectable 10 days post-injection; a single group of 13 cells were identified close to the femoral neck. Within 24 h, the cancer cells multiplied exponentially to 180 cells within 11 distinct clusters scattered across the BM length. By day 14, an average of 212 cells were observed, achieving 80% BM infiltration as confirmed by flow cytometry. Our data demonstrates the capability of our FWC to quantitatively track the growth and spread of leukemia in situ. Having the timeline of untreated AML establishment and proliferation determined, current efforts are now underway to test IL-12 effect on cancer development: 70Z/3-IL12 cells will be introduced 0 (co-injection), 4, 8, 11, and 14 days following 70Z/3-GFP injection, and the resultant degree of disease control imaged, as represented by the quantity of fluorescent cells visible within the BM relative to untreated controls. Based on prior work, we expect to see total rejection of leukemia following co-injection, while later time points induce various degrees of delayed disease onset. Experiments will then be repeated using varying quantities and administration frequencies of 70Z/3-IL12 cells to test for optimal treatment dosing and the benefit of repeated infusions, respectively.

Conclusion: Integration of molecular imaging into therapy development can provide invaluable biological readouts regarding treatment action in vivo. Our FWC model provides an innovative means by which to quantitatively study AML cell response to new therapies within the living BM for preclinical trials.

33. Integration of the human papillomavirus into the host genome promotes tumourigenesis by modifying the local epigenome and transcriptome

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Introduction. Human papillomavirus (HPV) drives almost all cervical cancers and 70% of head and neck cancers. The HPV double-stranded DNA viral genome integrates into the host genome in > 80% of cases. Upon viral integration, canonical upregulation of viral oncogenes drives tumourigenesis. We characterized the chromatin structure at HPV integration sites and examined the downstream effects on gene expression.

Methods. We investigated data of 353 HPV-associated cancers from The Cancer Genome Atlas (TCGA) and characterized the epigenome and transcriptome of 5 cancer cell lines with known integrated HPV16. We also assessed cell lines by ATAC-seq and CTCF ChIP-seq to evaluate chromatin structure.

Results. We observed unexpectedly low transcription of E6 and E7 in a group of HPV^+ patients. We hypothesized that changes in the epigenome and transcriptome contribute to tumourigenesis of HPV alongside its oncogenes. We found evidence for a change in local chromatin structure and gene expression in the vicinity of viral integration events. Gene expression changed in patients with low expression of E6 and E7 as well as patients with high expression of these viral oncogenes. We found that a conserved CTCF sequence motif within the HPV genome binds CTCF in cell lines without episomal HPV. In concert with these local changes in CTCF binding, we found consistent increase in chromatin accessibility of cell lines within a 100 kbp window of HPV integration sites. Changes in the chromatin landscape occurred concomitant with outlier expression and alternative splicing of genes at the vicinity of HPV integration sites. Some of these genes such as *FOXA1, SOX2, E2F1,* and *PBX1* among others have well-known roles in tumour development and progression.

Conclusion. Our results suggest that CTCF binding to the host-integrated HPV genome reorganizes chromatin and modifies the transcriptome in HPV-associated cancers. We consistently observed altered epigenome and transcriptome among cell lines and patients with matched RNA-seq and ATAC-seq data. These findings indicate that HPV integration directly contributes to tumour development.

Presenting author is a PhD Candidate at the Department of Medical Biophysics.

34. Elucidating the potential therapeutic opportunities of blocking DNA Repair Mechanisms during Oncolytic Virus Vaccination followed by Adoptive T-cell therapy

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**Presenting author is a MSc Student.*

Abstract:
One of immunotherapeutic approaches developed in the Wan lab is to combine adoptively transferred antigen-specific T-cells (termed ACT) with Oncolytic Virus Vaccines (OVV), which can completely regress well-established solid tumours. But in all models tested so far, we have also observed subsequent relapse of tumours that have genetically lost the antigen-encoding gene, as measured by gene-specific sequencing of genomic DNA. We hypothesize that this loss is due to the activity of DNA repair mechanisms.

The current mechanism of small molecules against proteins in the DNA damage and repair pathway are used to drive further instability in cancer cells. It is very effective in BRCA1/2 models due to synthetic lethality. The biggest challenge of this approach in other types of cancer is the sheer complexity and redundancy of the DNA Repair system. DNA repair inhibitors are traditionally combined with several rounds of chemotherapy and radiation. More recently, there has been an interest in combining them with checkpoint inhibitor blockade therapies in the context of several cancers.

We believe the repair inhibitors prevent the loss of neoantigens and thereby, allow unleashed or expanded T-cells to recognize their target for an extended period. By blocking the tumour cells ability to repair DNA, the loss of the target gene can be prevented. Our hypothesis is that DNA Repair inhibitors can synergistically couple with ACT/OVV therapy to achieve a two-fold effect, resulting in the potentiation of the ACT + OVV protocol. This approach can also augment the cytotoxic effects of endogenous T-cells by increasing the mutational load of the tumour.

This work is supported by the Terry Fox Research Institute.

35. Resistance to PD-L1 blockade in preclinical models of triple negative breast cancer

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Immunotherapy involving PD-1 or PD-L1 inhibition has seen remarkable successes in subsets of patients with certain cancers. In triple negative breast cancer (TNBC) atezolizumab (PD-L1 antibody) in combination with nab-paclitaxel has been FDA approved for the treatment of patients with PD-L1 positive advanced disease. Despite this, only minor subsets of patients respond favourably to this therapy and others can eventually become resistant. Here we investigate intrinsic and acquired resistance to PD-L1 blockade in preclinical models of TNBC using a preclinical version of atezolizumab. We have developed variant models of orthotopic EMT6 murine breast cancer that are resistant to PD-L1 blockade in comparison to the responsive parental line. These variant models express cell surface MHC class I and PD-L1 ruling out the loss of these molecules as the primary resistance mechanism. We used RNA-seq to investigate gene expression changes and whether such changes could be targetable. Using our preclinical data we plan to determine whether any changes found are predictive of TNBC patients that do not respond to PD-1/PD-L1 inhibition using recent clinical trial data. Furthermore, we investigate whether combinations of PD-L1 blockade with other therapeutic modalities such as metronomic chemotherapy can sensitise murine TNBC models to immunotherapy that are otherwise intrinsically resistant to PD-L1 monotherapy. These studies will likely shed light on resistance mechanisms of immunotherapy in TNBC, as well as inform new combination strategies that could be considered for evaluation in clinical trials.

36. Fragment-based drug discovery for the oncogenic protein KRAS

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RAS proteins are molecular on/off switches that control crucial cellular processes like proliferation and differentiation. The oncogenic role of RAS was discovered in 1982 in cancer cell lines. Due to the high frequency of mutations (30% of human cancers contain an RAS gain-of-function mutation) considerable effort has focused on development of an effective RAS inhibitor, with little success. RAS has proven to be an extremely challenging target due to the lack of deep binding pockets. Here, we performed a virtual screen of 10,000 'fragments', i.e. small molecules about half the size of typical oral drugs, for their potential to bind KRAS, the most frequently mutated RAS isoform. Promising hits from this in-silico screen were validated in-vitro using nuclear magnetic resonance (NMR) spectroscopy and surface plasmon resonance (SPR) to map their binding sites and determine binding affinities. The best-performing fragments were selected for follow-up and closely related analogues were analyzed. Leads with a ~4-9x improvement over the original fragment will be used in further rounds of optimization.

37. Evaluating the potential of tumour infiltrating lymphocytes for the treatment of adult sarcoma

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For sarcoma, due to their heterogeneity, conventional chemotherapy and molecular targeted therapies often have varying effects across individuals and subtypes. These methods often only provide short-term disease control and so, more effective treatments are urgently needed. Recent advances in immunotherapy strategies such as checkpoint inhibition or adoptive T-cell therapy (ACT) show potential towards providing a more personalized treatment for sarcoma patients, which could increase treatment efficacy. We hypothesize that some sarcoma subtypes contain tumor infiltrating lymphocytes (TILs) in the tumor microenvironment and that these TILs can be isolated and applied towards ACT. Our aims are to investigate the availability and viability of TILs from sarcoma tissue fragments, characterize the TILs, and assess the cytotoxicity of the TILs through functional assays.

We collected 190+ sarcoma tumor specimens from patients undergoing open biopsy or surgical resection without preoperative adjuvant treatment in order to isolate TILs. We compared different methods of TIL expansion and optimized a protocol specifically for efficacy in culturing TILs from sarcoma. The expanded TIL populations were characterized by flow cytometry analysis using CD3, CD4, CD8, CD14, CD19 and CD56 markers. The TIL populations were non-specifically stimulated to establish TIL reactivity and TILs were co-cultured with autologous and allogenic tumour cells to establish TIL specificity.

Through 87 cases, we found that the tumor fragment method was much easier to execute and yielded a more homogenous cell population. Additionally, there were relatively lower TIL yield from sarcoma compared to previous studies done on epithelial-based cancers, such as melanoma and ovarian cancer. Since we observed intratumoural differences in TIL growth, the modified protocol pools populations with similar growth densities to provide the higher cell counts needed for further experiments and also to reach a clinically significant amount for ACT. The flow cytometry results of 38 characterized cases show TIL cultures exhibiting varying ratios of $CD4^+$ to $CD8^+$ T-cells, both intratumoural and intertumoural. There also seems to be a population of CD56+ cells that hinders the growth of $CD3^+$ T-cells. Upon non-specific stimulation of 25 cases, through IFNy ELISA, TIL populations show reactivity, indicating a viable and functional population. Our preliminary results from tumour-TIL co-cultures show specific reactivity to autologous tumour cells through increase levels of IFNy and granzyme B.

With our modified protocol, 87 sarcoma cases have been cultured *in vitro* and TILs were successfully collected from over 40% of those cases. TIL populations from 38 cases have been characterized with flow cytometry and demonstrated variable ratios of $CD4^+$ and $CD8^+$ TILs with minimal association to the PDL1 expression levels of tumour cells. TILs from 25 cases have also shown reactivity and autologous tumour specificity. These initial studies will enable us to move forward with evaluating the potential of TIL-based ACT for patients with sarcoma.

38. Image Search for Digital Pathology

Digital pathology is referred to the study of the digitized image of biopsy tissues from a patient's organ, called whole slide images (WSIs). As the pathology is entangled with disease diagnosis such as cancer detection, it is considered a critical part of any healthcare system. Regarding the huge size of WSIs and their textural complexity, conventional methods may rather have unsatisfactory performance. As a result, this field requires knowledge adaptation for boosting the algorithmic performance. At KIMIA lab (Laboratory for Knowledge Inference in Medical Image Analysis), we are presently working on image identification using a multitude of AI algorithms. More particularly, we are focused on "Image Search" in large archives of histopathology images. Our current work is presenting a summary of the recent studies conducted at the KIMIA Lab.

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39. The impact of tumour burden on oncolytic virus vaccine-induced anti-tumour immune responses

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The use of oncolytic viruses (OVs) to selectively destroy cancer cells is poised to make a major impact in the clinic and potentially revolutionize cancer therapy. Pre-clinical and clinical studies have shown that OV therapy is safe, well-tolerated and effective in a broad range of cancers. In addition to directly infecting and lysing cancer cells, OVs represent a form of cancer immunotherapy, inducing potent anti-tumour immune responses that contribute to the eradication of tumours. Virus-mediated cell lysis results in the release of tumour-associated antigens (TAAs) in the context of highly immunogenic signals, resulting in an *in situ* vaccine effect that is essential for successful viral immunotherapy. Our lab has capitalized on the unique immunostimulatory properties of OVs such as vesicular stomatitis virus by encoding TAA peptides to enhance immune responses against specific TAAs (termed oncolytic virus vaccines or OVVs). This approach has been used in a syngeneic murine MC38 tumour model, resulting in robust CD8+ T cell responses and complete tumour regression. Interestingly, if tumour burden is intensified by allowing tumours to grow larger prior to treatment, CD8+ T cell responses are dampened and OVV therapy is no longer curative. In this context, combining OVV therapy with the epigenetic modifier entinostat (MS-275) is able to rescue the therapeutic effect of the OVV. Efforts are currently being made to understand the influence of tumour stage on CD8+ T cell responses and explore mechanisms by which the apparent immunosuppressive impact of tumours on T cells can be overcome. This project is supported by the Terry Fox Research Institute.

40. Identification of Cell Surface Proteins in Triple Negative Breast Cancer for the Development of Novel Targeted Therapies

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Abstract:

Background: Triple negative breast cancer (TNBC) stands out due to its aggressive course and high metastatic rates. No consistent protein biomarker has been described for TNBC, resulting in a scarcity of adjuvant therapies for the afflicted patients. Hence, there is an urgent need to determine novel TNBC-associated proteins for the development of new therapeutics and the identification of novel molecular mechanisms driving this malignancy. Notably, cell-surface proteins represent attractive targets for novel therapies, due to their easily-accessible localization and their involvement in essential signaling pathways.

Goal: We aimed to uncover novel TNBC-associated cell surface proteins involved in carcinogenesis and metastasis for the development of novel targeted therapeutics.

Methods: Taking advantage of the fact that cell surface proteins are often N-glycosylated, we employed hydrazide chemistry to isolate N-glycopeptides. The glycoproteome of six immortalized TNBC cell lines and five 'healthy' controls (HC) (the immortalized cell line MCF10A and four patient-derived human mammary epithelial cell lines) was analyzed using LC-MS/MS and label-free quantification. By applying a data mining strategy, five candidates of interest were selected for functional investigation. To further restrict the list of candidates, we knocked-down (k.d.) the proteins of interest (using siRNA technology) and evaluated cell growth using growth-curve analysis (Incucyte technologies). Plexin B3 (PLXNB3) was selected for subsequent functional validations. The effects of PLXNB3 k.d. (using siRNA and CRISPR technology) on cancer cell growth, apoptosis, senescence and adhesion were interrogated using western blotting and standard cell biology protocols.

Results: The N-glycoproteomics approach led to the identification of 1024 glycoproteins, with over 60% described as plasma membrane/secreted proteins. GO analysis revealed that the TNBC-associated glycoproteome was enriched in biological processes such as: cell adhesion, epithelial to mesenchymal transition, neuronal development and neuron projection extension.

Candidates of interest were selected from our list by focusing on those plasma-membrane proteins that were enriched in TNBC cells compared to HC according to our label-free quantified proteomics data. We further restricted the list of candidates to those proteins whose k.d. impaired cancer cell growth, with little effect on the HC. PLXNB3, an understudied protein typically expressed in healthy neuronal tissues, was selected for in depth functional analysis. PLXNB3 k.d. (using siRNA and CRISPR technologies) impaired TNBC cell growth (in both adherent and spheroid cultures) and was associated with elevated levels of cleaved-caspase 3 and cleaved-caspase 7 compared to scrambled controls. Furthermore, PLXNB3 k.d. negatively impacted TNBC cell adhesion to extracellular matrixes compared to scrambled controls. Preliminary data indicate that PLXNB3 CRISPR-ed cells undergo senescence at higher rates compared to parental cells (ß-gal senescence test). Little is known about PLXNB3 function in healthy and cancer cells. Subsequent tests will evaluate PLXNB3's role in tumor growth and metastasis *in vivo*, using orthotopic tumor models in immunocompromised mice.

41. Examining regional variations in somatic mutation rates in chromatin architectural elements of cancer genomes

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Tumorigenesis is driven by the accumulation of somatic mutations that allow cells to overcome natural growth checkpoints and acquire the hallmarks of cancer. The rate at which cells accumulate these mutations shows variability within and across multiple scales. At the megabase windows, somatic mutation rates correlate with transcriptional activity, replication timing and chromatin architecture. For example, regions of open chromatin are readily accessible to DNA repair machinery and in turn, accumulate fewer mutations than regions of condensed chromatin. At the nucleotide scale, variations are dependent on trinucleotide contexts and diverse factors such as nucleotide excision repair deficiencies and carcinogen exposure. While variation in mutation rates between orders of resolution has been established, the patterns and effects of intra-regional mutational variation remain largely unexplored.

We recently developed RM4RM, a statistical framework for detecting these differential mutation rates and patterns of mutational signatures in regulatory elements. Using a pan-cancer dataset of 2,500 whole cancer genomes, we confirmed a significant mutational enrichment in the binding sites of CCCTC-binding factor (CTCF) in several cancer types, a key regulator of three dimensional DNA conformation and looping interactions. Our model revealed that CTCF binding site mutations were enriched in aging-associated signature 5 and a rare signature 28 of unknown origin. Interestingly, this regional mutation enrichment became more pronounced in a subset of DNA sites co-bound by CTCF and additional architectural proteins such as RAD21 and topoisomerases. This pattern upheld when comparing triple sites, those bound by CTCF, RAD21 and TOP2B, to all combinations of double and single sites despite the decrease in statistical power retained in finer groupings. These proteins aggregate at loop boundaries and may act to cooperatively direct the folding of chromatin. Consequently, the significant increase in mutation burden at these sites suggests a novel mutational process and a potential cause of loop disruption and deregulation of gene expression. To investigate this in detail we integrated matched transcriptomic and clinical data of liver cancers from the PCAWG project. Finally, we performed driver discovery and found a subset of significantly mutated individual sites that showed signs of positive somatic selection. Our systematic approach helps characterize novel mutational processes that shape the genome and identifies functional gene regulatory and architectural DNA sites that act as non-coding drivers of cancer.

42. Protein Secretion Rates of Vascular Endothelial Growth Factor and Carbonic Anhydrase 9 in Normoxia and Hypoxia

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Tumor hypoxia results in poor patient outcome due to treatment resistance as well as biological changes that stimulate angiogenesis, vasculogenesis, migration, invasion and immune suppression. These hypoxia-induced adverse biological changes are often mediated by membrane bound or secreted proteins through transcriptional and translational upregulation. Thus, understanding the regulation of how secreted proteins in hypoxia can therefore reveal novel therapeutic targets. Proteins that traverse through the secretory pathway form disulfide bonds in the endoplasmic reticulum (ER). Recent data from our lab have demonstrated that disulfide bond formation remains incomplete in ER cargo proteins like LDLR and Flu-HA in the absence of oxygen. To address whether hypoxia-induced proteins were likewise impaired, radioactive pulse chase assays were performed to measure disulfide bond formation and secretion capacity under both normoxic and hypoxic conditions. Here, we demonstrate that both hypoxia induced proteins carbonic anhydrase 9 (CA9) and vascular endothelial growth factor (VEGF) complete disulfide bond formation and are secreted with equal kinetics under hypoxia and normoxia. These proteins hence have a superior ability to be expressed in the absence of oxygen. Additionally, in a global *in silico* analysis of all proteins that traverse through the ER, we discovered that hypoxia-induced proteins on average contain fewer free cysteines and shorter-range disulfide bonds in comparison to other proteins. These traits may contribute to their superior ability to form correct disulfide bonds in hypoxia. These data show that the ability of proteins to form native disulfide bonds in hypoxia varies widely which can ultimately contribute to their expression in the extracellular space.

43. Preclinical validation and clinical translation of transrectal diffuse optical tomography for monitoring prostate cancer photothermal therapy

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Abstract:
Background and Aim: Diffuse optical tomography in transrectal configuration (TRDOT) has been developed to monitor progression of the photocoagulation front in order to achieve complete tumor destruction while avoiding rectal damage during photothermal therapy (PTT) of focal prostate cancer. Based on previous feasibility studies, we focus here on system calibration, image reconstruction and steps towards clinical translation.

Material and Methods: Each component of the TRDOT system - diode laser power output, photomultiplier tube (PMT) sensitivity and transrectal probe channel - has been characterized by use of an integrating sphere power meter. Relationships between laser power (mW), laser driving current (mA) and DOT voltage signal (mV) were determined to obtain consistent calibration. Validation tests of the protocols were performed on optical calibration phantoms of known optical absorption and scattering properties. Furthermore, a novel shape-based DOT image reconstruction algorithm was assessed using customized tissue-simulating phantoms having fixed inclusions of known optical properties and size to simulate growing photocoagulation lesions, monitored by both magnetic resonance imaging (MRI) and DOT. Lastly, TRDOT measurements were acquired in 7 prostate cancer patients in an ongoing PTT clinical trial. Pre- and post-PTT results were compared. Safety, technical feasibility and consistency of the system in clinical use were also evaluated.

Results: The calibration protocol was validated. Tissue scattering property changes were mainly around +33% post PTT, and reconstructed coagulation lesion sizes in the phantoms were mainly within ±1 mm of the actual values in all cases. The reconstructed optical properties of the PTT lesions approximately matched with fitted values from diffusion theory: absorption coefficients were within 10% (e.g. 0.12 mm^{-1} fitted vs. 0.10 mm^{-1} reconstructed) and reduced scattering coefficients were within 3% (e.g. 0.73 mm^{-1} fitted vs. 0.70 mm^{-1} reconstructed). Real-time DOT image reconstructions were compared with MR-based thermometry monitoring. Observational data were assessed from the PTT clinical tests, including operational procedure, safety, fitness of the TRDOT probe in the patients, and compatibility of the system with MRI. Pre- and post-PTT DOT scans of the lesion were acquired for optical property assessment. Probe alignment artefacts due to intrinsic factors of the patients (e.g. length of the rectum and size of the prostate) were found, and distances between posterior PTT posterior lesion boundary and probe surface were approximately 1-2 cm measured from the MR images.

