January 2019 (INNOV-19) Competition
Awarded Innovation Grants
Listed in alphabetical order

I1a Biomarkers and Genomics

Foulkes, William
The Research Institute of the McGill University Health Centre (RI-MUHC)

Using mutational signatures and functional genomics to classify breast cancer gene variants

Background: The use of genetic testing in the clinic is increasing and the number of genes tested continues to grow. There is pressure to use the information not only for prevention purposes but also to inform treatment decisions. A problem emerges where the number of variants of unknown significance (VUS) is rapidly increasing, particularly for genes that are less well characterized. Lab-based methods to test the pathogenicity of variants are time-consuming and not readily accessible to clinical services. We propose that using mutational signatures derived from the molecular information contained in the tumours themselves can help determine whether a VUS may be pathogenic even in genes that have not been well studied.

Objectives: We will 1) analyse paired normal-tumour sequencing datasets from breast cancer patients to reveal mutational signatures that will allow us to reclassify VUS in clinically relevant HRD genes; **2)** use CRISPR/Cas9 genome editing technology and a PARP inhibitor sensitivity assay to identify candidate genes acting in the homologous DNA repair (HR) pathway relevant to breast cancer; **3)** perform functional assays to validate our findings in known and novel HR genes identified in Aims 1 and 2.

Methodology: Aim 1: Analyse paired normal-tumour sequencing datasets from breast cancer patients to reveal mutational tumour signatures to identify new disease-related genes/variants. For this aim, two complementary approaches will be used. 1) We will analyse existing WES datasets from various sources, including Montreal-based research WES datasets and a subset of 3000 whole genome datasets from normal/tumour pairs from women with breast cancer in the UK. 2) We will conduct WES on up to 25 normal/tumour pairs selected from women who received prior clinical testing and women who will participate in an on-going funded project where all women with incident cases of breast cancer at three McGill hospitals will be sequenced for these and other related genes. This aim will identify tumour variants that can be used to define tumour signatures associated with each tumour in these patients. Aim 2: Use CRISPR/Cas9 genome editing technology and a PARP inhibitor sensitivity assay to identify candidate HR pathway genes relevant to breast cancer. To realize this aim, we will employ targeted CRISPR-Cas9 editing and PARPi sensitivity selection assays to study genes identified as having co-evolved with *BRCA1* and validate candidate genes using functional tests to establish disease relevance. This aim will allow us to leverage existing, well validated functional assays in our laboratory to identify novel, clinically-relevant genes that contribute to the breast tumour phenotype based on their ability to respond to PARP inhibition. Aim 3: Perform HR deficiency (HRD) assays to test variants of unknown significance seen in patients in known HR genes and in genes identified in Aims 1 & 2. For this aim, we will review the sequencing data generated in Aim 1 from our normal/tumour breast cancer genomes to look for variants in known breast

cancer genes as well as in the new HRD candidate genes identified in Aim 2. We will re-create each VUS in a cell line and perform HRD functional assays on mutated lines to establish the pathogenicity of each variant. We will then compare the results to the predictions obtained in Aim1 using mutational signatures. In addition to generating new classification information for these VUS, our HRD assays will enable us to evaluate whether sequenced-based mutational signatures alone can reliably classify VUS in breast cancer genes, even in the case of genes not previously well characterized.

Significance: This project will provide clinically relevant information on the VUSs analysed in this proposal. In addition, developing an innovative classification strategy based on mutational signatures will enable classification of VUS in diverse or under-represented populations where variant frequency information is not easily available, which will significantly improve the power of clinical genetic testing to identify at risk individuals and guide the use of targeted therapies.