Discussion and Conclusions: DOT image reconstruction in phantoms is successful even though the optical contrast post PTT is limited. Reconstructed optical properties in patients might not rigorously correspond to the prostate because of the misalignment and separation distance. Design upgrades of the probe are needed. Initial guesses of optimization to the image reconstruction algorithm are key to generating adequate outcomes. Overall, with the standard calibration protocol being validated, the DOT system can accurately reconstruct the optical properties of coagulation lesions in tissue-simulating phantoms and their size within 1 mm, which is considered necessary for clinical translation and utility. Safety and technical feasibility of TRDOT in prostate cancer patients have been demonstrated for the first time. Further improvements in system performance, particularly to increase the scan speed to allow more rapid sampling during treatment progression, are in progress. *This work is supported by the Terry Fox Research Institute (grant #1075).*

44. Developing an efficacious chemoresponse assay for personalized CLL treatment using high-content screening

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Abstract

Nearly half of Canadians are estimated to be diagnosed with cancer in their life time and almost one out of four cancer patients will succumb to the disease. One of the most prevalent adult leukemia, chronic lymphocytic leukemia (CLL), has become the focus of this proposed research. Recently, new therapeutics such as ibrutinib (Bruton's tyrosine kinase inhibitor) and venetoclax (Bcl-2 inhibitor) are shifting the landscape of CLL treatment. Despite advance in targeted therapies, a cure for CLL patients remains elusive. Activation of patient-specific survival pathways, cancer clonal evolution and genetic heterogeneity likely contribute to the observed resistance and the factors behind the limited benefits for the other targeted cancer therapies. A previous study from Andrews laboratory utilizing high-content screening to identify the addition of Sunitinib being effective in promoting venetoclax killing the resistant leukemic cells from individual leukemic patients. Based on this project, we focus on the development and clinical validation of a rapid and versatile chemoresponse assay to guide personalized cancer treatment using high-throughput, high-content screening on primary patient samples that have been conditioned to approximate physiological relevant disease conditions. The aim is to rapidly (in days) identify effective drug or drug combinations from a large collection of clinically relevant compounds that increase patient response. Preliminary screen of 47 patients identified patient specific responses to screening compounds using proprietary machine learning software. In addition, our analysis revealed phenotypes that correlate with resistance to first-line treatment such as Chlorambucil and drug combinations that could overcome such resistance. The ongoing screening will collect more patients and the predicted results will be cross validated with patient outcomes.

45. Development of a bioprinting approach for automated manufacturing of multi-cell type biocomposite TRACER strips using contact capillary-wicking

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Key words: Tissue engineering, tissue mimetic, tumour model, biocomposite, co-culture, bio printing

Abstract
In many types of solid cancer, interactions between tumour cells and their surrounding microenvironment significantly impact disease progression, and patient prognosis. Tissue engineered models permit investigations of cellular behaviour and interactions in the context of this diseased microenvironment. Previously our group developed the Tissue Roll for Analysis of Cellular Environment and Response (TRACER) platform. To improve the manufacturing robustness of the TRACER platform and to enhance its use for studies involving multiple cell types, we have developed a bioprinting process that deposits cell-laden collagen hydrogel into a thin cellulose scaffolding sheet though a contact-wicking printing process. Printed scaffolds can then be assembled into layered 3D cultures where the location of cells in 3D is dependent on their printed position in the 2D sheet. After a desired culture time 3D TRACERs can be disassembled to easily assess printed cell re-location and phenotype within the heterogeneous microenvironments of the 3D tissue. In our bioprinting manufacturing process, cells are printed into scaffolding sheets, using a modified 3D bioprinter to extrude cells encapsulated in unmodified collagen hydrogel through a polydimethylsiloxane (PDMS) printer extrusion nozzle. This nozzle design reproducibly generated bioink deposition profiles in the scaffold without causing significant cellular damage or compromising scaffold integrity. We assessed print pattern quality and reproducibility and demonstrated printing of co-culture strips containing tumour cells and fibroblasts in separate compartments (i.e. separate TRACER layers). This printing approach will potentially enable adoption of the TRACER platform to the broader community to better understand multi-cell type interactions in 3D tumours and tissues.

46. RET Isoforms contribute differentially to invasive processes in Pancreatic Ductal Adenocarcinoma

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Abstract:
The Rearranged during transfection (RET) receptor tyrosine kinase is expressed as two protein isoforms, RET9 and RET51, which play critical roles during embryogenesis and are also expressed in a number of human tumour types. Although the RET isoforms are coexpressed, they differ both in cell surface localization and intracellular trafficking, and a growing body of evidence suggests major differences in their functional roles. However, the relative contributions of RET9 and RET51 isoforms to human tumours are not well defined.

We have shown that RET expression is linked to tumour invasion and spread in certain thyroid and lung cancers. More recently, RET expression has also been correlated with perineural invasion, a process that is frequent in pancreatic cancers, and is associated with poor response to therapy and reduced patient survival.

In this study we use pancreatic adenocarcinoma cell lines which endogenously express RET (MiaPaCa-2, PanC1) to explore the functional differences between the isoforms. Using co-immunoprecipitation and immunofluorescence techniques, we show that RET interacts with Src substrate and scaffold protein TKS5 to induce invadopodia formation and promote invasion. Additionally, we show that RET activates RhoA and Cdc42 kinases to induce cell polarization and mediate directional motility. Our data show that the RET isoforms differ in their abilities to mediate directional cell migration and invasion, and that RET51 may be the primary contributor to these processes. Overall, these findings suggest that RET expression in pancreatic ductal adenocarcinoma cells may be a marker for invasiveness, making RET a potential prognostic marker and therapeutic target.

47. Novel Preclinical Pig Model To Detect Early Mri Biomarkers Of Anthracycline Induced Cardiotoxicity

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Cardiotoxicity is a major complication of anthracycline-based cancer therapy, which leads in time to progressive electro-mechanical dysfunction, irreversible myocardial tissue remodeling and heart failure. We are developing novel preclinical large animal models to study early structural and functional cardiotoxic effects induced by doxorubicin (DOX) chemotherapy. In this pilot study, a weekly 1 mg/kg dose of DOX was injected intravenously in n=4 pigs, during a four-week treatment plan. MR imaging was performed pre-DOX and post-DOX therapy (i.e., at: 1, 5 and 9 weeks after ending DOX injections) using a scanning protocol at 3 tesla that included: left ventricle function quantification (i.e., ejection fraction), gadolinium-based contrast-enhanced T1-mapping MR methods at 1.4mm isotropic resolution for diffuse fibrosis detection, and T2-mapping of edema/inflammation. Our results showed that compared to mean baseline values pre-DOX, ejection fraction gradually decreased from 47% to 34% post-DOX, indicating that the cardiac biomechanical function started to deteriorate within 9 weeks post-DOX. The initially increased T2-derived edema at week 5 appeared to resolve by week 9, as confirmed by H&E histological stains. Furthermore, subtle patches of diffuse fibrosis were detected by contrast-enhanced MRI only at week 9 but not at week 5, and were confirmed by collagen-sensitive Masson Trichrome histological stains. Lastly, in two pigs we recorded endocardial bipolar voltage maps 9 weeks post-DOX and revealed the presence of small scattered tissue patches with low voltages (<1.5mV). In these 2 pigs, we also induced ventricular fibrillation via rapid pacing, suggesting that gradual deposition of DOX-induced diffuse fibrosis increases vulnerability to arrhythmia. These novel results will help us establish image-based biophysical models to predict early DOX-mediated cardiotoxicity and will help clinicians conduct studies on cardioprotective strategies to prevent malignant arrhythmia and heart failure progression.

48. E3 ubiquitin ligase Cbl-b deficient CD8+ T cells exhibit enhanced anti-tumour activity in an IFNy-dependent manner

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The success of current adoptive T cell therapy (ACT) is limited by factors within the tumour microenvironment such as regulatory T (Treg) cells. Therapeutic targeting of Treg cells would be ideal but challenging due to the lack of specific surface markers and potential clinical consequences of systemic Treg cell depletion. Another approach to enhance anti-tumour immunity involves rendering tumour-specific T cells refractory to the inhibitory effects of Treg cells. Literature suggests that T cells deficient in the E3 ubiquitin ligase Cbl-b, a negative regulator of T cell receptor signalling, can resist suppression by Treg cells *in vitro*. Thus, this project aims to validate the therapeutic potential of targeting Cbl-b in tumour-specific T cells to further enhance ACT. First, adoptive transfer of tumour-specific Cbl-b$^{-/-}$ CD8^{+} T cells provide better anti-tumour immunity than the wildtype counterpart in the transplantable E.G7-Ova thymoma and B16-gp33 melanoma murine model, both of which depend on Treg cells to evade anti-tumour immunity. In this study, we demonstrated that Cbl-b$^{-/-}$ CD8^{+} T cells distinctively upregulate IFNy in response to T cell activation. Additionally, their resistance to suppression by Treg cells is partially abrogated with the loss of IFNy. Using the B16-gp33 model, loss of IFNy in P14 Cbl-b-/- CD8+ T cells abrogated anti-tumour immunity while loss of IFNyR on the T cells had minimal effect. Thus, hypersecretion of IFNy by Cbl-b$^{-/-}$ CD8^{+} T cells serves as an important mechanism to counteract Treg cells and provide a more robust anti-tumour immunity

49. The mevalonate pathway as an actionable vulnerability of t(4;14)-positive multiple myeloma

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Multiple myeloma (MM) is a plasma B cell malignancy that affects nearly 3,000 Canadians per year. Despite the introduction of new therapies over the past decade, MM remains incurable. MM is a highly heterogeneous disease that is often characterized by one or more of several disease-associated chromosomal translocations. In particular, the 15-20% of patients with a translocation between chromosomes 4 and 14 have an overall worse prognosis compared to other MM patients. Recent work from our lab has identified an unexpected metabolic dependency of t(4;14)-positive MM cells on the mevalonate (MVA) pathway, which is responsible for the synthesis of cholesterol and other non-sterol isoprenoids. More importantly, we can demonstrate that inhibiting the MVA pathway with the statin family of cholesterol-lowering drugs induces apoptosis specifically in t(4;14)-positive MM cells and decreases disease burden *in vivo*. Furthermore, as a result of MVA pathway inhibition, statins activate the integrated stress response selectively in t(4;14)-positive MM cells, which can be augmented by co-treatment with the proteasome inhibitor bortezomib to potentiate apoptosis in t(4;14)-positive MM cells. Since statins are well-tolerated and already approved for the management of cardiovascular disease, they have the immediate potential to be repurposed for the treatment of MM patients with t(4;14)-positive disease.

50. Using cryo-EM to capture protein domain interactions in the 2.6 MDa fungal fatty acid synthase machine

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Invasive fungal infections are associated with high mortality rates on par with the global burden of tuberculosis and malaria. Certain cancers and procedures to treat cancers compromise the immune system, resulting in an increased risk of potentially lethal systemic fungal infections, most commonly caused by *Candida* species. Currently there is an unmet need for novel antifungal therapies to combat drug resistant fungal pathogens, such as the recently emergent *Candida auris* species which has caused outbreaks in hospitals over 35 countries, including Canada. A promising novel antifungal drug target is fatty acid synthase (FAS), an enzyme complex that catalyzes the *de novo* biosynthesis of fatty acids and has been shown to be essential for virulence of several pathogenic fungal species. X-ray crystallography first revealed that this 2.6 MDa complex has a barrel shape with two chambers, each containing 3 complete sets of 6 catalytic sites, remarkably divergent from the mammalian FAS structure. However, these structures failed to comprehensively capture the acyl carrier protein (ACP), a mobile domain that covalently shuttles substrates and fatty acid intermediates within the FAS chamber. During the iterative FAS catalytic cycle, ACP transiently interacts with six different domains; small molecules disrupting these interactions may be potent fungal FAS inhibitors. In order to characterize these weak ACP-domain interactions in fungal FAS, we have systematically introduced single point mutations at the catalytic residue of each of the six catalytic domains of FAS from the model yeast organism *S. cerevisiae* and expressed and purified these mutants in *E. coli*. Using electron cryo-microscopy, a technique capable of resolving high-resolution structures of proteins in a near-native environment, we observe a shift in the ACP population to the inactivated domain in the presence of substrates. The increased ACP density in the 3D cryo-EM maps allowed us to confidently dock a model of ACP backbone and define a novel binding interface involved in FAS catalysis. This strategy of systematically stalling ACP shuttling during FAS catalysis can be used to characterize transient protein interactions, which may inform design of antifungal drugs against fungal FAS.

51. Disrupted chromatin insulated interactions of long non-coding RNAs and protein-coding genes indicate transcriptional rewiring of oncogenic and tumor-suppressive pathways

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Long non-coding RNAs (lncRNAs) have diverse roles in gene regulation, but remain poorly annotated in the context of healthy human tissues and cancer. Transcriptional gene regulation involves interactions of transcription factors with gene promoters and distal enhancers, mediated by three-dimensional genome architecture. Enhancers are often transcribed, and enhancer RNAs have significant classification overlap with lncRNAs. Here, we analyzed 7277 cis-regulatory modules frequently bound by diverse transcription factors that encoded 1057 lncRNAs and interacted with protein-coding gene promoters through chromatin loop insulation across at least 5 unique Hi-C datasets. To analyze expression patterns of these interactions, we determined 11 pairs of tumor tissues and corresponding healthy tissues by annotating transcriptomics datasets from The Genotype-Tissue Expression Project (GTEx) and The Cancer Genome Atlas (TCGA). Stringent analysis of variance (ANOVA) tests were performed to identify significant putative regulatory links between lncRNAs and promoter regions in a tissue-specific manner. We identified 444 unique tissue-specific significant interactions between lncRNAs and promoters of protein-coding genes. Most significant interactions were present in tumor tissue (413) and much fewer in healthy tissue (113), indicating the formation of oncogenic regulatory interactions in cancer cells through remodelling of chromatin architecture. Conversely, 31 significant links in healthy tissue were non-significant in tumor tissue, suggesting the breakdown of tumor-suppressive interactions. Protein-coding genes in TCGA or GTEx exclusive significant interactions were enriched in cancer-related pathways such as DNA repair, apoptosis regulation, angiogenesis, and cell cycle regulation. We identified key disruptions in esophageal carcinoma involving tumor-suppressors *RNF43* and *FBXO11*, and their respective associations with lncRNAs *BZRAP1-AS1* and *AC079807.2*. Our results corroborate previous discoveries of lncRNA mechanisms of gene regulation mediated by chromatin loops and three-dimensional genome architecture. We demonstrate a broad network rewiring of these interactions in tumor-tissue through changes in chromatin structure and identify putative oncogenic links that may have prognostic and therapeutic potential.

52. MYC phosphorylation regulates oncogenic activity

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The oncogenic transcription factor MYC is deregulated in over 50% of human cancers and is often associated with aggressive disease and poor prognosis. Targeting MYC directly has proven difficult, therefore uncovering novel strategies to inhibit this potent oncoprotein are needed. MYC is highly regulated through a network of signaling cascades and is known to contain a many post-translational modifications (PTMs) as well as interact with numerous proteins belonging to a variety of complexes. Despite the important role of PTMs in regulating protein function, aside from two phosphorylation sites regulating MYC stability (threonine 58 and serine 62), the role of PTMs in regulating MYC function are poorly understood. Previous work in the Penn lab identified additional phosphorylation sites that are important for controlling MYC activity. Two of these sites, serine 71 (S71) and serine 81 (S81), when mutated together to alanine (S71A/S81A) and thus blocking phosphorylation, were found to be gain-of-function (GOF) for transformation when assayed in models of MYC-dependent transformation. These sites do not impact MYC stability, but are important for regulating MYC function, as the GOF mutant altered the expression of additional genes compared to wild-type MYC when assayed by genome-wide expression profiling. These results indicate that these residues are important regulatory sites, but the mechanism remains unknown.

Our hypothesis is that MYC PTMs can regulate protein-protein interactions, thereby contributing to the control of a specific subset of target genes. Some of these PTMs may be deregulated in cancer, such as through alterations in upstream signaling, leading to increased MYC activity as seen with the S71A/S81A mutant. Our goal is to use BioID, a proximity-based biotin labelling technique, to identify MYC interactors that are gaining or losing interaction with the S71A/S81A phosphorylation mutant when compared to wild-type MYC. Prioritized interactors will be validated and investigated for their role in promoting MYC oncogenic activity. We aim to identify protein partners that promote MYC oncogenic activity which represent potential novel therapeutic targets in cancers with deregulated MYC activity. Overall, by identifying new mechanisms controlling MYC activity, we can exploit insight gained to target these mechanisms, to advance cancer therapy and improve patient outcomes.

53. Identifying Tumor Initiating Cells and Subclonal Evolution in Human Multiple Myeloma

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Treatment failures in cancer types, despite intense therapeutic strategies, may suggest the existence of intra-tumoral heterogeneity and presumably rare subclones of minimal residual disease that are persistent to current therapies. These subpopulations are likely responsible clones to (re)initiate cancer and are often referred to as tumor initiating cells (TICs). A precise phyneotypic description of TICs is lacking in many malignancies. A reasonable method to identify TICs within a diverse population of cells is associated with those biological features that are clonal. In many cancers, particularly in Multiple Myeloma (MM) the most reliable clonal markers are chromosomal copy number variations (CNVs), many of which are clonal and reoccur frequently in patients. Therefore, it would be ideal if we can use single-cell RNA-sequencing (scRNA-seq) data to infer the underlying DNA CNV across the genome of those cells and thus identify tumor cells based on the presence of clonal chromosomal abnormalities, irrespective of whether or not those cells happened to be expressing any particular oncogene at the moment of their sequencing. Then having an optimal evolutionary dynamics of genetic pathways across diverse pheno/geno-types within a heterogeneous population of cells is a challenging question which can reflect clonal evolution of cancer in terms of time and space.

To address all these challenges, we developed a comprehensive method which covers a range of genetic-data-analysis from normalization to inferring CNV of RNA-seqs. More precisely, starting with a scRNA-seq data from a population of cells, our bioinformatics pipeline, called 'sciCNV', converts it into an iCNV curve across the genome. To validate our findings, we observed that result corresponds well with actual DNA CNVs, measured by whole exome sequencing of the same tumor. sciCNV package can also segregate tumor/malfunction individuals from normals and may offer a novel method to clone distinct CNV-compartments and to construct a phylogeny of subclonal structure and pathogenesis of the disease. Examining the CNVs and transcriptional profiles of cells, we deconvolute the evolution of subclones within MM samples at a CNV level and to establish the effect of recurrent CNV events on the transcriptional program of tumor cells. We have examined our methods against several patient samples to detect intra-tumor cellular diversity, not only in multiple myeloma but also in lung, brain and liver cancers suggesting that our techniques are general and can be applied to any other malignancies. To summarize, we believe that our sciCNV pipeline may reveal tumor dependencies and shed light on an important aspect of intra-tumor genetic heterogeneity present with MM and other cancer types.

54. Transcription factor drug sensitivity model based on master regulators and protein abundance

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Background
Transcriptomic modifications affect cellular functions and alter both protein activity and abundance in a cell. Proteomics complements genomics and they are the main sources of clinically-approved biomarkers. However, proteomics is not properly leveraged in pharmacogenomics because their coverage and quality remain limited [Chen, et al. 2016].

Objective
To develop robust companion tests for targeted and chemotherapies in multiple cancer types by integrating genomics, transcriptomics and proteomics features from large-scale preclinical pharmacogenomic data.

Methods
We built a model on top of *PharmacoGX* package [Smirnov, et al. 2015] that employs several proteomics *(RPPA)* signatures available from Cancer Cell Line Encyclopedia *(CCLE)* [Barretina, et al. 2012 & Ghandi, et al. 2019] together with the master regulators (namely, transcription factors) [Moran, et al. 2017] inferred using Virtual Inference of Protein activity by Enriched Regulon analysis *(VIPER)* [Alvarez, et al. 2016] and used modified concordance index (*mCI*) we developed for drug sensitivity analysis.

Results and Impact
The proposed model predicted comparable biomarkers from the pharcogenomics literature for instance *mCI* for *ERBB2* (a known gene expression biomarker for *lapatinib*) based on *RPPA, VIPER, RNASeq, CNV,* and *Mutation* was 0.84, 0.91, 0.85, 0.77, 0.5 respectively. The *FDR* was very significant (i.e, <0.05) for all omics except for mutation was at random.

55. High-throughput genome-wide phenotypic screening via immunomagnetic cell sorting identifies QPCTL as modulator of CD47

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Genome-scale loss-of-function screens using CRISPR or gene-trap technology have been previously used to determine genetic interactions and their modifiers in mammalian cells. However, screens for positive and negative regulators of protein expression have been hampered by the requirement of isolating small populations from large cell numbers by flow cytometry, which entails advanced instrumentation and is time consuming, limiting throughput, parallelization and recovery of live cells desired for such screens.

To address these challenges, we are employing microfluidics technology for identifying regulators of cellular markers of interest by immunomagnetic sorting. Combining antibodies against a given marker, coupled to magnetic beads, with genome-scale CRISPR screens generates a scalable, high-efficiency and high-throughput microfluidic sorting platform that is flexible with respect to the choice of cell lines, markers and type of CRISPR library.

We used this platform to perform genome-scale CRISPR-Cas9 loss-of-function screens as an unbiased and comprehensive method for finding modifiers of CD47 expression. CD47 plays a role in the myeloid-specific immune checkpoint and has been explored as a candidate for cancer immunotherapy. After two rounds of immunomagnetic live cell sorting, we identified the glutaminyl cyclase QPCTL as modifier of CD47 regulation through a little-studied N-terminal post-translational modification. Importantly, net sorting time compared to flow cytometry was shortened several fold, while preserving cell viability, recovery and accuracy.

In summary, we are presenting a flexible immunomagnetic sorting platform amenable to high throughput screening of rare cell populations, whose application yielded candidate genes regulating the cancer immunotherapy target CD47.

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56. TAK-243 in combination with standard of care therapies for small cell lung cancer.

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Background: Small cell lung cancer (SCLC) is an aggressive disease that accounts for ~15-17% of lung cancers, with a dismal overall five-year survival of 7%. The current standard of care prescribes a first-line therapy of chemotherapy+/- radiation, which has changed minimally over the past 20 years. TAK-243, an irreversible inhibitor of the ubiquitin activating enzyme (UAE) E1, holds promise as a novel SCLC therapy.

TAK-243 dysregulates many cellular processes, including DNA repair signaling and protein degradation by impairing the ubiquitin conjugation pathway. Thus, by dysregulating cancer-specific dependencies of UAE, TAK-243 may induce malignant cell death. TAK-243 is particularly effective for 2 SCLC cell-lines, compared to a variety of other cancer and normal tissues (n=31) (Hyer et al., 2018). However, TAK-243 has not been evaluated as monotherapy or in combination with standard chemotherapy and radiotherapy for SCLC.

Methods: The efficacy of TAK-243 as a single agent was assessed in normal lung cell-lines, and a subset of SCLC cell-lines. Cell-lines were treated with incremental doses of TAK-243 (0-1uM) and cell viability was determined using a resazurin conversion assay after 6 days to determine EC50 values. To evaluate combination chemotherapy, cisplatin and etoposide (C/E), were administered (1:1 ratio, 0-1uM) to cell-lines together with TAK-243 at cell-line specific doses yielding 30-35% kill by TAK-243 alone. EC_{50} values were compared between TAK-243+chemotherapy and chemotherapy alone groups using the Extra Sum-Of-Squares F Test. TAK-243 (0-1uM) was administered with radiotherapy to SCLC cell-lines and the dose modification factor was calculated at 37% survival.

Results:
Single-agent therapy. TAK-243 monotherapy elicited a range of responses in SCLC and normal lung cell-lines after 5 hours. EC50 values of normal lung cell-lines tested ranged from 47-260nM, whereas the EC50 values for the SCLC cell-lines evaluated ranged from 3nM to 120nM.

TAK-243+Chemotherapy. TAK-243 treatment with C/E revealed ΔEC50 values ranging from 0-10nM. NCI-H889 demonstrated chemosensitivity to combination treatment, with a significant change in EC50 ($p<0.0001$) compared to treatment with chemotherapy alone. However, TAK-243 appeared to elicit additive effects with C/E in other cell-lines.

TAK-243+Radiotherapy.
TAK-243 demonstrated potential radiosensitizing abilities after short-term evaluation of radiation response in a panel of 5 SCLC cell-lines. Dose modification factors calculated at 37% survival were between approximately 1.25-1.35 for all cell-lines.

Conclusions: SCLC cell-lines demonstrated a range of sensitivity to TAK-243 single agent therapy. Lower doses of C/E and radiation are required for certain SCLC cell-lines when used in combination with TAK-243. These preliminary findings hint that TAK-243 may have the potential to improve the current SCLC standard therapies. These results are being validated using the clonogenic survival assay and further interrogated in vivo, which may provide a basis to move TAK-243 to the clinic.

57. Mutation burden, methylation and mRNA analysis of Homeobox genes for selecting benefit from adjuvant radiotherapy or observation for IDH mutated lower grade glioma

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Purpose: Presence or absence of mutation in IDH gene defines prognosis in gliomas. Grade and 1p/19q codeletion status commonly correlate with prognosis and response to treatment. However, better understanding of the molecular characteristics of IDHmut low-grade gliomas (LGG), specially related to treatment response, is desired. We aim to analyze methylation, mRNA and mutation datasets of IDHmut LGG patients treated with adjuvant radiation (aRT) or those observed to discover treatment related prognostic markers.

Methods: We used 412 IDHmut LGG that were profiled by The Cancer Genome Atlas (TCGA) Research Network, utilising methylation, mRNA and mutation datasets for selecting benefit from aRT or observation.

Results: Overall, when analyzing 357 (treated or not with aRT) IDHmut LGG, tumour grade (2 versus 3) significantly correlates with survival (HR 2.3 CI95 1.3 - 4.2; p-value 0.0041) but not 1p/19q codeletion status (p-value=0.24). Inversely, for IDHmut LGG treated with aRT, tumour grade (p-value = 0.23) did not correlate with survival but 1p/19q codeletion status significantly correlates (HR 4.5, CI95 1.1 – 19; p-value=0.024). Importantly, median high mutation rate compared with low mutation rate predicts worst survival in IDHmut treated with aRT (HR 3.5, CI95 1.7 – 7.3; p-value = 0.0055) but not for those observed (p-value = 0.61). Overexpression and hypo-methylation of a set of 7 Hox genes were both associated with worse survival for IDHmut treated with aRT (HR 5.7 CI95 1.7-19; p-value = 0.0018) but only a trend was found for the observation group (p-value = 0.054).

Conclusion: High mutation rate and a 7 HOX-gene signature have strong and independent prognostic and predictive value in IDHmut LGG treated with adjuvant RT. For the IDHmut LGG observation group, a 7 HOX-gene signature has a potential to better stratify adjuvant treatment need however further evaluation in a larger sample is needed.

58. MICAL1-mediated protein oxidation in tumour cell motility

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Abstract:
The metastatic spread of tumour cells begins with changes in adhesion and motility, which are regulated by signalling events that affect protein activity and localization through post-translational modifications (PTM). An emerging PTM is cysteine oxidation, but technical challenges associated with studying protein sulfenylation, and a lack of convenient tools to measure this PTM, have resulted in there being little information on how oxidation may contribute to cell motility. We previously reported that the protrusions of migrating cells generate H_2O_2 to influence cell motility through oxidation of cytoskeletal proteins, and we have developed a cell-permeable variant of the strained bicyclo-[6.1.0]-nonyne (BCN-E-BCN) that enables tagging of intracellular sulfenylated proteins. From these studies we identified increased oxidation of proteins that influence actin cytoskeleton dynamics. Here, we describe how MICAL1, a H_2O_2 producing actin monooxygenase, affects cancer cell motility and invasion through its ability to oxidise actin in cancer cell models. We have identified that MICAL1 activity is regulated via RhoGTPase signalling, in which MICAL1 is phosphorylated by PAK1 in a CDC42 dependent manner. Phosphorylation of MICAL1 promotes its localisation to membrane compartments and increases its catalytic activity. Knockout of MICAL1 by CRISPR/Cas9 in MDA MB 231 breast cancer cells significantly alters cancer cell morphology, attenuates cancer cell migration *in vitro*, and reduces their capacity to form tumours *in vivo*. Furthermore, mass spectrometry of protein oxidation profiles in cells expressing a constitutively active form of MICAL1 provides evidence for the existence of additional MICAL1 oxidation targets. Taken together, MICAL1 and its targets play a significant role in cancer cell dynamics, linking growth factor signalling pathways and protein sulfenylation as an important PTM, which has promising avenues for future drug discovery efforts in developing novel cancer therapeutics.

59. A deep learning based computational pathology tool for the counting of CD138+ Plasma cells in bone marrow biopsies, to aid in the classification of hematopoietic neoplasms

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(Presenting author is a trainee.)

Abstract:

Background: Modern cancer therapeutics increasingly require the identification and quantification of precise biomarkers in order to target the right drug to the right patient. One common clinical example is in the evaluation of reactive and neoplastic plasma cell proliferations, which requires accurate counts and percentages to be determined. On bone marrow biopsies and clot sections, the percentages of CD138+ plasma cells are traditionally estimated by eye. While arguably accurate, this human-based method is by definition imprecise, which is potentially problematic when the "true" percentage is close to an important cut-off (e.g., ~10%, at the border between MGUS and myeloma). Computational pathology can potentially assist pathologists in such situations by providing rapid, accurate, and precise enumerations of all positive/negative cells on a specimen.

Methodology: The STTARR computational pathology core facility has developed a computational framework to enable quantitative analysis of pathology images, using either commercial software or deep learning based methods. Here we compare the commercial Definiens TissueStudio image analysis platform, against a custom-built deep learning based method, trained on patches from CD138 DAB immunostained bone marrow biopsy whole slide scanned images. This stain is challenging to analyze with standard software packages, requiring both fine tuning of the thresholds for positivity, and optimization of segmentation conditions for closely spaced cells.

Manual ("gold standard") annotations from pathologists on 101 patches / regions of interest within a set of patient samples produced 8210 CD138 negative bone marrow hematopoietic cells, and 1190 CD138 positive plasma blast cells. 80 patches with pathologist annotations were used for training a convolutional neural network, producing a pseudo-probability heatmap relating to negative cell, positive cell and background; automated cell labels were produced corresponding to peaks in these signals.

Results: We compared detected with predicted CD138- hematopoietic cell counts, and CD138+ plasma blast counts, to identify both false positives and false negatives in each case. Precision, recall and F-score were calculated, with deep learning reaching an F score of 0.894 for the detection of CD138- hematopoietic cells, and 0.834 for the detection of CD138+ plasma blasts. For comparison, Definiens TissueStudio reached a F-score value of 0.756 and 0.677 for CD138- and + cells, respectively; and the inter-observer variability / pathologist F score was determined as 0.925 and 0.840 for CD138- and + cells, between two independent pathologists annotating the same dataset.

Conclusions: This initial study indicates the potential for deep learning based classification to assist in the counting of plasma cell blasts, with performance reaching close to inter-observer variability, in this initial pilot study. This algorithm may eventually contribute to precision and standardization in hematopathology, potentially by acting as a digital "second opinion" tool for difficult cases.

60. Characterizing the innate immune response to oncolytic virotherapy with a novel zebrafish model

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Introduction/objectives: The use of oncolytic viruses (OVs) in the treatment of solid tumors represents an area of intense current research, with clinical successes for colorectal cancer remaining modest to date. As the ability of OVs to act as immunotherapeutic agents is increasingly recognized, and they are integrated into combination therapies in cancer management, novel approaches to model the therapeutic efficacy of these strategies are needed. Our objective was to develop a zebrafish model of colorectal cancer that allows in vivo quantification of OV, tumor, and immune cell interactions throughout the course of virotherapy.

Methods: Zebrafish embryos were injected with mammalian colon cancer cells 48 hours post-fertilization and oncolytic virus 24 hours later, after a period of tumor xenograft development and angiogenesis. Confocal fluorescent imaging was then used to quantify fluorescently-labelled virus, tumor, and immune cell dynamics, as well as anti-tumor response.

Results: Xenografts successfully recapitulated early colorectal tumor development and enabled real-time quantification of immune cell/OV/tumor dynamics. Using our model, we have demonstrated a significant anti-tumor response in vaccinia-treated fish.

Conclusions: Our protocol offers a powerful new approach to OV modeling. Potential future applications include evaluating anti-tumor and immunotherapeutic capabilities of novel oncolytic viruses, alone and with other therapeutic agents, in a high-throughput manner.

61. A Pan-Cancer Compendium of Genes Deregulated by Somatic Copy Number Alterations of Regulatory Regions

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Somatic mutations that cause aberrant expression of oncogenes and tumour suppressor genes can drive the process of oncogenesis. Gene expression is regulated by interactions between transcription factors bound to regulatory regions and the promoters of their target genes. Understanding how these interactions are affected by somatic mutations including somatic copy number alterations (SCNAs) is an active area of research. A catalogue of genes that are targets of transcriptional dysregulation by SCNAs of their regulatory regions has not been fully refined. We integrate an enhancer-gene interaction dataset with 232172 interactions of 13,691 interaction pairs between 103705 enhancers and 17985 genes with matched SCNA and gene expression data on 10425 patients across 27 cancer types in TCGA. Separately for each cancer type, we identified genes where modeling of the gene's expression is significantly improved by adding regulatory region copy number to a model including gene copy number, strength of association for regulatory regions covered by the SCNA, and mRNA molecular subtype. We further filter these results based on fold change and wilcoxon *q*-value. There were 64 genes associated with amplifications that passed filters and 16 genes associated with deletions that passed filters. Of the genes that passed filters, 29 are annotated to pathways involved in the hallmarks of cancer. Some cancers, termed C-class cancers, are driven primarily by copy numbers such as breast, endometrial, and ovarian cancers. These cancer types have more SCNAs and more genes with resulting expression changes potentially associated with SCNAs of regulatory regions. The results presented here improve our understanding of how copy number alterations of regulatory regions may contribute to the oncogenic process.

62. Development of multi-modal diagnostic imaging of breast cancer using needle micro-endoscopy

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Background and Objective

Breast cancer diagnostics via biopsy has limitations due to sampling error, which may lead to unnecessary additional biopsies causing tissue damage and patient suffering. We previously presented the development of an optical biopsy technique based on autofluorescence imaging using needle micro-endoscopy that reliably distinguished between normal and cancerous breast tissue in five mastectomy specimens. We now present a reanalysis of our data, as well as our preliminary work to add Raman spectroscopy to improve diagnostic accuracy.

Methods

For the initial study, five women undergoing mastectomy for palpable breast cancer were recruited, however, one patient had to be withdrawn because of insufficient data. The mastectomy specimens were taken directly to the pathology lab. After visual inspection by the pathologist, study procedures were performed on the breast specimen within 20 minutes. A biopsy trocar (BARD, 14-gauge) was inserted into the tumor via ultrasonic (US) guidance. The internal cannula of the trocar was removed and the micro-endoscope (PolyDiagnost, Germany, 550 microns outer diameter, 6000-pixel imaging resolution) was inserted into the trocar's hollow channel. Both the conventional white light and false color AF images (OncoLife, Xillix Technologies (now a part of Stryker Endoscopy), Canada) from a malignant spot (visually identified and US guided) were collected. Next, the micro-endoscope was removed and the biopsy gun (BARD Magnum, Bard Medical, USA) was attached to the trocar to collect a core needle biopsy of the cancerous breast tissue ex-vivo. Then, such a procedure was repeated for a normal/non-cancerous/benign region (visually identified and US guided) of the breast specimen. The collected images were compared to standard pathological examination of the cancerous and non-cancerous biopsy samples to validate the imaging results. Raman spectra were collected in non-contact mode using a custom designed collection setup connected to a Raman spectrometer (Hyperflux PRO, Tornado Spectral Systems) for healthy and tumor regions on the excised tissues. This clinical protocol was approved by UHN REB. Concurrently, the technology to add Raman spectroscopy to the micro-endoscope is in development.

Results

There was a consistent contrast observed via the AF images between cancerous and non-cancerous tissue, which has been proven by the histopathology. For cancerous tissue, the AF images appeared reddish in colour, whereas for non-cancerous tissues, they were predominantly green/cyan. It is important to note the first patient received neo-adjuvant treatment prior to surgery. Therefore, pathological evaluation showed no traces of malignancy and this was also demonstrated in patient's AF image (true negative case). Preliminary Raman spectra collected from healthy and tumor tissues are also presented.

Discussion and Conclusion

It has been demonstrated that the AF micro-endoscope can be applied for interstitial needle endoscopy in breast, facilitating contrast between cancerous and non-cancerous breast tissue. The number of patients was limited; the next step is to recruit more patients and combine the AF optical biopsy along with Raman Spectroscopy. Such a multi-modal technique is expected to potentially provide more accurate results and ultimately lead to image-guided biopsy procedures in the future which improve breast cancer diagnostics; reduce unnecessary patient biopsy procedures, cut time and clinical costs.

63. Genome-wide CRISPR screens identify REV3L as a target in *BRCA1*-deficient ovarian cancer

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Introduction: Ovarian cancer is currently the foremost cause of gynecological cancer related death in the developed world. The most aggressive subtype of ovarian cancer, high-grade serous ovarian cancer (HGSOC) represents roughly 70% of all ovarian carcinomas. Although considered a curable disease when discovered early, HGSOC is typically diagnosed at an advanced stage due to late presenting symptoms. Approximately 80-90% of all patients who receive treatment at an advanced stage of disease will relapse, virtually all of whom will become resistant to chemotherapy.

The loss of the tumor suppressor *BRCA1* is one of the most significant risk factors for the development of HGSOC. Loss of *BRCA1* function and the subsequent inability to maintain genomic stability by repairing DNA double stranded breaks through homologous recombination (HR) has been associated with the development of primary and metastatic malignancies in various cancers, including ovarian, breast and prostate. Considering that reduced *BRCA1* activity is highly associated with HGSOC cancer development, research surrounding *BRCA1* has potential implications in the context of ovarian cancer therapeutics. This potential has recently been highlighted through the clinical utility of PARP inhibitors (PARPi), an inhibitor that specifically kills *BRCA1* mutated and HR defective tumours. Despite an initial response and greater progression free survival, resistance to PARPi arises in advanced disease, highlighting the necessity for additional therapeutics.

Methods and Objective: Here we employ genome-wide CRISPR-cas9 screens in a *BRCA1* deficient and *BRCA1* wildtype ovarian cancer cell line to identify genes, that when depleted, result in a lost of fitness in *BRCA1* deficient cell lines. To provide a more robust analysis, publically available genome-wide CRISPR-Cas9 screens were further analyzed to find a candidate target in *BRCA1* mutated tumours. Through functional genomics, we report REV3L, a DNA polymerase and putative R-loop factor that is involved in the repair of DNA damage as a novel candidate for the targeting of BRCA1 mutated cancers. Through the proposed area of research, this project focuses on expanding the basic understanding of *BRCA1* mutated ovarian cancer, with the end goal of highlighting a novel targetable gene.

Keywords: BRCA1, REV3L, cancer, CRISPR-cas9, synthetic lethality, DNA repair, R-loops, replication stress, genome stability

64. Development of a 3D microfluidic pancreatic tumor micro-environment for the investigation of immunosuppression and chemoresistance

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Abstract content:
Pancreatic ductal adenocarcinoma (PDAC) has proven to be one of the most lethal solid malignancies due to the heterogeneous and hostile nature of the tumor micro-environment (TME), with a 5-year survival rate of 6% and still less than 25% after surgery. Drug development takes an average of 12 years and 1.8 billion dollars and even with immune treatments, remains perpetuated by a lack of *in vitro* models for patient-specific testing and development. The objective of my work is to develop a 3D microfluidic culture that recapitulates the immunosuppressive and chemoresistive nature of the TME. In this work, preliminary data regarding device design and cell culturing will be discussed. The device consisted of three channels separated by phase guides to allow for fluid pinning. Minimization of the phase guide height allowed maximum interaction between cells in adjacent channels while also maintaining pinning of the gels. Devices were micro-milled over multiple iterations, with various heights of phase guides used to test for pinning until an optimal height was obtained. The central channel was used for collagen which would serve as the tumor stroma mimic and the side channel was coated with fibronectin and seeded with endothelial cells for monolayer formation, to serve as the vasculature of the system. Live/dead and VE-cadherin staining were then carried out on the monolayers to obtain preliminary visual data on the quality of the endothelial barrier. The completed platform will present a patient-specific *in vitro* drug testing system with engineering control over essential tumor characteristics.

65. The tumorogenic effect of the oncometabolite 2-droxyglutarate in low-grade gliomas

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Glioblastomas are brain cancers known for their aggressiveness and their fast progression. Recently, studies have shown a clear connection between the oncogenic isocitrate deshydrogenase (IDH1/2) mutations and the formation of secondary glioblastomas (GBM; World Health Organization [WHO] grade IV). In gliomas, a lower grade and less malignant brain tumors, mutations in IDH genes are thought to have at tumorogenic initiation effect.

Proteins encoded by the IDH genes are involved in the acid citric cycle by synthesizing a-ketoglutarate (a-KG), which is also an important co-factor in numerous enzymes. We found that affecting some of these a-ketoglutarate-dependent enzymes impacted the regulation of the mechanistic Target of Rapamycin (mTOR) pathways. mTOR is the core enzyme of two distinct complexes (mTORC1/2) involved in multiple cellular processes, namely protein translation, cell proliferation, cell survival, cytoskeleton reorganization, and cellualr migration. Mutation on IDH genes confers a enzymatic gain of function that sequentially transforms a-KG into 2-hydroxyglutarate (2-HG), leading to a cellular build-up of the secondary metabolite. Since 2-HG inhibit many a-ketoglutarate-dependent enzymes known to maintain cellular integrity, 2-HG was pointed-out as a tumorogenic metabolite (oncometabolite).

As part of my master's degree, I am characterizing the ability of 2-HG to promote gliomas progression by studying its effect on both mTOR complexes. Despite the impact of 2-HG being still misunderstood, my preliminary results suggest that 2-HG instigates the constitutional activation of mTOR, of which results an uncontrolled proliferation, increased cell survival, which are two major hallmarks of tumorigenesis. These findings could be crucial for the development of new therapeutic targets regarding highly refractory cancers like brain tumors.

66. Patient-Derived Organoid Models at Princess Margaret Living Biobank

Christine Ng, Nikolina Radulovich, Laura Tamblyn, Ni Liu, Molly Udaskin, Jennifer Cruickshank and Ming-Sound Tsao

Princess Margaret Living Biobank (PMLB) is a repository for patient-derived organoid models. PMLB organoids have been established from primary, metastatic and patient-derived xenograft tissues from lung, pancreas, colon, ovary, esophageal and breast tumour tissues obtained by surgical resection or biopsies at University Health Network, Toronto, Canada. We have deposited over 100 long-term, renewable and revivable PDOs to the UHN Biobank with links to other patient banked materials, as well as clinical and molecular profiling data. All deposited models have passed standard quality checks including Short Tandem Repeat (STR) genotyping for parental cell line identity matching, mycoplasma testing, growth analysis, and pathohistological identification of organoid tissues using a panel of tissue specific biomarkers reviewed by UHN pathologist. In addition, we have subjected a panel of pancreatic and lung organoid models with known patient response to treatments with Standard of Care (SOC) chemotherapy and molecular-targeted agents and have correlated organoid/patient responses to SOCs.

PMLB organoids closely recapitulated the histology, the mutation and copy number landscape of their parental tumors and were tumorigenic in mice. The majority of organoid drug responses were similar to that of the matched patient/PDX. Thus, PMLB organoids appear to be good surrogates for clinical tissue for drug screening and biological studies.

67. Investigating the role of DCAF1 as a therapeutic target for lung cancer

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DDB1-CUL4 Associated Factor 1 (DCAF1) is the substrate receptor of the Cullin4-RING (CRL4) E3 ligase complex that provides the substrate specificity to the complex. DCAF1 also belongs to the WD40 repeat family of scaffolding proteins, and has a wide range of cellular functions in cell cycle control, lipid metabolism and gametogenesis. DCAF1 protein expression is often up-regulated in many cancer types, with multiple studies linking DCAF1 to cancer development through its interactions with oncogenes and tumor suppressors. After mining genome-wide shRNA dropout screen data, lung cancer was determined to be one of the most sensitive cancers to DCAF1 knockdown. Therefore, we hypothesize that DCAF1 may be a potential drug target for lung cancer. Lung cancer accounts for the most cancer-related deaths worldwide and there is a need for better treatment options. Doxycycline (DOX) inducible DCAF1 shRNA constructs were generated to validate the dependency of DCAF1 in a panel of lung cancer cell lines. DCAF1 protein knockdown correlates with growth suppression in NCI-H460, SW1573 and NCI-H358 cells after DOX induction. Furthermore, cell cycle defects in DCAF1 deficient SW1573 cell lines were observed, resulting in the accumulation of cells in the G1-phase. Based on these results, DCAF1 appears to be important for cell proliferation in these lung cancer cell lines. Future studies will focus on understanding the mechanism responsible for the observed phenotype by identifying and characterizing DCAF1 interacting proteins in DCAF1 sensitive lung cancer cell lines.