Li, Yingfu
McMaster University

Investigating novel biomarkers of pancreatic cancer

Pancreatic ductal adenocarcinoma (PDA) is the most lethal type of common cancer, with a 5 year survival rate of only 6%. Its poor prognosis is attributed largely to late diagnosis. Yet, no screening program for pancreatic cancer currently exists, and evidence suggests early detection can save lives. Therefore, we propose to pursue an unconventional, high-risk study aimed to identify novel serum tumour markers of pancreatic cancer, with the goal of incorporating them into screening and diagnostic assays for early detection and screening. We plan to pursue this goal through the use of a powerful test-tube selection technique to search for, from an extremely a large synthetic DNA library, catalytic DNA molecules (DNAzymes) to be activated solely in PDA serum. We will then use them to work backwards to identify the activating biomarkers present in PDA serum, thus uncovering novel PDA serum biomarkers.

Our lab focuses on the derivation and application of RNA-cleaving DNAzymes (RCDs) designed to cleave a substrate at a specific RNA dinucleotide junction embedded in the DNA sequence. We have developed effective methods of deriving RCDs from large random sequence DNA libraries consisting of 1016 sequences of DNA. The sequences of these libraries contain a random sequence domain needed to produce such a high sequence diversity. For the purpose of RCD selection, a library will be ligated with an RNA substrate to create the RNA cleavage site. The ligated library will undergo RNA cleavage-based selection to identify biomarker-responsive DNAzymes. RCDs that have undergone self-cleavage will become shorter than their pre-cleavage size and will travel farther on gel electrophoresis, which is simultaneously used to purify the sequence, concluding one round of selection. Purified RCDs are then subjected to amplification via polymerase chain reaction (PCR) and go on to further rounds of selection.

Our study has three Aims. Aim 1 is RCD isolation. This Aim will begin with acquiring preoperative/pretreatment serum samples from the Ontario Tumour Bank. The samples will consist of 20 pancreatic cancer sera (PDA+ samples), and 10 serum samples each of colon, breast, prostate, lung cancer and healthy people (PDA- samples). The DNA library will be subjected to a series of counter-selection, positive-selection, and amplification steps to identify RCDs solely active with PDA+ samples. In each selection cycle, the PDA- samples will be used in the counter-selection step to remove any active DNAzymes with cross-activities. The remaining pool will be incubated with PDA+ samples in the positive-selection to enrich RCDs with the PDA+ serum. These steps serve to uncover RCDs that are highly selective for PDA+ sera. Aim 2 is probe validation in which we examine diagnostic utility of

individual RCD probes (sensitivity and specificity) using sera from PDA patients and healthy and other disease controls. We will recruit 50 patients undergoing pancreaticoduodenectomy for PDA at Juravinski Hospital in Hamilton. Selection criteria for pancreatic cancer patients include: age >18 years, no history of pancreas-associated genetic conditions, and no exposure to neoadjuvant therapy. Additionally, we will obtain 50 serum samples from healthy patients, patients with benign hepatopancreatobiliary pathologies, patients with acute pancreatitis, and patients diagnosed with other cancers, such as colorectal, breast, prostate, and lung. We aim to establish a RCD-based diagnostic assay with sensitivity and specificity greater than 80%. Aim 3 is biomarker discovery in which we will apply a combination and proteomic and molecular biology techniques to identify the interacting biomarkers for the best performing RCDs established in Aim 2.

This project aims to uncover both serum pancreatic cancer biomarker(s) and associated RCD probe(s). By taking advantage of the power of in vitro selection and stringent experimental design, we expect to derive RCDs that are sensitive and specific to early stage, resectable pancreatic cancer. This will enable future construction of cost-effective diagnostic assays for life-saving screening and early diagnosis of pancreatic cancer.

Liu, Geoffrey
Princess Margaret Cancer Centre - UHN

Breathomics as a non-invasive, inexpensive, point-of-care predictive test for immune checkpoint inhibitor efficacy

Background: Immune checkpoint inhibitors (ICIs) offer substantial life-prolonging benefits to a subset of patients with advanced stage melanoma and non-small cell lung cancer (NSCLC). However, the subset benefitting is a minority of patients. The remaining patients do not receive substantial clinical benefit from ICIs yet remain at risk for ICI toxicity, and burden the healthcare system with cost and resource utilization. Improved selection of patients for ICI therapy is critically needed.