68. Identification of a Novel YAP-TEAD Inhibitor for Cancer Therapy by High Throughput Screening with Ultrasensitive *YAP/TAZ–TEAD* Biosensors

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The Hippo pathway has emerged as a key signaling pathway that regulates a broad range of biological functions, and dysregulation of the Hippo pathway is a feature of a variety of cancers. Given this, some have suggested that disrupting the interaction of the Hippo core component YAP and its paralog TAZ with transcriptional factor TEAD may be an effective strategy for cancer therapy. However, there are currently no clinically available drugs targeting the YAP/TAZ–TEAD interaction for cancer treatment. To facilitate screens for small molecule compounds that disrupt the YAP–TEAD interaction, we have developed the first ultra-bright NanoLuc biosensor to quantify YAP/TAZ–TEAD protein–protein interaction (PPI) both in living cells and also in vitro using biosensor fusion proteins purified from bacteria. Using this biosensor, we have performed an in vitro high throughput screen (HTS) of small molecule compounds and have identified and validated the drug Celastrol as a novel inhibitor of YAP/TAZ–TEAD interaction. We have also demonstrated that Celastrol can inhibit cancer cell proliferation, transformation, and cell migration.In this study, we describe a new inhibitor of the YAP/TAZ–TEAD interaction warranting further investigation and offer a novel biosensor tool for the discovery of other new Hippo-targeting drugs in future work.

69. Predicting regional mutational variation in cancer using the chromatin accessibility landscape of primary tumours

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Cancer genomes are characterized by a diversity of somatic mutations, a majority of which are passenger mutations while a minority drive cancer by enabling oncogenic properties. Open chromatin is known to accumulate fewer mutations in cancer at a megabase-pair scale due to the differential activity of mutational and DNA-repair processes. Previously, healthy cell line chromatin organization has been shown to outperform cancer cell lines in predicting cancer mutational density. This implies that the mutational landscape of cancer is defined by the healthy cell-of-origin epigenome and potentially that most of the somatic mutations occurred prior to or early during tumourigenesis. Until recently, no primary tumour chromatin organization datasets were available to test this theory. Here we leverage the TCGA ATAC-Seq dataset to show that the primary tumour epigenome from the matching cancer type, not the healthy cell-of-origin, defines the mutational landscape of cancer in most cancer types.

We trained random forest models on either primary tumour or healthy cell line chromatin accessibility to predict regional mutational variation (RMV) in 26 different cancer types on a megabase-pair scale. We found that the models trained on primary tumour epigenomic data outperformed the models trained on healthy cell lines in 20 out of 26 cancer types. Furthermore, we trained models on a combined set of primary tumour and healthy cell line predictors to predict RMV in 9 cancer types which had both matched primary tumour and matched healthy cell line chromatin accessibility data. 5 out of these 9 cancer types showed the matched primary tumour epigenome as the top predictor. Interestingly, melanoma was the only cancer type that showed its healthy cell-of-origin (melanocyte) epigenome as the top predictor of its RMV. This indicates that melanoma, unlike other tumours, accumulated most of its passenger mutations prior to tumourigenesis, possibly from years of UV exposure. Furthermore, the top predictor of RMV in glioblastoma multiforme (GBM) was consistently the chromatin accessibility derived from low-grade glioma tumours (LGG) (as opposed to chromatin accessibility from GBM tumours). As LGG often develops into the more aggressive GBM tumour, this finding indicates that the mutational landscape of GBM is potentially established when the tumour is still in the earlier, less malignant LGG stage. Taken together, this study provides insight into the relationship between the mutational and epigenomic landscape of 26 different cancer types as well as into the timing of the accumulation of passenger mutations within a tumour.

70. oncoReactome: Cancer Hallmarks View of Reactome Pathways for Visualization and Analysis of Cancer Omics Datasets

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Reactome is an open source, manually curated and peer-reviewed database of human biological pathways. Currently, Reactome covers 2287 human pathways, composed of 12608 reactions that feature 10860 unique human proteins (>50% of predicted human proteome), over 1856 small molecules and 222 drugs. This knowledgebase was assembled by curating over 30000 published research articles.

oncoReactome is a cancer hallmarks based Reactome web-based portal that is currently under development. Compared to generic Reactome, oncoReactome will provide cancer hallmarks-oriented pathway views and enable advanced analyses of cancer omics datasets. In oncoReactome, biological pathways will be organized under cancer hallmark topics (Hanahan and Weinberg 2011), and these topics will be further grouped according to the role they play in cancer progression phases (Vogelstein and Kinzler 2015). Oncogenes and tumor suppressor genes that are part of the COSMIC's Cancer Gene Census (CGC) and the biological processes they regulate will be manually assigned to cancer hallmarks, thus linking the Reactome pathways that contain them to these hallmarks. Clinically relevant cancer biomarkers, many of which are already part of Reactome, will also be assigned to cancer hallmark topics and flagged in Reactome pathways. Reactome pathways currently provide information on the function of 556 of the 723 CGC genes (September 2019 releases of Reactome and COSMIC), while 674 CGC genes are part of Reactome's functional interaction (FI) network, available via the Reactome FIViz Cytoscape app. In addition, over 200 cancer variants of more than 20 CGC genes have been functionally annotated in Reactome's cancer-specific pathways, including, when available, information on variant-specific drug interactions. The oncoReactome portal will allow exploration of publicly available cancer datasets and customized analysis and visualization of user-owned omics data, including pathway enrichment and pathway modeling, as well as the comparison between user-owned and public datasets. oncoReactome will be established and distributed as a community resource for cancer researchers.

Reactome provides a solid foundation for computationally accessible organization of the existing knowledge on cancer biology and for building a web-based, high performance, researcher friendly visualization and analysis tool specifically intended for cancer research. The comprehensiveness and accessibility of oncoReactome will make it uniquely suited to be a catalyst for hypothesis generation in the search for novel cancer targets and therapies.

71. Metabolic control of integrin membrane traffic by AMP-activated protein kinase

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Cancer cells rely on their diverse cell surface proteins to sustain growth, survival, invasion, and migration. These proteins are dynamically regulated by endocytosis, sorting, and recycling pathways. The poor vascularization of tumors along with other metabolic alternations lead to metabolic stress in many cancer cells. How metabolic stress controls the membrane traffic at the surface of cancer cells, which in turn impact critical parameters such as cell adhesion and migration, is poorly understood. AMP-activated protein kinase (AMPK) is a cellular energy sensor that is activated upon cellular ATP insufficiency. We previously uncovered that AMPK broadly regulates the cell surface proteome, including proteins that impact cell adhesion and migration such as B1-integrin. We now aim to resolve how AMPK regulates the cell surface membrane traffic and endocytosis of B1-integrin in order to control functions such as cell migration. Using a FRET-based biosensor, we uncovered that AMPK controls GTP-binding by the GTPase Arf6, known to regulate endocytosis. Using siRNA gene silencing coupled to measurement of cell surface B1-integrin levels, and other methods to systematically probe regulation of endocytosis, we found that Arf6 and the GAP protein ArfGAP3 are required for AMPK to regulate cell surface membrane traffic of B1-integrin and clathrin-mediated endocytosis. Our results suggest a new signaling pathway linking regulation of membrane traffic and metabolic stress sensing by AMPK through control of ArfGAP3 and Arf6 to control B1-integrin. This suggests a novel mechanism by which metabolic alterations in cancer cells impact cancer cell growth, survival, and migration.

72. Quantitative comparison of anti-cancer BH3 mimetic drugs using FLIM-FRET microscopy

Elizabeth Osterlund, Nehad Hirmiz, Qiyin Fang, David W Andrews

To maintain the balance of cells in the body, each cell has a "self-destruct" program, called apoptosis, which is activated when a cell gets too old, redundant or damaged. A key event in cancer development is turning off this protective death response. The Bcl-2 family of proteins regulate cell death from within the cell. Some Bcl-2 proteins promote cell death (pro-apoptotic proteins), while others prevent cell death (anti-apoptotic proteins). Tipping the balance of pro- and anti-apoptotic signalling in the cell determines cell fate. Cancer cells grow rapidly, have mutations and are stressed and therefore should activate pro-apoptotic signalling pathways. To avoid cell death, some cancer cells become addicted to the expression of one or more anti-apoptotic protein for survival. Small molecule drugs designed to inhibit anti-apoptotic proteins are called, BH3 mimetics. The idea is, that these drugs displace pro-apoptotic proteins from one or more anti-apoptotic protein. In 2016 Venetoclax, which targets the anti-apoptotic protein Bcl-2, became the first BH3 mimetic to receive FDA approval for treatment of chronic lymphocytic leukemia. More work is needed on small molecules that target other anti-apoptotic proteins, such as Bcl-XL, Bcl-W and Mcl-1. Many companies have developed BH3 mimetics, but these have only been tested for selectivity using peptides and truncated anti-apoptotic proteins. We screened the top BH3 mimetics available for disruption of 24 unique Bcl-2 family protein-protein interactions in live cells using FLIM-FRET microscopy. Our measurements are quantitative and allow us to directly compare the efficacy of these inhibitors in live cells. We find that some compounds are highly functional, yet many exhibited unsuspected cross-reactivities and/or poor function.

73. A trillion nanoparticles overwhelm liver clearance and enhance tumour delivery

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Abstract

Nanoparticle delivery to solid tumours over the last ten years has remained stagnant at a median 0.7% of the injected dose. The liver is the principal delivery barrier because it sequesters most of the nanoparticles. Although its clearance ability is finite, the effect of high nanoparticle doses on liver sequestration and subsequent tumour delivery is unclear. Here we showed that nanoparticle bolus doses exceeding 1 trillion nanoparticles disproportionately reduced liver clearance and resulted in increased tumour delivery. By administering high numbers of nanoparticles, up to 12% of the injected dose delivered to tumours and 93% of tumour cells took up nanoparticles. Using this principle, we improved the efficacy of a clinically-used nanoparticle formulation Caelyx® (Doxil®) by artificially boosting the bolus dose using empty drug-free liposomes without active drug. Finally, we performed a meta-analysis and found that half of the reports published between 2005-2015 used doses below this trillion threshold and had significantly less tumour delivery than studies that used doses above the threshold. Our results define a nanoparticle number dose threshold that can be used to increase nanoparticle delivery to solid tumours.

74. Brain tumor assembloids as personalized avatars for glioblastoma drug discovery

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Abstract
Glioblastoma (GBM) is the most aggressive brain tumour with a survival of <15 months despite multi-modal therapy. Thus far, most drug discovery efforts focus on culture systems where GBM cells are grown/profiled in isolation, or on xenograft models. However, these systems suboptimally recreate GBM's complex infiltrative interaction with normal brain tissue elements seen histopathologically. Advances in tissue engineering now allow scientists to generate three-dimensional, brain-like structures called "cerebral organoids" from human pluripotent stem cells. Here we report co-culturing of cerebral organoids with patient-derived glioblastoma stem cells (GSCs) to form "brain tumour assembloids". This system highlights spatially compartmentalized human brain tissue elements that, once formed, are merged with GSCs to create expandable in vitro patient avatars for chemical profiling. Preliminary results indicate that the introduced GSCs display an infiltrative pattern that is a hallmark of GBM. As such, we hypothesize that this more pathologically faithful culture system will help better prioritize promising agents for personalized care. We will use this miniaturized and accelerated model of tumour growth to correlate a larger panel of assembloid cultures to highly specific kinase inhibitors in terms of their impact on GSC infiltration within the organoid. This project aims to fill the need for laboratory tools/methods to help us better understand GBM's pathology and refine personalized therapeutic research.

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75. Understanding the role and regulation of DICER in cervical cancer under conditions of hypoxia

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Hypoxia develops frequently within tumours in regions where oxygen metabolism exceeds oxygen delivery. Hypoxic regions are highly heterogenous in both space and time and are strongly associated with poor prognosis. In cervical cancer, an influential clinical trial conducted at Princess Margaret concluded that patients with more hypoxic tumors had reduced disease-free survival when treated with curative intent using radiotherapy due to increased metastasis. Recently, our lab discovered that hypoxia can promote metastasis through the epigenetic regulation of the *DICER* gene. DICER, is a RNase enzyme, responsible for cleaving precursor microRNA (miRNA) into mature miRNA. The process of miRNA biogenesis is an important regulatory mechanism influencing the overall capacity of cells to generate miRNA, and consequently influencing gene expression and cellular phenotype on a broad scale. Our findings in breast cancer models suggest that hypoxia induced repression of DICER can selectively reduce specific miRNAs that regulate the epithelial-mesenchymal transition (EMT) and acquisition of stem cell properties. Consequentially, miRNA alterations during hypoxia in breast cancer promote cancer initiation, progression, and survival. In this study, we will explore the underlying significance of miRNA regulation by DICER in response to both hypoxia and radiation exposure, and its consequence on progression and survival of cervical cancer. We will study this regulation using a range of *in vitro*, *in vivo*, and clinical models that capture the cellular and patient heterogeneity that is critical to this disease. Our initial experiments in cervical cancer models indicate that DICER expression decreases in an oxygen-dependent manner. Additionally, DICER knockdown *in vitro* models have shown to decrease the processing of the miR-200 family of miRNAs. Taken together, these findings could indicate that EMT induction in hypoxic tumors is mediated by DICER suppression and loss of the miR-200 family. The findings of this study may help us determine if DICER expression can serve as prognostic marker in cervical cancer.

76. RNF168 confers synthetic lethality in *BRCA*-deficient tumors through impairment of R-loop resolution

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Introduction: Tumor suppressor BRCA1 and BRCA2 are involved in DNA double strand break (DSB) signaling and homologous recombination (HR) repair. As a result, women who inherit harmful *BRCA* mutations are at a higher risk of developing breast (≥60%) and ovarian (≥40%) cancers compared to those with wildtype *BRCA* (12%) genes. Similar to BRCA1, RNF168 is an E3 ubiquitin ligase, which orchestrates the recruitment of effector proteins to DSB sites, allowing their repair. While research has led to discovery of drugs like Olaparib to target *BRCA*- and HR-deficient tumors, optimal therapeutic targets have not been established for majority of patients carrying *BRCA* mutations.

Methods: Using mouse models coupled with cell-based assays including proximity-dependent biotin identification (BioID), immunoprecipitation (IP), chromatin IP (ChIP), immunofluorescence, and western blotting, DNA-RNA IP (DRIP), we have examined the impact of RNF168 loss in a BRCA1-deficient setting. We have also utilized publicly available datasets on The Cancer Genome Atlas (TCGA) and collaborated with the Consortium of Investigators of Modifiers of BRCA1/2 (CIMBA) to assess the essentiality of RNF168 in the absence of functional HR.

Results: Our data indicates that mice with mammary-specific deletion of *Rnf168* and *Brca1* are significantly protected from mammary tumors when compared to mice with mammary-specific deletion of *Brca1*. Depletion of RNF168 results in accumulation of triple-stranded nucleic acid structure or R-loops which leads to compromised genomic integrity, replication fork instability, and senescence in *BRCA1*-deficient cells and tumours; thus, resulting in synthetic lethality and protection from BRCA1-associated mammary tumorigenesis. Furthermore, our data indicates that depletion of RNF168 in deficient human tumors impairs their *in vivo* and *in vitro* growth and increases their sensitivity to radiation and Olaparib. Mechanistically, we have discovered a novel role of RNF168 in regulating RNA:DNA helicase DHX9 through K63-linked ubiquitination. In the absence of RNF168, DHX9 is not recruited to R-loop prone genomic loci, leading to R-loop accumulation, genome instability, and synthetic lethality. Furthermore, analysis of the TCGA breast cancer (provisional) dataset indicates that homologous-recombination (HR) -deficient tumors express higher levels of RNF168 compared to other tumor types. Patients with HR-deficient tumors that express low levels of RNF168 have improved survival outcome compared to patients that express high levels of RNF168. Collectively, our data supports targeting RNF168 for treatment of *BRCA1*-deficient cancers and potentially for other HR-deficient tumors, including tumors with mutations in BRCA2, RAD51, and FANCD2.

Keywords: BRCA1, BRCA2, RNF168, cancer, senescence, synthetic lethality, DNA repair, R-loops, replication stress, genome stability

77. Differential Regulation of Glycogen Metabolism in Mutant IDH Chondrosarcomas and Chondrocytes

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Introduction: 38-70% of chondrosarcomas (CSAs) harbor somatic mutations in the isocitrate dehydrogenase genes (*IDH1* and *IDH2*).[1] *In vivo*, mutant *IDH1* and its gain of function metabolite, D-2HG, were found to inhibit growth-plate chondrocyte differentiation, driving enchondromatosis, the benign precursor to malignant chondrosarcomas.[2] For these reasons mutant *IDH* has been identified as a driver mutation of CSAs. Upon our characterization of metabolomic activity in CSAs, electron microscopical examination of patient CSA cells revealed glycogen deposition exclusively in mutant *IDH* cells. Thus, we plan to uncover why this phenomenon is present in mutant *IDH* CSAs and how mutant *IDH* may regulate glycogen metabolism in CSAs to drive tumor growth.

Methods: With institutional review board (IRB) approval, human CSA tumor samples were obtained fresh from surgery. Tumors were implanted in interleukin-2 receptor gamma chain (gamma)-null NOD/SCID (NSG) mice and excised once tumor capacity size was reached. Patient derived xenograft (PDX) CSA tumors were dissociated for usage in *in vitro* experiments. PDX tumors were pulverized and glycogen was quantified by Abcam's Glycogen Assay Kit II. To examine the effects of mutant *Idh* in glycogen metabolism and its role in the development of cartilaginous neoplasia, *Col2a1-Cre*, *Idh1 R132Q-KI*, and *Hif1a*$^{fl/fl}$*; Hif2a*$^{fl/fl}$ mice were used. Pharmacological blockade of *PYGL* enzymatic activity was achieved by usage of CP-91149 drug from Selleckchem Inhibitor.

Results: Significant elevation of glycogen in mutant *IDH* CSAs was confirmed using patient derived xenograft (PDX) tissues in a quantitative glycogen assay, electron microscopical examination, and Periodic acid-Schiff (PAS) staining. Upon investigation of glycogen in early cartilage lesion formation, *Col2Cre*; *Idh1*-KI mouse growth plates displayed positive PAS staining for glycogen accumulation from hypertrophic and to resting zones; however, this phenotype was absent in wildtype growth plates. Immunostaining of glycogen synthase enzyme, *GYS1*, was significantly elevated in *Col2Cre*; *Idh1*-KI growth plates confirming glycogen accumulation upon mutation of *Idh*. RT-qPCR experiments show that D2HG and *HIF* are a potential regulators of glycogen metabolism. X-ChIP of *HIF1a* in CSA tumor cells confirm that *HIF1 a* is a transcriptional regulator glycogen metabolism enzymes, *GYS1*, *PYGL*, and *PGM1*. *PYGL* inhibitor drug (CP-91149) induced sensitivity in CSA cells and significantly reduced cell viability.

Discussion: Mutant *IDH* CSA tumors display enhanced glycogen stores and sensitivity to glycogen synthase inhibitor drug. D2HG has been identified to function via *HIF1a* and *HIF1a* has been identified as a transcriptional regulator of glycogen metabolism enzymes in mutant *IDH* CSAs. Glycogen metabolism's role in CSA tumorigenesis is currently being investigated by blockade of *PYGL* and assessing its effects in cell proliferation, apoptosis, and viability. *Idh1* R132Q-KI mouse growth plates also display glycogen deposition, suggesting that mutant *Idh* is a regulator of glycogen metabolism via *HIF1a*. Targeting glycogen metabolism in CSAs holds as a promising therapeutic for a disease resistant to chemotherapy and radiation.

78. Mutation burden in a MMTV-PyMT mouse model of breast cancer with RHAMM loss during tumorigenesis and metastasis shows evidence of microenvironmental purifying selection

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The MMTV-PyMT transgenic mouse is increasingly used as a model of aggressive luminal B mammary tumorigenesis. We used this model to assess the consequence of loss of a cancer antigen, RHAMM, that is associated with poor prognosis in breast cancer. Loss of RHAMM is expected to contribute to genomic instability given that RHAMM is a mitotic spindle protein. RHAMM loss in the MMTV-PyMT mouse model of breast cancer is associated with a seemingly unchanged phenotype of mammary primary tumors but a significant increase in the number of metastases in the lung. The *de novo* mutation burden in primary mammary tumors and lung metastases in MMTV-PyMT wildtype and *Rhamm*$^{-/-}$ mice was examined for associations between genome instability and cancer phenotype. Here, *de novo* mutation burden was assayed using the Mouse Diversity Genotyping Array (MDGA) to detect two types of mutations, base substitutions at single nucleotide polymorphic (SNP) loci and copy number variants (CNVs). These mutations were examined for enrichment in genes and pathways to identify novel cancer targets. Compared to spontaneous germline mutations in this mouse genetic background, the MMTV-PyMT mice had a CNV burden up to 3.2 fold greater. The CNVs in MMTV-PyMT mice are on average 1.7 to 5.2 fold longer than the germline background. The total point mutation burden of *Rhamm*$^{-/-}$ mice including primary tumors and metastases is 3.6 fold lower than the wildtype comparators. However, *Rhamm*$^{-/-}$ metastases have a 7.4 fold increase in the point mutation burden compared to the primary tumors. In contrast, the point mutation burden in wildtype mice was 1.4 fold greater in the primary tumors compared to the metastases. There is also strikingly low inter-animal variation in point mutation burden in *Rhamm*$^{-/-}$ primary tumors and metastases. The top Ingenuity Pathway Analysis [IPA] network enriched with *de novo* mutations at SNP loci shared across all three *Rhamm*$^{-/-}$ mice is 'cancer, gastrointestinal disease, organismal injury and abnormalities', with five known cancer drivers identified in this network that are affected by the point mutations. The cancer samples have more copy number losses than gains and the CNVs overlap or encompass genes. The distribution of CNVs across the genome resembles a mutator phenotype for all cancer samples. In *Rhamm*$^{-/-}$ primary tumors, 10 recurrent genic CNVs were identified. In *Rhamm*$^{-/-}$ metastases, five recurrent genic CNVs were identified. IPA analyses grouped these on the same pathways, with the top being 'connective tissue development and function, tissue morphology, cellular growth and proliferation'. A principal component analysis using both point mutation and CNV data captured the similar mutation burden across the *Rhamm*$^{-/-}$ mice in contrast to the heterogeneity observed in wildtype mice. This homogeneity in *Rhamm*$^{-/-}$ tumors is consistent with a purifying selection that favors metastatic growth but not primary tumor growth. Further study will reveal critical microenvironmental factors that are responsible for the increased metastasis of *Rhamm*$^{-/-}$ tumor cells and is likely to be critical in increasing mechanistic understanding of how the microenvironment promotes tumorigenesis.

79. mTOR Inhibition via Rapamycin Modulates DNA Damage Response Following Radiation in Canine Mast Cell Cancer Cells

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Background: Mastocytosis, a rare disease in humans, is common in canines. Canine mastocytosis is spontaneous and occurs in immunocompetent patients who share our living environment, making it a valuable translational model. Using a comparative medicine approach, this project examined mTOR signalling, a conserved pathway upregulated in cancer cells that effects several essential cell functions. We evaluated its relationship to ionizing radiation induced DNA strand break repair in two canine mast cell cancer cell lines (MCT-1, MCT-2) derived from naturally occurring tumours in dogs. mTOR inhibition was achieved using Rapamycin, a cytostatic compound demonstrating potential radiosensitizing effects in other cancers. Our prior work with MCT-1 cells revealed Rapamycin pre-treatment led to sustained DNA damage foci after radiation compared to untreated cells. Here we examine the kinetics of this response.

Results: Canine specific plasma steady-state doses of Rapamycin (5.5 nM, 11 nM and 16.5 nM) were combined with 3, 6 or 10 Gy of radiation. MCT-1 and MCT-2 cells were treated with Rapamycin for 4 or 7 days post-radiation. Clonogenic survival of MCT-1 decreased significantly after 4 days of 5.5nM of Rapamycin treatment following 10 Gy. Western blots demonstrated a dose-dependent inhibition of S6K beginning at 30 minutes for MCT-1, and at 15 minutes for MCT-2. Radiation alone activated mTOR in MCT-1 and MCT-2 in a dose-dependent manner. The comet assay suggests mTOR inhibition enhanced severe DNA damage but allows repair of low levels of DNA damage in cells. RNF168, a ubiquitin ligase involved in the initiation of DNA damage repair, does not appear to play a role in the modulated response caused by Rapamycin.

Conclusions: Rapamycin modulated DNA strand break repair in canine mast cell cancer cells following radiation. Inhibiting mTOR is a possible avenue for enhancing the effectiveness of radiation therapy. Targeting mTOR might also be an effective route for radio-resistant cancers.

80. Microfluidic Arrays of Breast Tumour Spheroids

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Abstract:

Extracellular matrix stiffness and interstitial flow impact the response of malignant tumours to anticancer drugs1–3, however many of the in-vitro cancer models used for drug screening, including 3D organoid and spheroid models, fail to consider these key features4,5. Here, we report a microfluidic spheroid-on-a-chip platform for the generation of massive arrays of breast tumour spheroids that are grown in a biomimetic nanofibrillar hydrogel under physiological flow conditions. We show the applications of this platform for time- and labor-efficient drug testing. Using our platform, we investigate how interstitial flow influences the chemosensitivity of tumour spheroids. The microfluidic spheroid-on-a-chip platform enables screening of the efficacy of individual drugs, the optimization of drug formulations, and can facilitate fundamental cancer biology research.

81. Integrating Systems Biology and Machine Learning to Partially Overcome Limitations of Anti-PD-1 Immunotherapy

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The response rate to immunotherapy has historically had a ceiling of approximately 10% of patients. However, when favorable response is exhibited in a patient, it tends to be durable and sustained for several years, meaning that immunotherapy can significantly improve patient prognosis. Anti-PD-1 immunotherapy has produced the highest response rate of any single-agent immunotherapy and is the first immunotherapy to break through the 10% response rate ceiling. It has recently shown promise for the treatment of several aggressive cancers including melanoma, non-small-cell lung cancer, bladder, and head and neck cancers. However, there is high variability and unpredictability in the treatment outcome, which is thought to be driven by patient-specific biology, particularly, the interactions of the patient's immune system with the tumor.

Here I will introduce an integrative experimental and theoretical approach which was developed to study the patient-specific interactions between immune cells and tumor cells, in order to capture the variability in patient response to anti-PD-1 immunotherapy. This integrative approach utilizes clinical data from an ex vivo human tumor system that incorporates fragments from tumor biopsies in co-culture with patient-matched peripheral immune cells and plasma. The patient-derived cytokine expression levels and immune cell populations under control and Nivolumab treatment conditions were used to develop and calibrate a multi-scale systems biology model of the immune system which includes interactions of immune cells with the tumor cells and cytokine signaling. I will illustrate how the patient data was integrated into the model to capture the variability in patient response to treatment. Then I will show how the application of machine learning approaches to a simulated patient data set obtained from the calibrated model can be used to stratify features of response from non-response to anti-PD-1 immunotherapy. This approach has the potential to identify novel therapeutic targets that may sensitize otherwise non-responsive patients to anti-PD-1 immunotherapy.

*Michelle Przedborski is a post-doctoral fellow at the University of Waterloo.

82. Interstitial Fluid Pressure Induces Phenotypic Plasticity in Breast Cancer Cell Lines, in Presence or Absence of Drugs

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Abstract

Tumors are highly dynamic systems characterized by complex interactions among the vascular, interstitial and cellular compartments. A collection of distinct cell types constitutes the tumor microenvironment. The cellular heterogeneity within a tumor has been shown to influence resistance to standard treatments and metastasis formation. In addition, tumor microenvironment that arises from abnormal angiogenesis is characterized by hypoxia, acidosis, and interstitial hypertension, which strongly contribute to inefficient drug delivery, drug resistance, invasion and metastasis. There has been a number of studies to investigate the effect of hypoxia and acidosis on cellular heterogeneity and the development of the drug resistance, however, less attention has been made to the effect of the high interstitial fluid pressure (IFP) within solid tumor.

We have developed an integrative approach that combines experimental and theoretical studies to investigate the effects of the IFP on phenotypic plasticity in breast cancer cell lines. We have shown that the expressions of CD24/CD44 significantly depend on the IFP. In addition, we have discussed on the results which have been influenced in the presence of the drug. Our computational simulations have shown that the change in the expression of CD24/CD44 is associated with gaining a stem cell like phenotype, which are more resistance to drugs. Moreover, we have shown that cells may have gone under epithelial to mesenchymal transition in the presence of interstitial hypertension.

83. Novel combination therapy for the treatment of metastatic pancreatic cancer using repurposed drugs

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Pancreatic cancer is the only malignancy with a 5-year survival rate still in the single digits at only 8%. Surgery is the only curative option; however, less than 25% of patients are eligible for surgery, due to the nature of the disease at the time of diagnosis. As a result, the standard remains to be palliative chemotherapy, to which patients invariably develop resistance and succumb to disease progression and metastatic burden. Thus, there is an urgent need for a therapy that simultaneously targets the complex genomic landscape, multiple overactivated parallel pathways characteristic of pancreatic cancer, and sensitizes cells to the cytotoxic effects of chemotherapy. We have previously reported on the crucial role of mammalian lysosomal enzyme neuraminidase-1 (Neu-1) in regulating the activation of several receptor tyrosine kinases (RTKs), including the epidermal growth factor receptor (EGFR) that is upregulated on the surface of malignant cells. Neu-1 cleaves terminal a-2,3 sialic acid residues on the ectodomain of the EGFR to relieve steric hindrance and allow for receptor dimerization and downstream signaling following ligand binding. We have uncovered that antiviral oseltamivir phosphate (OP) acts as a structural analog to a-2,3 sialic acid, preventing Neu-1-mediated activation of the EGFR, ultimately shutting down downstream signaling that is implicated in multistage tumorigenesis. However, redundancy in cellular signaling underscores the need for additional components for the effective targeting of this complex disease, leading to the proposed use of non-steroidal anti-inflammatory drug (NSAID) acetylsalicylic acid (aspirin) and antidiabetic metformin due to their recently reported chemoprotective roles in addition to conventional chemotherapeutic agent gemcitabine. Sialidase assays on live aspirin-treated, EGF-stimulated PANC-1 pancreatic cancer cells have demonstrated a previously unknown effect of aspirin shutting down Neu-1 activity. Immunocytochemistry analysis of PANC-1 cells revealed each drug as a single agent alters markers of epithelial-to-mesenchymal transition (EMT) characteristic of metastatic disease. This work demonstrates promise in the efficacy of this novel drug cocktail to specifically target and kill pancreatic cancer cells *in vitro* and *in vivo*.

This project was supported in part by the 2017 Terry Fox Research Institute Transdisciplinary Training Program in Cancer Research.

84. Investigating radiation response of pancreatic tumors and their microenvironment using *in vivo* optical imaging to identify new treatment strategies

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*Presenting author is post-doctoral fellow. Project is supported by the TFRI New Investigator's Grant.

Introduction and Aim. Despite therapeutic advances, the 5-year survival rate for locally advanced pancreatic cancer (PC) is ~4%. Recently, stereotactic body radiation therapy (SBRT) of PC is being explored clinically to maximize therapeutic efficacy by delivering a large and precise radiation dose to the tumor with minimal toxicity to surrounding tissues. However, we and others have shown that high-dose SBRT induces acute, yet transient vascular damage, increasing tumor hypoxia and, subsequently, the expression of hypoxia-inducible factor-1a (HIF-1a). As a result, tumor survival is promoted and metastasis is induced, leading to treatment resistance. New therapeutic approaches that can overcome treatment resistance and reduce metastases are therefore urgently needed. Using a novel optical intravital imaging platform and orthotopic mouse model of a multi-reporter human PC cell line (BxPC3), we aim to investigate which SBRT-induced changes in the tumor microenvironment (TME) lead to tumor hypoxia and whether we can address tumor hypoxia by decreasing the tumor's O2 consumption. This can be achieved by using a HIF-1a inhibitor, such as the FDA-approved diabetes drug metformin.

Materials and Methods. With a transparent window chamber placed over the pancreas, we were able to non-invasively and longitudinally image orthotopic PC (DsRed-BxPC3 cells transfected with luciferase and a GFP-labeled hypoxia response element) and its TME simultaneously at cellular resolution using in vivo fluorescence confocal microscopy (Zeiss LSM710). Imaging was performed before (D0) and after (D1, D4, D7, D14) fractionated, high-dose SBRT (5x8Gy) with (*n*=5) or without (*n*=5) the addition of metformin (5mg/ml in drinking water for 5 days). A small animal irradiator (X-Rad225Cx) equipped with cone beam computed tomography and electron multiplying CCD for bioluminescent imaging was used to target and irradiate the orthotopic tumor. A control group (*n*=5) and metformin-treated group (n=5) were imaged as well. The level of tumor hypoxia was quantified at each time point as the ratio of GFP/DsRed intensities for each pixel and plotted relative to baseline values. Tumors where harvested after the final imaging time point for *ex vivo* immunohistological analyses.

Results and Conclusion. Elevated GFP/DsRed ratio as well as pimonidazole staining was observed in SBRT-treated mice 2 weeks after treatment **(Fig. 1)**. In contrast, SBRT-treated tumors co-treated with metformin did not become hypoxic within the same time period. Metformin treatment alone reduced the GFP/DsRed ratio over time. Preliminary *in vivo* and *ex vivo* results support the hypothesis that SBRT induces hypoxia, and that the addition of metformin mitigates this response. To confirm this, we are currently increasing sample size and performing additional immunohistological evaluations. Our ongoing research will address key areas of hypoxia-associated treatment failure in PC and could potentiate the design of a new pilot clinical study in PC combining SBRT and metformin.

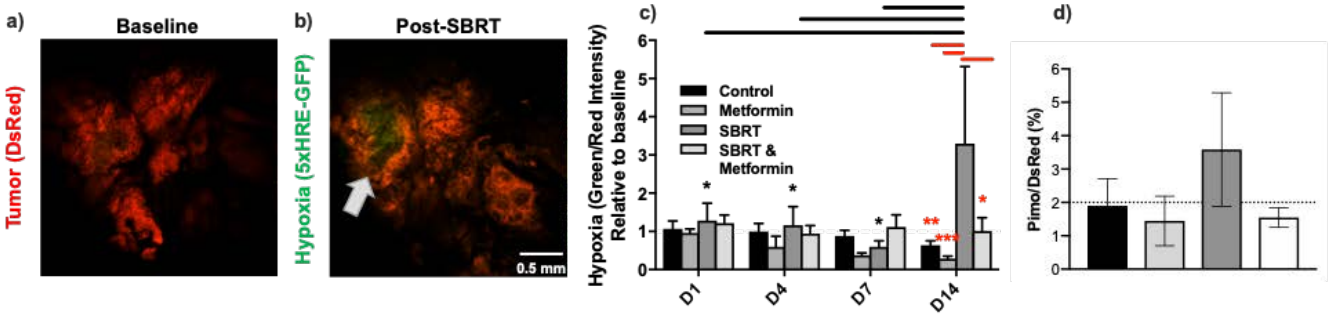

Figure 1 a,b) Quantification of hypoxia in orthotopic PC multi-reporter tumor using intravital fluorescent imaging shows SBRT-induced hypoxia (green, *arrow*) 2 weeks post-treatment, which is confirmed by *in vivo* **(c)** and D14 *ex vivo* **(d)** quantification, while co-treatment with metformin did not change the level of hypoxia.

85. Promoting MYC hyperphosphorylation through disrupting the MYC:PNUTS interaction as a promising anti-cancer agent

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MYC is an oncogenic driver that regulates transcriptional activation and repression and is itself regulated by interactors that deposit post-translational modifications to mediate MYC's turnover. One of these MYC modifiers is protein phosphatase 1 (PP1) that with its regulatory subunit PP1 nuclear-targeting subunit (PNUTS) interacts with MYC. The MYC:PP1:PNUTS complex co-occupies MYC target gene promoters, stabilizing the MYC protein and preventing MYC proteasomal degradation. Inhibition of PP1, genetically or pharmacologically, leads to hyperphosphorylation of MYC and detachment from chromatin, while maintaining the interaction with MYC's obligatory partner protein MAX. Therefore, we propose that developing a dominant negative to the MYC:PNUTS interaction will pose a promising tool to inhibit MYC-driven cancers.

86. Tumor cell heterogeneity and dynamics in colorectal cancer response to therapy

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Sumaiyah Rehman – Post-doctoral Fellow

Abstract:
Colorectal cancer (CRC) remains the second leading cause of cancer mortality in North America, despite advances in our understanding of CRC tumorigenesis. Sequencing and phenotypic analyses revealed extensive cellular heterogeneity, 'subclones', within developing CRCs, representing a major hurdle in understanding tumorigenesis and therapeutic response. The cancer stem cells (CSCs) subpopulation is known to mediate tumor progression and therapeutic resistance, though their role in CRC has not been studied at the clonal level. Cellular lentiviral DNA barcoding is a powerful tool that can track tumor cell subclones, number and size, within a tumour xenograft during tumorigenesis and therapy. We labeled patient tumor-derived CRC cells lines with $1.2x10^6$ unique barcodes and conducted in vivo tumor limiting dilution assays (LDAs). Resulting tumors contained multiple barcodes (polyclonal) with tumors at limiting dilutions harboring fewer barcodes. Secondary *in vivo* tumor LDAs enriched for CSC frequencies and produced dominant barcoded tumors with a few single barcoded tumors (monoclonal; established as cell lines). *In vitro* LDAs revealed enriched CSC-frequencies of the monoclonal tumors compared to the parental polyclonal population. Furthermore, we investigated whether monoclonal tumors **a)**have inherent dominant tumor-initiating capacity and/or **b)**cooperate to drive tumorigenesis. Preliminary experiments in which we mixed a monoclonal with a polyclonal cell line demonstrated clonal cooperation to gain growth advantage. While studies with defined genetic manipulations showed interclonal cooperation as essential for tumor maintenance, to date, no one has observed interclonal tumor cell dependence without genetic manipulation. Further analysis (transcriptomic, proteomic) will provide insight into the sources of intratumoral heterogeneity and guide treatment.

87. Personalized predictive biomarkers of therapeutic response and recurrence for individualizing ovarian cancer treatment

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Problem: Ovarian cancer (OC) is most lethal gynecological cancer in developed countries and survival rates has remained relatively unchanged over the past 30 years. Although complete remission is observed in most patients after 1st-line therapy of surgery and chemotherapy, 95% of advanced cases will relapse within 3-5 years. Unfortunately, the traditional OC biomarker, serum CA125, is controversial for monitoring relapse as initiating 2nd-line therapy sooner based on CA125 levels alone does not impact survival. CA125 is also elevated in only 70% of patients with confirmed relapse and not elevated in 20% of advanced disease. Clinical decision based on CA125 alone is thus difficult in many cases and urgent intervention may sometimes be missed. With up-and-coming immunotherapies and precision cancer medicines, there is a timely need for novel biomarkers of relapse in order to find the optimal time to start 2nd-line therapy and enhance survival outcome in each patient.

Hypothesis and Rationale: Owing to tumour heterogeneity, we hypothesize that each unique tumour secretes a distinct array of proteins into the blood, which if quantified could serve as personalized biomarkers that complement CA125. Future screening against a panel of personalized markers could identify the best biomarkers for each patient to guide individualized treatment strategies.

Methods and Results: To discover personalized OC markers, we used Proseek® panels (Olink, Sweden) to simultaneously measure 1,104 proteins (including CA125) in sera of 30 OC patients collected pre-operation, 3 weeks post-operation, 5 months and 11 months post-operation. CA125 was non-elevated upon relapsed in 8/25 patients with confirmed relapse. Non-cancer and healthy controls (N=21) were used. By choosing proteins which significantly decreased post-op and increased upon relapse (reflecting tumour burden) in each patient, we identified 21 personalized proteins (including CA125) that together is predictive of recurrence in 95% of patients (compared to 68% with CA125 alone). Our panel was informative in 88% of the low CA125 patients.

Conclusion: With future validation, personalized markers can be used to sensitively track OC tumours in each patient, ultimately playing key roles in customizing patient management and treatment.

88. Opposing effects of NPM1wt and NPM1c mutants on AKT signalling in AML

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Email: mark.minden@uhn.ca

While direct physical interaction with AKT/PKB protects nucleophosmin (NPM1) from proteolysis, it is not known whether wild-type or mutant forms of NPM1 such as NPM1c, which frequently occur in acute myeloid leukemia (AML), can affect AKT signalling. Here we show that human AML samples with NPM1c mutations as well as bone marrow and spleen cells from NPM1cA/+ knock-in mice exhibit significantly higher AKT phosphorylation relative to their NPM1 non-mutant counterparts. Mechanistically, we show that NPM1 inhibits AKT phosphorylation and downstream signalling, and that NPM1c can reverse the inhibitory effect of NPM1 on AKT phosphorylation. Moreover, NPM1c robustly binds AKT in the cytoplasm. Lastly, NPM1c expressing AML cells were significantly more sensitive to a small molecule AKT inhibitor compared with NPM1 non-mutant cells. These findings uncover an inhibitory effect of NPM1 on AKT, which is countered by NPM1c in AML, suggesting selective targeting of AKT signalling as a novel therapeutic modality for NPM1c-driven AML.

Zhuo Ren is currently a postdoctoral fellow in Minden lab at Princess Margaret Cancer Centre.

89. Development of a micro-ultrasound endoscopic imaging device for improved detection of esophageal cancer

C.F. Roa[1], J. Yin[2], A. Boyes[2], N. Singh[1], B. Motlagh[2], E. Chérin[2], F.S. Foster[1,2], C.E.M. Démoré[1,2]

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(Presenting author is a graduate student. This project was supported by TFRI New Frontiers Program Project Grant in *Porphysome Nanoparticle-Enabled Image-Guided Cancer Interventions,* project lead Dr. GANG ZHENG)

Improving survival rates of diseases like esophageal cancer (EC) strongly depends on early detection of tumors, especially when minimally invasive therapy is advisable and has positive outcomes. Current techniques to detect small malignant lesions in patients at risk of EC are limited in terms of specificity and ability to assess invasion depth. Endoscopic microultrasound (micro-US) and photoacoustic imaging (PAI) have the potential to give useful diagnostic information, including the visualization of changes in microvasculature, or upregulated molecular biomarkers such as epidermal growth factor receptor (EGFR) when combined with targeted contrast agents. We present ongoing work to develop a high frequency US mini probe for *in vivo* intraluminal imaging.

We have recently shown, in *ex vivo* tissue specimens, the feasibility of using a photoacoustic targeted contrast agent to visualize neoplastic lesions. We have also shown that the combination of photoacoustic imaging and micro-US can visualize the microvasculature and tissue structures at greater depths than conventional optical endoscopic techniques. The combination of the ultrasound and photoacoustic images could serve to characterize tissue and enable early stage treatment of disease.

We present the design for a micro-US array packaged in an endoscopic form factor. The device considers space for future addition of optical fibres for photoacoustic imaging. Fabrication approaches for defining array elements in PZT piezoceramic plates and electrode tracks on polyimide flexible substrate for cabling are explored. An excimer laser was used to separate individual elements in the ceramic. A novel photolithography process incorporating laser machining was used to pattern conductive traces on a flexible polyimide substrate without damaging the polymer; this forms the cabling connection to the 25 µm wide elements of the array. Metal sputtering followed by laser machining was used to connect element electrodes to copper traces on the flexible cabling. Electrical impedance, pulse-echo response and acoustic pressure fields were measured.

A prototype transducer array operating at 35 MHz center frequency with 32 elements was fabricated and characterized. Flexible circuit cabling with 60 µm pitch tracks was patterned and used to connect to individual elements. Electrical connections with metal vapor deposition on a loaded epoxy matrix could be reproduced reliably and are suitable for housing in 3¬¬ mm catheter. Electrical and acoustic characterization results show expected performance. Extension to a 64-element array with improvements in design (e.g. matching layers, improved material selection) to optimize performance will be discussed.

90. KSR1 potentiates mTORC1 signaling upon amino acid stimulation

Manipa Saha[1], Jose La Rose[1], Etienne Coyaud[1], Estelle M. N. Laurent[1], Brian Raught1[,2], ROBERT ROTTAPEL[1, 3, 4, 5]

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[5] Ontario Institute for Cancer Research

A hallmark property of cancer is deregulated signaling. Among the major signal transduction pathways utilized by cancer cells are the MAPK and PI3K-Akt-mTORC1, to generate proliferative signals that control entry into the cell cycle, protein synthesis and metabolic reprogramming. The present study investigates the kinase suppressor of Ras 1 (KSR1), a pseudokinase, which was originally identified as a scaffold protein required for optimum flux through the MAPK cascade. Recent studies have however shown a link between KSR1 and members of mTORC1 signaling, namely Raptor and S6K. Since mTORC1 is instrumental in relaying cues of amino acid availability to the cellular protein translation machinery, we attempted to understand whether KSR1 has a role in this function. In order to do so, we subjected cells to amino acid deprivation followed by stimulating with amino acids. Our data suggest that KSR1 depletion impairs mTORC1 signaling downstream of amino acid stimulation. We also uncover that KSR1 interacts with multiple members of this signaling pathway. The present study aims to understand mechanisms of KSR1-mediated regulation of mTORC1 signaling and therefore to unravel the biological significance of this novel interaction. We aim to elucidate a previously unanticipated mechanism of signal diversification through KSR1, potentially integrating mitogenic and biosynthetic pathways. This putative KSR1-mediated coupling between the two signaling pathways is expected to be a novel and attractive target to exploit in the context of cancer combat.

91. KSR1 potentiates mTORC1 signaling upon amino acid stimulation

Manipa Saha[1], Jose La Rose[1], Etienne Coyaud[1], Estelle M. N. Laurent[1], Brian Raught[1,2], **ROBERT ROTTAPEL[1, 3, 4, 5]**

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A hallmark property of cancer is deregulated signaling. Among the major signal transduction pathways utilized by cancer cells are the MAPK and PI3K-Akt-mTORC1, to generate proliferative signals that control entry into the cell cycle, protein synthesis and metabolic reprogramming. The present study investigates the kinase suppressor of Ras 1 (KSR1), a pseudokinase, which was originally identified as a scaffold protein required for optimum flux through the MAPK cascade. Recent studies have however shown a link between KSR1 and members of mTORC1 signaling, namely Raptor and S6K. Since mTORC1 is instrumental in relaying cues of amino acid availability to the cellular protein translation machinery, we attempted to understand whether KSR1 has a role in this function. In order to do so, we subjected cells to amino acid deprivation followed by stimulating with amino acids. Our data suggest that KSR1 depletion impairs mTORC1 signaling downstream of amino acid stimulation. We also uncover that KSR1 interacts with multiple members of this signaling pathway. The present study aims to understand mechanisms of KSR1-mediated regulation of mTORC1 signaling and therefore to unravel the biological significance of this novel interaction. We aim to elucidate a previously unanticipated mechanism of signal diversification through KSR1, potentially integrating mitogenic and biosynthetic pathways. This putative KSR1-mediated coupling between the two signaling pathways is expected to be a novel and attractive target to exploit in the context of cancer combat.

92. Overcoming challenges in longitudinal mouse intravital imaging

This project is sponsored by the TFRI New Investigator's Grant.

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Presenting author is a graduate student.

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Abstract:
Intravital microscopy is an extremely powerful tool that enables imaging of dynamic biological processes *in vivo*. Ongoing development of novel genetic tools, fluorescent probes, and optical imaging modalities have provided a valuable toolset for investigating several aspects of cell biology (both healthy and pathological) in live animals. To visualize the same tissue over a time-span of days to months, imaging windows must be used to expose the organ of interest. This allows access to tissues such as the brain, mammary glands, liver, kidney, pancreas, small intestine, and bone. In addition to surgical complexity, several practical aspects of these imaging windows can impede research involving live animal imaging. Spending time to troubleshoot these problems can not only slow research productivity but also valuable research funding.

Through our experience in developing intravital models, we share some common challenges that researchers may face in implementing a mouse-intravital model for their imaging experiment and provide strategies to overcome them. (1) Surgically implanting and maintaining imaging windows with the utmost care is imperative to their overall longevity. While different types of imaging windows will have their own considerations, minimizing animal discomfort is the main priority. If direct care is not taken to control analgesia and sterile procedures, this may result in damage to the imaging window, severe pain for the animal, infection, or death. (2) Once you have a stable imaging window, it essential to choose the microscope that best suits your animal model. Various light microscopy techniques such as wide-field fluorescence, laser scanning confocal, multiphoton, and spinning disk microscopy can be implemented. Certain variables such as imaging depth are imperative to visualize the tissue and maximize the amount of data being collected. (3) Depending on the location of the organ being visualized, motion artifacts can also significantly hinder data acquisition. Motion from breathing and heartbeat can compromise image quality and render quantitation impossible. Designing a custom animal restrainer can be a useful aid to immobilize certain tissues. (4) Finally, depending on the duration of each imaging session, animals should be sedated and continuously monitored for normal breathing and body temperature. Outfitting the microscope stage with anesthesia as well as proper heating and air circulation is necessary to ensure normal physiological conditions.

Intravital imaging has huge potential to help uncover dynamic physiological processes in vivo. However, there are several challenges that researchers may face. While technical skill and first-hand experience are both helpful when implementing a procedure for intravital imaging, understanding the major hurdles and how to overcome them can save valuable research effort.

93. Potential Role Of The Mitochondria-Associated Membrane In Protein Folding

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Background:
The tumor microenvironment is characterized by poor oxygen availability, known as hypoxia. Hypoxia-induced biological processes such as angiogenesis, invasion and migration are mediated by proteins that are secreted or expressed on the cell surface. Oxidative disulfide bond formation in the endoplasmic reticulum (ER) plays a critical role in the protein folding and stability of secretory proteins and is largely thought to depended on oxygen availability. However, our group has found that some of the hypoxia-induced secretory proteins including vascular endothelial growth factor (VEGF) are able to utilize a hitherto unidentified oxygen-independent pathway to complete their disulfide bond formation and traverse the secretory pathway under hypoxic conditions. In mammalian cells, in addition to the ER, mitochondria are also capable of forming disulfide bonds into proteins de novo. Protein oxidation in the ER and mitochondria is mediated by different folding factors but with a similar biochemical mechanism. Mitochondria and the ER maintain close structural and functional contacts via formation of mitochondria-associated membrane (MAM). Several mitochondrial and ER-resident protein folding factors as well as mitochondrial metabolites required for protein folding are enriched at the MAM. Data from our group suggests that VEGF may undergo ER-localized protein folding in proximity to the MAM. Intriguingly, mitochondria have been described to re-localize closer to the ER nuclear domain area in hypoxia.

Hypothesis:
Based on the unique environment at the MAM, rich in folding factors and mitochondrial metabolites required for protein folding, we hypothesize that the MAM supports protein folding in the ER.

Methods:
We apply pharmacological and genetic approaches to disturb the integrity of the MAM and assess its effects on protein folding. Alteration of ER-Mitochondria juxtaposition via the MAM is determined by transmission electron microscopy as well as confocal microscopy. Additionally, we investigate the effects of knockdown of mitochondrial protein oxidation factors on protein folding in the ER. We also isolate the MAM under normoxia and hypoxia and apply quantitative mass spectrometry-based proteomics technique to identify and assess potential MAM-resident protein folding factors under normoxia and hypoxia.

Results:
Disruption of the MAM integrity and knockdown of mitochondrial oxidoreductases lead to ER stress and unfolded protein response, supporting a possible role for the MAM in ER-localized protein folding. We were able to isolate the MAM and validate its purity by detecting reliable MAM protein markers.

Conclusion:
Our results suggest that the interaction of the ER and mitochondria may be essential for protein folding. Better understanding of protein folding pathways under hypoxic condition may help in the design of novel therapeutics that target protein folding and cell survival in hypoxic cancer cells.

94. Elucidating isoform specific roles for calpain-1 and calpain-2 in breast cancer

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Breast cancer is one of the most frequently diagnosed cancers worldwide. Despite progress in the early diagnosis and treatment, breast cancer still often reaches stages III and IV, when the expected overall survival significantly decreases.

Clinical analyses have shown that high expression of calpain-1 and calpain-2, prototypic members of a calcium-dependent subfamily of cysteine proteases, correlates with poor survival in breast cancer. Calpains act through proteolytic cleavage of their substrates; many of which regulate actin reorganization, cell survival signaling, invadopodial dynamics and resistance to chemotherapeutics. Calpain-1 and calpain-2 are ubiquitously expressed heterodimers consisting of catalytic subunits CAPN1 and CAPN2, respectively, and a common CAPNS1 regulatory subunit. We hypothesize that calpain-1 and calpain-2 play isoform-specific essential roles in breast tumor growth, metastasis and resistance to specific chemotherapeutics.

We developed a complete isogenic panel of MDA-MB-231 triple negative breast cancer cell lines which are CRISPR-Cas9 knocked out for individual *CAPNS1, CAPN1 or CAPN2* genes, and then rescued by transduction with GFP-expressing lentiviral vectors encoding corresponding Myc-epitope tagged recombinant proteins (both wild type and catalytically inactive in the case of CAPN1 and CAPN2), or empty vector. This panel of cells is being used to elucidate the individual and combined roles of calpain-1 and calpain-2 on *in vitro* migration, invasion, and sensitivity to paclitaxel, doxorubicin, and docetaxel; and in vivo tumor growth, metastasis and drug sensitivity.

95. Identification of novel epigenetic and druggable radiosensitizing targets in small cell lung cancer with an EpiDrug CRISPR screen

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Background: Small cell lung cancer (SCLC) is a highly aggressive type of lung cancer. Patients are treated with different DNA damaging agents, such as chemotherapy and radiotherapy. Due to rapid disease relapse caused by resistance, there is a need for novel treatments. Epigenetic mechanisms may be a targetable therapeutic vulnerability of SCLC.

Objective: This study aims to identify epigenetic and druggable targets that could sensitize SCLC cells to ionizing radiation and understand the mechanisms underlying these targets.

Method: A CRISPR knockout screen was conducted on the SBC-5 cell line using a custom EpiDrug CRISPR sgRNA library targeting ~1,000 genes: 334 genes are involved in epigenetic pathways and 657 genes with FDA approved drugs. Candidate genes with a statistically significant false discovery rate adjusted p-value were orthogonally validated using shRNA knockdown, sgRNA knockout, and pharmacological approaches with chemical probes depending on availability. Resazurin conversion assay and clonogenic assays were performed to quantify cell viability. Dose modification factor (DMF), defined as the ratio of radiation doses that achieve the same level of cell survival in a radiation alone group as compared to a radiation plus drug group, was calculated at 75% and 37% survival for the resazurin conversion assay and clonogenic assays respectively.

Results: In addition to known radiosensitizing genes, such as *ATM* and *BRCA2*, the screen has identified several promising candidates such as *HDAC3*. The impact of HDAC3 inhibition by suberoylanilide hydroxamic acid (SAHA) was quantified by a resazurin conversion assay in 9 SCLC cell lines and 1 *ex vivo* derived PDX cell line. Clonogenic assay experiments are ongoing. Cell viability results indicate that as a single agent, SAHA affected all 10 cell lines with an IC50 value in the range of 200 nM – 3 μM. SAHA significantly radiosensitized 8 cell lines with a DMF of 1.7 – 4.3. A SBC-5 clonogenic assay confirmed the radiosensitizing effect of SAHA with a DMF of 1.4.

Conclusion: Several potential radiation sensitizing targets have been identified using a custom EpiDrug CRISPR screen that may have clinical application and should be further investigated. One of these candidates is *HDAC3*.

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96. Cancer cell genotype as a determinant of response to STING agonist based combination chemo-immunotherapy

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High grade serous ovarian carcinoma (HGSC) is the most lethal gynecologic malignancy with high rates of chemotherapy resistance and poor outcomes. We previously showed that tumors from chemotherapy resistant patients show an immunologically cold phenotype, exhibiting lower density of CD8+ T cells and low expression of interferon genes. Subsequently, using the ID8-Trp53-/- murine model of HGSC, we further demonstrated the potential of **St**imulator of **In**terferon **G**enes (STING) pathway activation in enhancing response of HGSC to carboplatin chemotherapy. Currently, we aim to better characterize the effect of cancer cell genotype on response to treatment in HGSC.

The evolution of the tumor immune microenvironment (TIME) as immunologically hot or cold can be to some extent dictated by cancer cell specific genetic alterations. While loss of the tumor suppressor TP53 is a universal mutation (>96% of cases), previous reports in HGSC have shown that patients with ***BRCA1*** mutations (~25% of cases) have higher $CD8^+$ T cell infiltration and higher chemosensitivity. In contrast, loss of ***PTEN*** (seen in ~20% of cases) is associated with poor outcomes and chemoresistance.

We hypothesized that HGSC tumors with loss of ***PTEN*** expression can be treated via activating the STING pathway with STING agonist to increase immune activation and synergistically enhance the effect of chemotherapy. C57/BL6 mice were injected with either ID8-*Trp53*$^{-/-}$; *Brca1*$^{-/-}$ cells or ID8-*Trp53*$^{-/-}$; *Pten*$^{-/-}$ cells and subjected to one of three treatment groups: vehicle, carboplatin, or carboplatin + STING agonist. Characterization of the TIME of tumors generated from ID8-*Trp53*$^{-/-}$; *Brca1*$^{-/-}$ cells, and those from ID8-*Trp53*$^{-/-}$; *Pten*$^{-/-}$ cells, through local and systemic immune profiling, showed significant immunologic differences between different genotypes. Addition of STING agonist significantly increased chemosensitivity and improved overall response in ID8-*Trp53*$^{-/-}$; *Pten*$^{-/-}$ injected mice compared to those treated with carboplatin alone.

This study helps to determine the potential of STING pathway activation in inducing an activated tumor immune state in genotypes of HGSC that result in a "cold" TIME, such as loss of PTEN, to augment responses to chemotherapy and prolong survival.

Supporting Agency: Canadian Institutes of Health Research (CIHR) and the Early Researcher Award from the Ontario Ministry of Research, Innovation and Science

97. Transurethral light delivery for photoacoustic imaging of porphysome contrast agent in the prostate: Evaluation in a tissue mimicking phantom

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Background, Motivation and Objective

Prostate cancer (PCa) is a focal disease that has the potential to be successfully treated with focal or targeted therapies. However, these treatment approaches need accurate index lesion delineation and real-time image guidance. We are investigating photoacoustic (PA) imaging combined with a transrectal micro-ultrasound (micro-US) as a means of achieving both high optical contrast and high spatial resolution for guiding focal therapies in the prostate. Prior work indicated deeper illumination is required to obtain useful PA images of the prostate. The PA image can also be enhanced with improved imaging depth and by using PA contrast agents, for example organic porphyrin-lipid porphysome (PS) nanoparticles that have enhanced uptake in tumours. Here we introduce and evaluate a transurethral (TU) illumination system combined with PS contrast agents for improved PA imaging depth in the prostate.

Statement of Contribution/Methods

Side-fire TU optical fiber tip has been designed to illuminate the prostate from the urethra in large animal disease models. A 1.5 mm core multimode optical fiber was flat cleaved and polished with a right-angled prism to divert the light at 90° and held in a 5 mm diameter case. A tunable (680 to 970 nm) laser (Vevo LAZR, VisualSonics, Toronto, Canada) was coupled into the TU fiber. The fiber was evaluated with a cylindrical PVC-Plastisol prostate mimicking phantom with optical absorbers and acoustic scatterers; the fiber was placed in the center of the lumen mimicking the urethra. Polyurethane (PUR) tubes were inserted at up to 15 mm radially from the central lumen, approximating the distance between urethra and rectum, and filled with 35 μM -135 μM solution of PS contrast agent. A commercial 256-element, 20 MHz micro-US array was used for PA mode receive and B-mode image acquisition. The acquired PA signal was spectrally unmixed to separate PS from other optical absorbers in the phantom.

Results/Discussion

In air, the TU fiber generated 160 mJ/cm^2 fluence at 2 mm with an input laser pulse energy of 5mJ. PA and B-mode images localized PS contrast to all PUR tubes for contrast agent concentration > 135 μM. We were able to detect 35 μM concentration at 10 mm but SNR at lower concentrations needs more investigation and optimization of the phantom. These results are promising for TU illumination of the prostate and future translation to imaging PS nanoparticles in tumours to delineate lesions for focal therapy.

This project was supported by: TFRI New Frontiers Program Project Grant in P*orphysome Nanoparticle-Enabled Image-Guided Cancer Interventions* (Program Lead: Dr. Gang Zheng)

98. ICOSL.COMP: A Powerful Enhancer of Anti-Tumoral Responses Restored by Immune Checkpoint Inhibition

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The Inducible T cell Costimulator (ICOS) is a receptor in the CD28 family of B7-binding proteins expressed mostly by activated T cells. Upon binding to its ligand, ICOSL, a co-stimulatory pathway is engaged resulting in the induction and regulation of effector T-cells. Multiple lines of evidence suggest that the activation of the ICOS:ICOSL pathway is essential for an optimal anti-tumour response to anti-CTLA4 immunotherapy. Current agonistic strategies rely on the generation of Fc-ligand fusion constructs and/or monoclonal antibodies. These modalities however have several drawbacks such as Fc-mediated cytotoxicity and/or the necessity for Fc-mediated cross-linking *(in vivo)*. Our laboratory has recently observed that, higher-order, avidity-based engagement of other IgV-containing immune checkpoint receptors represents a key factor in agonizing signal transduction. Accordingly, we have designed a high avidity pentavalent form of the human ICOSL extracellular domain by fusing it to a short -helical peptide from the human cartilage oligomeric matrix protein (COMP), termed ICOSL.COMP. ICOSL.COMP, spontaneously assembles into stable pentamers, binds tightly to both human and murine ICOS (K_d <10 nM) and co-stimulates (with anti CD3 mAb) both murine and human CD4+ and CD8+ T cells when added as a soluble factor (unlike ICOSL.Fc). Impressively, most mice treated with ICOSL.COMP combined with an immune checkpoint inhibitor (anti-CTLA4 mAb), were either cured or displayed a stable tumor burden in contrast to checkpoint monotherapies (anti-CTLA4 mAb or anti-PD1 mAb or ICOSL.COMP). In summary, our results suggest that ICOSL.COMP represents a new and powerful biologic to be used in combination with existing immune checkpoint inhibitors.

99. Predictive Modelling of Cancer-Type in Li-Fraumeni Syndrome

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Objective: Li-Fraumeni syndrome (LFS) is an autosomal dominant cancer predisposition syndrome associated with a germline *TP53* mutation. Individuals with LFS are prone to developing a wide spectrum of tumours at varying ages. However, germline *TP53* mutations themselves do not explain specific cancer phenotypes in LFS, nor the collective clinical heterogeneity. Hence, it is important to consider genomic and epigenomic data in the development of predictive algorithms in these patients.

Methods: This study consists of blood-derived methylation and whole genome sequencing from LFS patients (n=134). A generalized linear model was implemented to estimate the probability of getting a particular cancer-type based on methylation patterns. The cross-validation hold out randomization test (CV-HRT) was used to identify the most predictive features. Enrichment of these features for transposable elements (TE) was evaluated in WGS using RepeatMasker and MELT.

Results: The classifier can accurately predict cancer-type in LFS (auROC=0.963;F1=0.808). CV-HRT identified differential methylation of endocytosis, TGF-B signaling and axon guidance as predictive of cancer-type. These predictors show an enrichment for TE, which are hypomethylated in LFS patients with cancer. Stratifying patients into a high and low risk by burden of cancer-type specific genic TE shows a significantly earlier age-of-onset for the high-risk group ($p<0.0001$).

Conclusion: This project illustrates the contribution of genetic changes in LFS beyond TP53 and highlights the existence of molecular differences within LFS patients that contribute to phenotypic differences. Ultimately, it will allow for the early detection of tumour-onset in LFS patients to assist clinicians in developing personalized surveillance protocols.

100. RTK expression in canine mast cell cancer: influence on disease outcome in a model of human Mastocytosis

S. Syed, B. Knight, R. Foster, G. Wood, B. COOMBER

Canine mast cell tumours (MCTs) are a potentially useful preclinical model of human mastocytosis. These spontaneous and common tumours are frequently driven by the receptor tyrosine kinase (RTK) KIT, but also express other RTKs, the significance of which for disease progression is unclear. To assess this, a tissue microarray (TMA) was constructed with 0.6mm cores taken from paraffin blocks of canine MCTs. Immunohistochemistry for VEGFR2 and PDGFR-beta was performed and slides were scanned using the Digital Image Hub hosted by Vanderbilt University Medical Center. H-scores were generated for cores based on cytoplasmic IHC intensity. Cores were excluded from analysis due to loss, technical artifact, or less than 30% neoplastic cells. In total, 208 tumours (including 94 dermal, 94 subcutaneous, and 14 metastatic lesions) from 189 dogs were analyzed. H-scores were stratified into high and low score groups using X-tile, for analysis. Kaplan-Meier curves and log-rank analysis for progression free survival and overall survival showed that a higher VEGFR2 H-score was associated with better overall survival compared to lower VEGFR2 H-score in dermal tumours only. In contrast, higher H-scores for PDGFR-beta showed a worse overall survival, again only for dermal tumours. Our findings suggest that low VEGFR2 and high PDGFR-beta MCT expression may impact aggressiveness of dermal MCTs. Two RTK inhibitors are used clinically for this disease: toceranib (Palladia, which inhibits cKIT, VEGFR2 and PDGFR-beta) and masitinib (which inhibits cKIT and PDGFR-beta but not VEGFR2). Our findings support the advantage of treating aggressive dermal MCTs with masitinib rather than toceranib

101. Cancer stem cells and disease progression in multiple myeloma: fact or fiction?

Ines Tagoug, Natalie Erdmann, John Konda, Ali Mahdipour, Michelle Chattaragada, and Rodger Tiedemann

Multiple Myeloma (MM) is a mature B cell neoplasm characterized by the accumulation of immunoglobulin-secreting plasma cells (PC) within the bone marrow. A remarkable therapeutic development, including immunomodulatory drugs (thalidomide, lenalidomide and pomalidomide), proteasome inhibitors (bortezomib and carfilzomib), alkylators, steroids, monoclonal antibodies, and histone deacetylase inhibitors has been made in the treatment of MM. Despite the remarkable progress in MM drug discovery showing a significant prolongation of patient's survival, relapses remain a major impediment to the complete remission. The failure to cure MM, despite deep treatment responses, suggests the existence of intra-tumor heterogeneity and that at least some cells are not targeted by current PC therapies. The persistent cells, which possess the capacity to re-initiate MM are often referred to as tumor-initiating cells or myeloma steam cells. Unfortunately, the identification of the cell types responsible for MM progression remain unclear and introduced confusion. Understanding the cell hierarchy of MM is fundamental for the cure.

Our group identified a clonal tumor cell subpopulation within the bone marrow of MM patients that recapitulate the physiologic maturation stages between post germinal centre B cells and plasma cells. These clonal MM cells include tumor cells that resemble $CD20^{+}$ $CD27^{+}$ $CD38^{-}$ $CD138^{-}$ $Irf4^{-}$ $Xbp1s^{-}$ memory B cells, $CD38^{+/-}$ $CD138^{-}$ $Irf4^{-}$ $Xbp1s^{-}$ pre-plasmablasts, $CD38^{+}$ $CD138^{-}$ $Irf4^{+}$ $XBP1s^{+}$ plasmablasts and tumor-bulk $CD38^{+}$ $CD138^{+}$ $IRF4^{+}$ $Xbp1s^{+}$ plasma cells. In the current study we are investigating the functional phenotype, genomic profile, gene expression, cellular hierarchy and stemness of intra-tumor MM subpopulations using IF-FISH, in vitro differentiation, engraftment in mice, whole exome sequencing and 10X single-cell RNA-sequencing. Using IF-FISH, a clonal tumor progenitor cells were detected in the clonal MM cells supporting the hypothesis that MM progenitor cells may be a mediator of relapse in patients. To further clarify whether these immature MM cells actually cause disease, we used next generation sequencing and 10X single-cell-RNA-sequencing to characterize SNVs and copy number variants CNV in these clonal MM cells. Towards this goal we have performed preliminary whole exome sequencing of several tumor samples and control germ line cells and have developed a pipeline for variant calling. As the frequency of MM progenitor cells in clinical samples is often low, we optimized the sequencing strategies by designing a custom capture targeting only tumor SNVs regions. Significantly, we find that genetic diversity and SNVs identified in tumor bulk PCs, are also identified within pre-PC tumor cells suggesting that rare immature MM cells may play a role in the genetic evolution of MM and may represent a reservoir for subclonal relapse.

Using in vitro assays, primary MM $Xbp1s^{-}$ pre-PC appear to possess the ability to differentiate into mature $Xbp1s^{+}$ MMPCs, supporting a model in which these may act to replenish MMPCs. Notably, however, MMPCs do not appear to be continuously replenished by pre-PC as in some cases we have identified genetic events that appear to show restricted representation amongst MMPCs, suggesting that MMPC comprise a self-sustaining tumorigenic population in their own right. To better understand cellular hierarchy in MM we transplanted primary MM subpopulations into NSG mice bearing human bone chips. These studies suggest that CD138+ MMPC are plastic and under some conditions may dedifferentiate to generate CD138- Xbp1s- cells. However, tumor development was infrequent; we are therefore currently developing a humanized (IL6, BAFF, APRIL, GMCSF, IL3, SCF) NSG mouse that we hope will better support human hematopoietic cell and B cell engraftment.

Overall, our studies suggest that rare immature MM cells may survive standard treatments in the clinic and that substantial genetic diversity can exist within these subpopulations. As the immature MM progenitor cells can mature into tumor-bulk plasma cells, at least in vitro, they likely contribute to disease persistence, evolution and relapse in patients. These findings imply that treatment strategies in MM need to better address rare tumor progenitor cells whilst also erradicating tumor-bulk MMPCs to achieve deeper treatment responses and cure for patients.

102. Novel immunotherapeutic targeting of Carbonic Anhydrase 9 (CA9) in Glioblastoma using Dual Antigen T-cell Engagers (DATEs)

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Glioblastoma (GBM) is the most common malignant primary adult brain tumor, characterized by extensive cellular and genetic heterogeneity. Even with surgery, chemotherapy with temozolomide (TMZ), and radiation, tumor re-growth and patient relapse are inevitable. This poor patient survival is correlated with increased presence of chemo- and radio-resistance brain tumor-initiating cells (BTICs). Tumor microenvironment is considered as one of the main factors that can promote stem cell maintenance and therapeutic resistance.

To study the BTIC chemo-resistance, we developed a novel *in vitro* stem cell model to test the effect of TMZ on primary treatment-naïve BTIC enriched GBM samples. We observed that expression of Carbonic Anhydrase 9 (CA9), a hypoxia and acidic stress induced enzyme, was increased in TMZ resistant cells. We also observed that CA9+ GBM BTICs have increased self-renewal and proliferation properties when compared to CA9- cells. This indicates that CA9 plays an important role in treatment resistance and thereby recurrence, underscoring that CA9 is a potential therapeutic target in GBM.

To target CA9+ GBM BTICs, we constructed CA9 DATEs which consist of two arms for CA9 and T-cells CD3 antigen recognition. The dual specificity of DATEs for CD3 of T cells and CA9 on GBM cells were confirmed using flow cytometry. We observed that in the co-culture of T cells and GBM cells, $CA9^{high}$ GBM cells were killed in the presence of CA9 DATEs, validating their specificity to target CA9+ BTICs. Incubating T cells with CA9 DATEs and GBMs resulted in increased surface expression of T-cell activation markers CD69 and CD25 in both, CD4+ and CD8+ T cells. Moreover, activated T cells showed higher release of pro-inflammatory cytokines including TNF-a and IFN-y. Treatment of GBM tumor-bearing mice with CA9-specific DATEs yielded extended survival in mice and significant reductions in brain tumor burden.

Targeting CA9+ GBM BTICs provides us with the opportunity of killing GBM BTICs which eventually reduces cancer progression. This rigorously obtained data suggest that DATE-mediated cytotoxicity against treatment-resistant and evasive CA9+ GBM BTICs could provide a novel therapeutic strategy for GBM patients.

*The presenting author is trainee (PhD Candidate). The project is supported by the TFRI.

103. Investigating the Status of the Tumor Microenvironment in Li-Fraumeni Syndrome

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Li-Fraumeni Syndrome (LFS) is a highly penetrant cancer predisposition syndrome associated with germline mutations of the *TP53* tumor suppressor. LFS patients face a significantly elevated lifetime risk of developing a broad spectrum of early-onset malignancies including sarcomas, breast, and brain cancers. Emerging evidence has implicated p53 as a mediator of immune responses. Of particular relevance for anti-tumor immunity are proposed roles for p53 in the regulation of immune checkpoints and natural killer (NK) cell activation. However, the immunological status of LFS patients, in whom p53 function is reduced or altered, is unclear. Given the prognostic significance of intra-tumoral immune infiltration and interest in cancer immunotherapy, including immune checkpoint inhibition, as a treatment modality for clinically challenging malignancies such as those characteristic of LFS, study of the immunological status of these patients is imperative. The objective of our study was to clarify how p53-dependent processes in immune cells might regulate the composition of the tumor microenvironment in LFS-associated cancers. We analyzed 51 unique tumors of various histological types procured from pediatric LFS and non-LFS cancer patients. These tumors were subjected to bulk RNA-sequencing and various immunogenomic analyses - namely, immune contexture deconvolution with the quanTIseq and EPIC modules and single sample gene-set enrichment analysis with the xCell and MCPcounter modules, to qualify and quantify the tumoral immune cell composition. Additionally, blood-derived DNA methylation analysis was performed on 134 samples procured from LFS patients either affected or not affected with various LFS spectrum cancers. Differential expression of key immune-related gene promoter regions was observed in LFS patients affected versus unaffected with cancer. Specifically, *FOXP3, CTLA4,* and *ICOS*, were hypomethylated in LFS patients affected with cancer, and *ULBP1* was hypermethylated in these patients. Additionally, analysis of methylation-based immune contexture deconvolution revealed NK cells to be associated with cancer status in LFS patients – LFS patients affected with cancer had a lower normalized fraction of NK cells versus those who were unaffected. Preliminary transcriptomics-based immunogenomic analyses have suggested that certain LFS-associated tumors may have impaired anti-tumor immunity, possibly associating with the prognosis of the tumor type in question. NK cells, $CD8^+$ T cells, and endothelial cells were significantly depleted in LFS patient tumors relative to their quantified proportions in sporadic pediatric tumors of related histological types. Further characterization of p53-dependent processes in immune cells and their relevance in LFS could help to inform future study and use of immunotherapeutic agents in this clinical context with the ultimate goal of improving the clinical management and survival of LFS patients with cancer. This project is the first comprehensive analysis of the immunological status of LFS and utilizes the largest LFS cohort to-date. Our findings illustrate the contribution of genetic changes in LFS to the composition of the tumour immune microenvironment and highlight how these may be associated with cancer status.

104. A Low Carbohydrate Diet orchestrates with pks+ E.coli to induce Colitis-Associated Colon Cancer

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Over the past century, a dramatic increase in microbiota-associated diseases has been observed which is likely due to changes in dietary habits associated with the industrial revolution. Colitis-associated cancer (CAC) is one such disease and has been found to be strongly associated with dietary habits in terms of incidence and mortality. There is limited evidence for the existence of specific interactions between the gut microbiota and dietary components which influence host and/or microbial metabolism to either predispose to or protect from CAC. Clinical studies have identified pathobionts associated with CAC but their role as driver and/or passenger in CAC pathogenesis needs further investigation. Since, pathobionts are only expected to cause disease in a specific setting, and specific diets are frequently associated with increased colorectal cancer incidence, we investigated the effects of dietary components in microbial-induced CAC pathogenesis. Using colitis susceptible mice models, we investigated the effect of diets with reduced fiber on CAC initiation by various microbes that secrete genotoxins. While *E.coli* NC101 infection does not appreciably increase polyps in $IL10^{-/-}$ mice, a low carbohydrate diet (LC diet), but not the western diet, enhanced *E.coli NC101*-induced CAC and the effect was dependent on the genotoxin gene colibactin that is expressed by *E.coli NC101*. Furthermore, the effect of the LC diet on microbial-induced CAC was specific for *E.coli NC101* since CAC-induced by other genotoxin-secreting microbes (i.e. *Enterotoxigenic Bacteroides fragilis (ETBF) and Helicobacter hepaticus*) was unaffected by LC diet. We found that the LC diet degrades the colonic mucus barrier and maintains increased colonization for promoting *E.coli NC101* pathogenesis. Since the LC diet leads to a reduction in microbial-produced short-chain fatty acids (SCFAs), we supplemented the LC diet with exogenous SCFAs, and this significantly rescued the LC diet-mediated effect on *E.coli NC101*-induced CAC. This study highlights the specific interaction of dietary components with particular microbes during CAC initiation which could lead to reduced CAC susceptibility through dietary and/or microbial intervention.

105. Patient Derived High Grade Serous Ovarian Cancer Organoids in Princess Margaret Living Biobank

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University Health Network, Princess Margaret Living Biobank

High grade serous ovarian cancer (HGSOC) is the most common and deadly ovarian cancer, with little improvement in survival rates over the past twenty years. Patient derived organoids (PDO)s are more readily generated and experimentally representative than traditional cell lines. The Princess Margaret Living Biobank (PMLB) is a repository core of PDOs and patient derived xenografts generated from normal and various cancer type tissues, including ovarian. PDOs and drug screening services are available through PMLB.

Surgical resections and ascites samples were collected from HGSOC patients through the UHN Biobank. Cells were dissociated from tumor tissues and/or enriched from ascites fluid, embedded in a Matrigel dome and overlaid with HGSOC organoid specific media. Resulting PDO cultures were passaged every 1-4 weeks. Histopathological identification of ovarian organoids was confirmed by p53, PAX8 and AE1/AE3 staining. Short tandem repeat (STR) genotyping was used to match cultures to their parental tissue.

From a total of more than 100 samples, 78 short term (1-4 passages) and 3 long term (passage 6 and beyond) models were generated, characterized and biobanked. The long term cultures were subjected to quality control tests including STR. STR comparisons between established models and the patient ranged in similarity from 91-100%. These long term cultures expressed similar immunohistochemical staining to their parental source/tissue and were able to propagate for upwards of 300 days or 18 passages. HGSOC organoids differ in morphology; OCAD36.g1 presented predominantly as cystic organoids with clearly defined lumens while OCAD55 were morphologically dense structures with aggregates of cells. The heterogeneity of the organoid morphology demonstrates the heterogeneity of the disease.

The long term HGSOC organoids banked were histopathologically and genetically very similar to their parental match. Although HGSOC is an aggressive disease, ovarian organoid cultures are difficult to generate and slow to propagate. Further optimization of the growth and media conditions is likely needed.

106. Computational pharmacogenomics screen identifies synergistic statin-drug combinations as anti-breast cancer therapies

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Triple negative breast cancer (TNBC) is an aggressive subtype of breast cancer that has the worst prognosis amongst major breast cancer subtypes. Current therapy is ineffective as TNBC responds initially to chemotherapy but tends to recur quickly after treatment. Statins are traditionally used to treat high cholesterol but have been re-evaluated for their role as an anti-cancer agents. Epidemiological studies, clinical trials and pre-clinical studies conducted in breast cancer that have demonstrated efficacy for the re-purposing of statins. Statins inhibit the rate limiting enzyme, HMG-CoA reductase of the mevalonate pathway. In response to statin treatment, the cholesterol levels in the cell decrease and the cells trigger a feedback response through the transcription factor family sterol regulatory element-binding proteins (SREBPs). The SREBPs upregulate mevalonate specific genes to restore homeostasis to the pathway. Previous work in our lab has shown that inhibiting this feedback response with dipyridamole, an FDA-approved anti-platelet agent, can potentiate the anti-cancer activity of statins.

By identifying additional potentiators of statins that act similar to dipyridamole using computational pharmacogenomic screen we can provide a novel and effective therapeutic to inhibit TNBC progression.

107. Developing a platform for "off-the-shelf" CAR-T cell therapies for cancer using double negative T cells

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Acute lymphoblastic leukemia (ALL) accounts for 19-25% of all cancers in patients under the age of 20. B-cell (B)-ALL specifically is responsible for most ALL cases. With induction chemotherapy, lasting remissions are uncommon, and long-term survival for relapsed patients is below 10%. Recently, highly effective autologous chimeric antigen receptor-modified T cell (CAR-T) therapies targeting the B-cell lineage antigen, CD19, have been FDA-approved for B-ALL. Clinical trials treating relapsed and refractory B-ALL patients reported remarkable results, with remission rates above 80% and 5-year survival rates above 78%. However, this novel immunotherapy still faces many challenges such as high treatment costs, patient ineligibility, and cell manufacturing failures, making autologous CAR-T therapy inaccessible to many patients. One approach to overcome these challenges is to use allogeneic donor-derived CAR19-T cells. However, serious toxicities can arise from graft-versus-host disease (GvHD), and host-versus-graft (HvG) rejection, counteracting the effectiveness of the treatment. $CD3^+CD4^-CD8^-$ double negative T cells (DNTs) are a rare subset of mature T cells that can be expanded ex vivo under clinically compliant conditions from the peripheral blood of healthy donors. DNTs fulfill the requirements of an off-the-shelf (OtS) adoptive cellular therapy (ACT) including donor-independent anti-leukemic activity, cryo-preservability, resistance to HvG rejection and no observed off-tumor toxicity, thus increasing their clinical utility at a lower cost. Incorporating CAR19 technology onto DNTs will create a potent OtS-ACT against $CD19^+$ B-ALL. Here we show that DNTs can be successfully transduced with a 2^{nd} generation retroviral anti-CD19-CAR construct with a transduction efficiency above 50%. CAR19-transduction does not affect the normal DNT phenotype, expansion or endogenous cytotoxic function against CD19- leukemia. CAR19-DNTs induce superior cytotoxic function against the CD19+ B-ALL cell line, NALM-6, as well as B-ALL patient samples compared to non-transduced (NT)-DNTs. The *in vitro* efficacy of CAR19-DNTs against $CD19^+$ leukemia is comparable to that of CAR19-transduced conventional $CD4^+/CD8^+$ T (T_{conv}) cells. Importantly, in contrast to CAR19-Tconv cells, CAR19-DNTs showed no alloreactivity against normal allogeneic cells. Moreover, NALM-6 engrafted mice treated with CAR19-DNTs show significant reduction in leukemia load in a dose-dependent manner, with near-complete leukemia eradication at the highest dose. Also, CAR19-DNT treated mice exhibited prolonged survival as well as superior overall health status compared to untreated mice. Collectively, the findings from this study demonstrate the potential of using DNTs as a platform for CAR-technology to provide a novel therapeutic treatment option for B-ALL patients that is equally effective, but safer, more accessible, and more affordable. Importantly, this study will also provide a deeper insight into the generalized application of DNTs as an OtS CAR-vehicle, not only for other $CD19^+$ hematological cancers, but for all CAR-based cancer immunotherapies.

108. ^{177}Lu-Chelated Nanotexaphyrins for PDT/SPECT Theranostics

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Texaphyrins are macrocyclic molecules capable of stably chelating large metal ions that have been clinically investigated for precision imaging and ablative therapies (e.g. computed tomography, magnetic resonance imaging, and photodynamic therapy (PDT)) [1]. We recently developed nanotexaphyrin with texaphyrin-lipid conjugates that self-assemble into liposomal nanoparticles. These nanoparticles exhibit good biocompatibility and are produced with high metal ion loading capacity [2]. The ^{177}Lu-texaphyrin complex is both a singe-photon emission computed tomography (SPECT) contrast agent and a potent photosensitizer [1], presenting us with a unique opportunity for image-guided PDT in the treatment of oligometastatic diseases.

To optimize Lu-nanotexaphyrin for sensitive SPECT imaging and potent PDT, a hybrid ^{177}Lu/^{175}Lu-nanotexaphyrin will be synthesized by relying on a previously developed "mix-and-match" chelation strategy [2]. This strategy allows us to chelate the vast majority of texaphyrin-lipid monomers with stable ^{175}Lu for photodynamic activity, while only needing to post-insert a small fraction of radioactive ^{177}Lu to generate SPECT contrast. Having already developed 100% ^{175}Lu-chelated nanotexaphyrins, we are now focused on assessing the photo-properties of these particles before beginning radiolabeling studies. In doing so, we hope to achieve an optimal balance between imaging sensitivity and radiotoxicity by modulating the ratio of stable and radioactive lutetium isotopes. Finally, we will evaluate the *in vivo* characteristics of this particle following the strategy in Figure 1. We hope to harness the safety profile of metallo-nanotexaphyrins to enhance therapeutic efficacy by SPECT-guided PDT in order to meet clinical needs for precision imaging and therapy of oligometastatic diseases.

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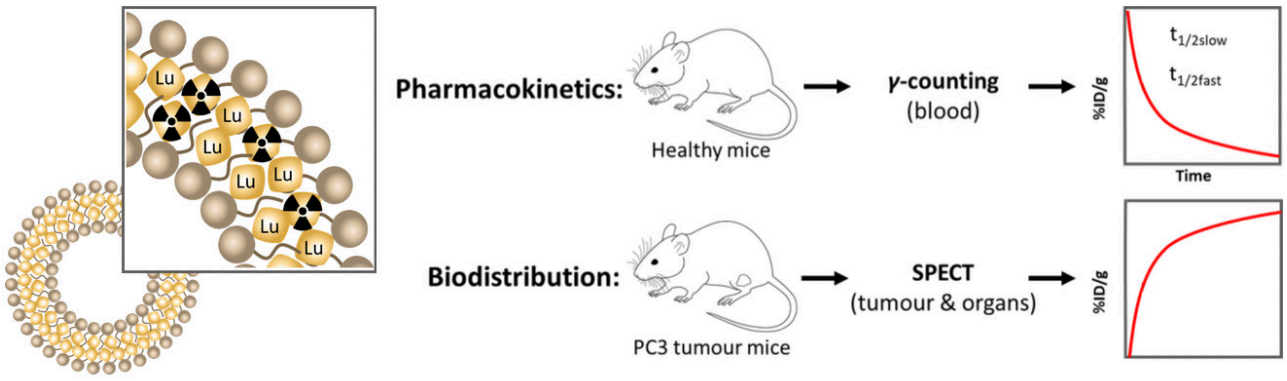

1: Hot/cold Lu-nanotexaphyrin will be **Figure** assessed in rodents.

109. Targeting Epigenetic Regulation in Clear Cell Renal Cell Carcinoma Reveals PRMT1 as a Novel Target

Sporadic Renal Cell Carcinoma (RCC) is dominated by the clear cell subtype (ccRCC) and overwhelmingly associated with a biallelic inactivation of the von Hippel-Lindau (VHL) gene leading to constitutive activation of the cell's hypoxia response and deleterious alterations to gene expression, metabolism and growth characteristics. However, VHL inactivation alone is insufficient to cause tumourigenesis. Other key genetic players identified through sequencing of ccRCC patient cohorts include frequent inactivating mutations in epigenetic regulatory enzymes- proteins that mediate the accessibility of transcription machinery to specific areas of the genome. The high frequency of these alterations in ccRCC implicate epigenetic vulnerabilities that may be exploited to develop new therapies.

Accordingly, our lab has completed a proliferative screen of the Structural Genomics Consortium's (SGC) epiprobe library in patient derived ccRCC models and a particularly favorable growth inhibition profile was noted for the probe MS023, which inhibits members of the type I protein methyltransferase (PRMT) family, including the canonical PRMT1. PRMTs transfer methyl groups to both nuclear histones and cytoplasmic targets, influencing gene expression, cell signaling, growth and viability. Evidence is mounting that the dysregulation of these enzymes is implicated in the biology of cancer, but they have not been investigated in the context of ccRCC. The aim of this project is to validate, characterize and further delineate the biological relevance of PRMT1 inhibition as a potential therapeutic approach.

As our nascent understanding of the regulation, function and clinical relevance of arginine methylation continues to expand, this project represents an exciting opportunity to contribute to this body of knowledge, while at the same time describing novel therapeutic approaches to ccRCC with the potential for rapid clinical translation.

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110. NPM1c Impedes CTCF Functions through Cytoplasmic Mislocalization in Acute Myeloid Leukemia

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Acute myeloid leukemia (AML) is not a single disease but rather a collection of diseases caused by a variety of mutations. About 30% of AML cases are characterized by a TCTG insertion in the exon 12 of Nucleophosmin 1 (NPM1c); the resulting frameshift creates a nuclear export signal (NES) and cytoplasmic localization of NPM1c. However, how NPM1c causes AML is not completely understood. NPM1 participates in multiple protein-protein interactions one of which involves the CCCTC binding factor (CTCF). Through binding of CTCF binding sites (CBS), CTCF mediates nuclear functions including DNA looping, regulation of gene expression and RNA splicing. Based on this observation, we hypothesized that mislocalization of CTCF into the cytoplasm by NPM1c reduces the functional level of nuclear CTCF and so alters gene expression.

To demonstrate that NPM1c could mislocalize CTCF to the cytoplasm we used confocal microscopy. While CTCF was completely nuclear in cells expressing NPM1, CTCF was present in the nucleus and the cytoplasm of cells expressing NPM1c. The cytoplasmic interaction of NPM1c and CTCF was confirmed by immunoprecipitation and western blotting.

Increased expression of HOXA9 is a characteristic of NPM1c AML and has been shown to be regulated by CTCF binding between *HOXA9* and *HOXA7* in IMR90 cells. As the sequestration of CTCF into the cytoplasm should mimic CTCF knockdown, we predicted that expressing NPM1c would similarly induce HOXA9. Upon transduction, there was increased expression of HOXA9 in the NPM1c, but not NPM1 modified cells. Moreover, the NPM1c IMR90 cells became immortal. We found that there was reduced binding of CTCF to the CBS 2kb downstream of HOXA9 NPM1c IMR90 cells. Reduced CTCF binding to this site was also observed in other NPM1c expressing cell lines including NPM1c AML cell line OCI-AML-3. Functionality changes resulting from loss of CTCF binding in NPM1c cells was further demonstrated by changes in CTCF mediated alternate splicing and enhancer blocking activity using reporter assays. Finally, interrogation of the TCGA AML data set showed increased methylation of CBS in NPM1c AML. This is in keeping with de novo methylation that occurs when CTCF no longer binds to its cognate site. Moreover, the addition of frequently co-occuring epigenetic mutations affects the degree of methylation in CBS: the presence of a mutant IDH increased the proportion of methylated CBS while there were fewer methylated CBS with DNMT3A.

Finally, we determined that CTCF a.a.1-265 interacts with the C-terminus of NPM1c and NPM1wt a.a. 244-298. As this interaction occurs with both forms of the NPM1 protein, it suggests that the region of contact is immediately N-terminal of the mutation insertion site in the 12th exon. Using the N-terminal fragment of CTCF, we showed that its introduction into cells expressing CTCF and NPM1c resulted in CTCF nuclear relocalization. Moreover, the repression of HOXA9 expression through restoration of nuclear CTCF from the cytoplasm in NPM1c cells further confirms that disruption of the NPM1c:CTCF interaction can reduce HOXA9 expression.

Based on these studies we propose that the transforming effect of NPM1c is due, in large part, to its ability to mislocalize CTCF and suggests that targeting the interaction between these two proteins could be of potential therapeutic benefit.

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111. Characterization of the role of Tandem Ubiquitin Binding Motif (tUBM) of E3 ligase HUWE1

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Abstract

HUWE1, also named MULE and ARF-BP1, is an E3 ligase of ubiquitination. It regulates various cellular processes including apoptosis, DNA repair, and transcription regulation through ubiquitinating its substrate proteins such as p53, c-Myc, Mcl-1 and BRACA112345. HUWE1has reported to be involved in tumorigenesis and is considered as a potential therapeutic target of multiple cancers67891011. However, the structure and function of HUWE1 protein has not been fully revealed. As one of HUWE1 domains, the tandem ubiquitin binding motif (tUBM) locates upstream to HECT catalytic domain containing three repeats that each repeat can recruit one ubiquitin molecule. Through recognizing ubiquitin or ubiquitin chain and non-covalently binding to ubiquitin, tUBM domain modulates E3 ligase activity of HUWE1. Since different ubiquitin linkages determine the distinct fates of substrate protein leading to different corresponding cellular responses, it is important to find out the preference and specificity of ubiquitin linkages recognized by tUBM. The previous data (Sheng lab) showed that linear (M1) and K63 di-ubiquitin linkage are preferred by tUBM over K48 di-ubiquitin linkage. In this project, the atypical di-ubiquitin linkages such K6, K11, K27, K29 and K33 interacting with tUBM were also tested. The result from affinity binding experiment through bio-layer interferometry and native gel electrophoresis implicates that tUBM domain interacts all eight types di-ubiquitin linkages with different affinity. Moreover, the effects of tUBM domain on HECT domain catalytic activity were also characterized. In-vitro ubiquitination assay results demonstrated the tUBM domain enhances HECT domain catalytic activity leading to higher auto-ubiquitination level. This conclusion applies to all eight types of mono-ubiquitin molecules and eight types of di-ubiquitin linkages. Therefore, tUBM domain can be potentially targeted to reduce certain E3 ligase activity of HUWE1 especially on the signaling aspect. In the future work, ubiquitin variants will be tested as the inhibitors of tUBM domain.

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112. GLUT1 inhibition as a therapeutic strategy for RB1-positive Triple Negative Breast Cancer

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Abstract

Triple negative breast cancer (TNBC) is a deadly form of breast cancer due to the development of resistance to chemotherapy affecting over 30% of patients. New therapeutics and companion biomarkers are urgently needed. Recognizing the elevated expression of glucose transporter 1 (GLUT1, encoded by *SLC2A1*) and associated metabolic dependencies in TNBC, we investigated the vulnerability of TNBC cell lines and patient-derived samples to GLUT1 inhibition. We report that genetic or pharmacological inhibition of GLUT1 with BAY-876 impairs the growth of a subset of TNBC cells displaying high glycolytic and lower oxidative phosphorylation (OXPHOS) rates. Pathway enrichment analysis of gene expression data implicates E2F Targets pathway activity as a surrogate of OXPHOS activity. Furthermore, the protein levels of retinoblastoma tumor suppressor (RB1) are strongly correlated with the degree of sensitivity to GLUT1 inhibition in TNBC, where RB1-negative cells are insensitive to GLUT1 inhibition. Collectively, our results highlight a strong and targetable RB1-GLUT1 metabolic axis in TNBC and warrant clinical evaluation of GLUT1 inhibition in TNBC patients stratified according to RB1 protein expression levels.

113. Magnetic resonance guided focused ultrasound generating localized hyperthermia in combination with thermosensitive liposomal doxorubicin for treating a rhabdomyosarcoma murine model

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Introduction: Rhabdomyosarcoma (RMS) is a tumor of skeletal muscle that most commonly affects children and young adults. Current treatment involves surgery, chemotherapy, and/or ionizing radiation with overall survival remaining around 30% in the most aggressive form of the disease. This number has stagnated in the last two decades. Current chemotherapy regimens are vincristine based but most patients develop resistance long-term. Thus, new treatment methods and drugs need to be developed for more efficacious treatment with reduced toxicity in this patient population. Magnetic resonance guided high intensity focused ultrasound (MRgHIFU) is an emerging therapy that combines real-time visualization of the tumor using magnetic resonance imaging (MRI) and analytics that calculate temperature and control the operation of the ultrasound.

Rationale: In cases of relapsed or metastatic RMS, alternate lines of chemotherapy include doxorubicin, which can cause severe cardiotoxicity. To minimize these effects and to target drug delivery to the tumor, our team seeks to develop a use of MRgHIFU, which generates mild hyperthermia (~41 C) in tissues, in combination with a clinically approved thermosensitive form of liposomal doxorubicin for regional disease. Our specific hypothesis is that the drug will have increased uptake in the tumor due to increased permeability of the dilated blood vessels under hyperthermia as well as localized release in the ultrasound beam path secondary to the generated heating. The project will also determine if this combination therapy impacts tumor growth and thus improves survival.

Results: Our lab previously generated an RMS mosaic mouse model by FGFR4 overexpression in immunocompetent mice. We have optimized delivery of hyperthermia to the RMS tumors in the mouse hindlimb by MRgHIFU on a preclinical 7T MRI system with a 2.5mHz small animal lab focused ultrasound system. Next, we performed high pressure liquid chromatography to analyze the percent of the initial dose found in the tumors and plasma after either ten or twenty minutes of hyperthermia to evaluate the role that heating duration played in drug delivery. We are currently performing survival studies of mice treated with thermosensitive liposomal doxorubicin compared to non-liposomal doxorubicin. We will also be analyzing accumulation of the drug in other organs including the liver, heart, and kidneys for toxicity in long-term survival.

Summary: We have developed an immunocompetent murine model of RMS that is able to be targeted with MRgHIFU. As doxorubicin is an effective treatment in RMS, we propose that this treatment combination of MRgHIFU generated hyperthermia in combination with thermosensitive liposomal doxorubicin will allow for a more targeted drug delivery with decreased systemic accumulation and toxicity in this susceptible population.

114. Identifying the Key Epigenetic Partners of the Polycomb Repressive Complex in Regulating B Cell Lymphoma

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Lymphoma is the one of the most common cancers worldwide, where a subtype known as diffuse large B cell lymphoma (DLBCL) is highly prevalent. In DLBCL, dysregulation of key molecular mechanisms involved in B cell differentiation leads to the malignant transformation of B cells and lymphomagenesis. In B cell development, the Polycomb Repressive Complex 2 (PRC2) is a crucial epigenetic regulator. Its enzymatic subunit, the Enhancer of Zeste Homolog 2 (EZH2) enzyme catalyzes chromatin methylation, a function aberrantly reinforced in DLBCL. Given the key role of EZH2 in regulating lymphomagenesis, we aimed to identify its presently unknown epigenetic partners through which it cooperates with to mediate gene silencing. Our lab has performed an unbiased CRISPR-Cas9 knockout screen in DLBCL cell lines treated with an EZH2 inhibitor. Through this preliminary screen, we were able to identify genes of interest implicated as epigenetic regulators in B cell development, where we observed reduced cellular proliferation significantly associated with their depletion. Subsequently, we have utilized genetically engineered DLBCL cell line models to validate our findings. For this validation, we have quantitated the cell proliferation of isogenic knockout DLBCL cell lines treated with EZH2

inhibitor. Lastly, to comprehensively identify the interacting partners of EZH2, we will utilize BioID technology to further examine its protein-protein interactions. Our research aims to elucidate the key epigenetic partners through which EZH2 cooperates with to regulate gene expression in B cell lymphomagenesis. With this, we hope to further understand the regulatory biological mechanisms underlying B cell lymphoma.

*Presenting author is a trainee (graduate student)

115. Screening for Agonistic and Antagonistic Antibodies to the T cell-suppressing VISTA Immune Checkpoint Ig V domain

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VISTA (V-domain immunoglobulin suppressor of T cell activation) is a recently discovered immune checkpoint ligand that suppresses T cell activity [1, 2]. An analysis of immune infiltrates of pancreatic cancer unresponsive to immune checkpoint inhibitors (ICIs) identified VISTA as a potential cause for the resistance to therapy [3], suggesting that antagonistic antibodies to VISTA may represent important and novel biologics for cancer immunotherapy. Herein, we screened and characterized libraries of monoclonal antibodies targeting the extracellular IgV domain of human and mouse VISTA. Our lab had recently developed a stable pentameric form of the VISTA IgV domain, termed VISTA.COMP, that is capable of functioning as a potent soluble immunosuppressor of both T cell activation and proliferation. VISTA.COMP was shown to rescue mice from acute concanavalin A-induced hepatitis [4]. To produce monoclonal antibodies targeting human and murine VISTA, we vaccinated mice with human VISTA.COMP, while rats were immunized with mouse VISTA.COMP. Following hybridoma selection screens, monoclonal antibodies displaying high affinity for the VISTA IgV domain, determined by surface plasmon resonance, were further expressed and purified to establish if they had agonistic or antagonistic properties in T cell assays. Seventeen monoclonal antibodies targeting human VISTA.COMP were identified, with some of them functioning as antagonists that countered the suppression of human T cell proliferation by human VISTA.COMP, and others acting as putative agonists able to suppress T cell proliferation. Furthermore, of the 7 purified monoclonal antibodies that bound tightly to mouse VISTA.COMP, 5 of them also bound strongly to human VISTA.COMP, making them potentially cross-species anti-VISTA antibodies that will function in both mice and humans. Preliminary data suggests that some of these cross-reactive antibodies rescue the IL-2, IFNy, and TNFa production suppressed by VISTA.COMP. These antibodies will be further tested for their ability to overcome VISTA-mediated suppression of human and murine T cell proliferation as well as other cytokine production. While antagonistic antibodies to human VISTA serve as an invaluable tool for cancer immunotherapy, cross-species antibodies that target both human and mouse VISTA will also allow us to test their *in vivo* potency in mouse tumor models. Overall, we have now established hybridoma cell lines that express high affinity monoclonal antibodies recognizing both human and mouse VISTA extracellular IgV domain; and plan to better characterize their agonistic and antagonistic properties in hope to pair them with existing ICIs in terms of augmenting the clinical impact of cancer immunotherapies to target cancers that are currently resistant to ICIs.

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116. Quantitative digital pathology to evaluate hypoxia and proliferation in the tumor microenvironment relative to perfused vasculature

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The tumor microenvironment is highly heterogeneous and contributes to significant differences in tumor cell phenotype. A major contributor to this heterogeneity is variable oxygenation, particularly moderate to severe hypoxia, which has been shown to increase metastasis, radiation resistance, and contribute to an overall poor treatment response. It is therefore crucial to understand the spatial and molecular mechanisms that contribute to tumor hypoxia formation to improve the efficacy of radiation treatment, develop hypoxia-directed therapies, and increase patient survival. Here, we present a quantitative digital pathology pipeline for analyzing hypoxia and proliferation gradients relative to morphological elements in the tumor, such as perfused blood vessels and necrosis. In this pipeline, we utilize multiplexed immunofluorescence staining to generate whole slide scanned image datasets. This allows us to visualize 5 or more different markers, without encountering spectral overlap between fluorophores. Not only do we use single cell segmentation, but the distance of each segmented cell to nearest blood vessel in order to incorporate spatial information into the analysis. Because a high spatial resolution is required for accurate single cell segmentation, we explore the use of Expansion Microscopy (ExM) to separate closely-spaced structures such as cells. ExM is performed by embedding and binding the stained tissue in a swellable acrylamide gel. The swelling of the gel will isotropically stretch the embedded tissue by a factor of 4-5 times. By performing whole slide scanning of the gel and a digital downsizing to the unstretched dimensions, we can effectively enhance the spatial resolution proportional to the extent of swelling. Thus, we allow this methodology to be translated into whole slide imaging technologies that are currently limited by poorer spatial resolutions, such as imaging mass cytometry. This methodological pipeline can serve to further elucidate the relationship between vessel distance and microenvironment-linked markers such as hypoxia and proliferation, can help to quantify parameters relating to oxygen consumption and hypoxic tolerance in tissues, as well as potentially serve as a hypothesis generating tool for future studies testing hypoxia-linked markers.

117. Nanoparticle Size Influences Antigen Retention and Presentation in Lymph Node Follicles for Humoral Immunity

Yinan Zhang

Lymph node follicles capture and retain antigens to induce germinal centers and long-lived humoral immunity. However, control over antigen retention has been limited. Here we discovered that antigen conjugated to nanoparticle carriers of different sizes impacts the intralymph node transport and specific cell interaction. We found that follicular dendritic cell (FDC) networks determine the intralymph node follicle fate of these nanoparticles by clearing smaller ones (5−15 nm) within 48 h and retaining larger ones (50−100 nm) for over 5 weeks. The 50−100 nm-sized nanoparticles had 175-fold more delivery of antigen at the FDC dendrites, 5-fold enhanced humoral immune responses of germinal center B cell formation, and 5-fold more antigenspecific antibody production over 5−15 nm nanoparticles. Our results show that we can tune humoral immunity by simply manipulating the carrier size design to produce effectiveness of vaccines.

118. Understanding the Role of Hippo Signalling in Regulating Tumour Heterogeneity and Drug Resistance

Tumour heterogeneity poses one of the greatest challenges in cancer therapy as it gives rise to drug-resistant tumour cells. While a plethora of signalling pathways have been implicated to mediate resistance to different cancer therapeutics, few have tackled regulators of tumour heterogeneity as a potential target for therapy. Here, we show that the Hippo pathway effector, YAP, is able to regulate breast cancer cell transcriptional heterogeneity at the single cell level and contribute to the overall resistance of the bulk tumour. Upon activation of YAP, breast cancer cells adopted diverse morphological changes *in vitro* and exhibited differences in tumour growth and metastasis *in vivo*. Using the 10x Genomics single cell RNA-sequencing platform, we analysed breast cancer cells with inducible YAP activation, and demonstrated that YAP amplifies transcript heterogeneity in cancer cells. We found that YAP drove diverse expression patterns of genes pertaining to drug resistance, suggesting that heterogeneity translates into multi-drug resistance. The impact of YAP-driven heterogeneity on tumour heterogeneity in human disease and in drug resistance will be discussed. Our work not only allows us to understand how YAP mediates tumour heterogeneity and contributes to drug resistance, but also suggests a new target class for cancer therapy.

119. Pannexin 1 channels modulate tumorigenic properties of melanoma

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Pannexin 1 (PANX1) is a channel-forming glycoprotein expressed in many mammalian tissues. The amplification or upregulation of Panx1 seems to be a predominant feature in a variety of cancer cell lines and tumours when compared to normal tissues. We have found high PANX1 levels in human melanoma at all stages of this deadly skin cancer. Preventing PANX1 function using shRNA-knockdown, CRISPR/Cas9-mediated knockout, and known PANX1 channel blockers -Carbenoxolone (CBX) and Probenecid (PBN)-, significantly reduced cell growth and migration of A375-MA2 human melanoma cells. Using a chicken embryo xenograft model, PANX1 channel blockers significantly reduced tumour weight and invasiveness of A375-MA2 cells. Probenecid treatment reduced ATP release of melanoma cells, suggesting that cell surface PANX1 channels may play a role in purinergic signalling. We have crossed Panx1-global knockout mice with the tri-allelic mouse melanoma model ($BRaf^{CA}$, $Pten^{loxP}$, Tyr::$CreERT^{2}$). Although previously we have seen that the global Panx1-deletion significantly decreases the spontaneous melanoma tumour rate observed in these mice, upon tumour induction with 4-hydroxy-tamoxifen, we have not seen a significant difference in survival or tumour incidence in Panx1-KO compared to Panx1-WT counterparts. Collectively, our findings suggest that PANX1 promotes the tumorigenic properties of human melanoma. However, in the inducible mouse model of melanoma, the activation of driver mutations (BRAF and Pten) alone seems to override the effects of Panx1 in melanoma progression. We propose that blocking PANX1 channels could be a promising adjuvant therapeutic strategy for the treatment of melanoma that requires further investigation.

No conflict of interest to declare by the authors.

Presenting author: Rafael Sanchez-Pupo – Graduate Student
Project leader: Dr. Silvia Penuela

120. Generation and bio-banking of pancreatic ductal adenocarcinoma patient-derived organoids in the Princess Margaret Living Biobank

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Princess Margaret Living Biobank, University Health Network

Pancreatic cancer affects 1 in 64 people with an average patient survival of 5 months. There is a tremendous need for research models of this disease to improve treatment options and outcomes. Princess Margaret Living Biobank (PMLB) Organoid Centre is a repository for patient-derived tumour fragments established as three dimensional organoids (PDO) in gel matrices. PMLB has generated a variety of tumour organoid models, including many from pancreas, that are available to researchers.

PMLB organoids are established from primary, metastatic and patient-derived xenograft tissues obtained by surgical resection or biopsies at UHN. Long-term, renewable and revivable PDOs are deposited in the UHN Biobank and linked to other patient banked materials, as well as clinical and molecular profiling data. All deposited models have passed standard quality checks including Short Tandem Repeat genotyping for parental cell line identity matching, mycoplasma testing, growth analysis, and histopathological identification of organoid tissues using a panel of tissue specific biomarkers reviewed by site specialist UHN pathologist. PMLB offers distribution of organoid models and services for drug screening to researchers.

PMLB has processed over 150 surgical resections and needle biopsy cores from patient pancreatic ductal adenocarcinomas (PDAC). To date, 58 PDAC PDO's have been established, characterized, quality checked and deposited into the UHN Biobank with a current success rate of 83% from resections and 70% from biopsies. PMLB has also banked 21 PDAC xenograft-derived organoid models. Established PDAC organoids can be passaged at a ratio of 1:3-1:6 every 10-14 days. Although maximum passage is not determined, several models have been propagated 15-20 passages. Histopathological analysis confirms CK19 expression in PDAC organoids, and conserved tumour characteristics from tissue of origin to organoid model are observed. PMLB has distributed 33 PDAC organoid models to research investigators and has used every PDAC organoid model for contracted research projects.

The PMLB aims to provide researchers at the University Health Network, external academic institutions and industrial collaborators with tools to answer many basic oncology research questions with hopes to positively impact cancer treatment. PMLB's PDAC organoid models and linked data are regarded as a valuable resource for pancreatic cancer research.

121. Targeting ALK2: An Open Science Approach to Developing Therapeutics for the Treatment of Diffuse Intrinsic Pontine Glioma

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Diffuse Intrinsic Pontine Glioma (DIPG) is an aggressive cancer arising from glial cells in the brainstem. There are no effective chemotherapeutic drugs that have been approved for this indication. As such, radiation therapy continues to be the only treatment option for children diagnosed with DIPG. Analysis of the genomic landscape of this disease has led to the identification of the serine/threonine kinase ALK2 as a potential target for therapeutic intervention. In this work, we adopted an open science approach to develop a series of potent ALK2 inhibitors that are orally bioavailable and brain penetrant. Initial efforts resulted in the discovery of **M4K2009**, an analog of the previously reported ALK2 inhibitor **LDN-214117**. Although **M4K2009** is highly selective for ALK2 over the TGF-ßR1 receptor ALK5, it is also moderately active against the hERG channel (IC¬50 = 8 µM). Varying the substituents of the trimethoxyphenyl moiety gave rise to an equipotent benzamide analog **M4K2149** with reduced off-target affinity for the ion channel (hERG IC50 > 50 µM). Additional modifications to the benzamide and piperazine motifs yielded 2-fluoro-6-methoxybenzamide derivatives (**26a-c**) which possess high inhibitory activity against ALK2, excellent kinome-wide selectivity and superior pharmacokinetic profiles.