Given the complex nature of the immune system, single molecule biomarkers, such as PD-L1 immunohistochemistry (IHC), may be inadequate to predict for therapy response. Recent research suggests that analyzing multiple factors such as tumour mutation burden, interferon gamma-related gene expression signatures, and immune infiltrates may be more effective. However, the logistics and cost in terms of finance and time for analysis are high, and none have shown strong predictive power (AUC>0.80) to date.

Instead, we propose using the SpiroNose device, an array of metal oxide sensors, to detect volatile organic compounds (VOCs) released in exhaled breath. VOCs found in exhaled breath can originate from biological processes such as inflammation as well as microorganisms within the body. The study of these VOCs in exhaled breath to detect health conditions is known as breathomics.

Preliminary research using the SpiroNose has demonstrated its ability to differentiate between patients whose NSCLC has progressed or not progressed early while on ICI therapy more effectively than PD-L1 IHC, with an AUC of 0.84. However, this study was small, exploratory, and unconfirmed using rigorous methodologies. A rigorous study design and sophisticated machine learning methods, as proposed in this study, could allow us to generate robust VOC-based classifiers to predict ICI efficacy. This classifier could better stratify patients for treatment and may help monitor their responses non-invasively. Furthermore, as both cancer cells and immune cells may contribute to VOC readings, the SpiroNose may also

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non-invasively capture information regarding the tumour-immune microenvironment, which could help identify further therapeutic strategies.

Aims:
1. Investigate the ability to predict response to ICI therapy by developing and refining VOC-based classifiers.
2. Explore whether exhaled VOCs can be used to monitor response to ICI treatment.
3. Explore whether exhaled VOCs partially reflect tumour microenvironment characteristics such as type of immune infiltrates and their polarization.

Methods: Patients with metastatic NSCLC or melanoma about to start ICI treatment at the Princess Margaret Cancer Centre will have their exhaled VOCs measured using the SpiroNose device. We will build a VOC-based classifier for ICI efficacy, refining the method from the preliminary study and explore more advanced machine learning techniques (Aim 1). To determine if our classifier can be used to monitor response to ICIs, we will longitudinally measure exhaled VOCs from a cohort of patients responding to ICIs and compare our classifiers measured serially with the timing of clinical progression (Aim 2). Finally, we will perform multiplexed immunofluorescence studies on archival pre-ICI tumour tissue to determine whether and how immune infiltrates (in regards to cell types and polarization) are associated with exhaled VOC signals (Aim 3).

Impact: Breathomics has the potential to impact hugely how we select patients for ICI therapy and monitor their responses, ultimately improving clinical care for two deadly cancers while potentially decreasing healthcare costs. With this device, we can capture important predictive information simply and non-invasively through exhaled breath, without needles or biopsies. Once appropriate classifiers have been developed, patient exhaled VOC data can be uploaded immediately to a secure cloud server, analyzed and results reported back within a few minutes. Additionally, if exhaled VOCs can capture information regarding the tumour-immune microenvironment, this may be useful in understanding ICI host-environment interactions scientifically.

Lupien, Mathieu
Princess Margaret Cancer Centre - UHN

Pancancer analysis to discover the epigenetic determinants of tumour initiating cells (TICs).

Rationale & Hypothesis
Tumour initiating cells (TICs) are cells with self renewal and tumour initiating capacity. Identified in multiple malignancies including leukemia, brain and breast cancer among others, these cells are resistant to conventional chemo and radiation therapies and implicated in metastasis, drug resistance and relapse in cancer patients. While current therapeutics can effectively decrease tumour size, therapies targeting TICs are needed to provide a lasting cure to cancer.

TICs were thought of as dedicated cells lying at the apex of tumour hierarchy generating rest of the differentiated tumour bulk. But characterisation in solid cancers revealed that under appropriate stimuli, cell-state transitions can occur in committed progenitors to generate TIC like phenotypes. Studies in leukemia, colon and breast cancer identified distinct TICs with varying tumour initiation potential within the same tumour. This suggests that TIC capacity is a functional state arising from plasticity and subclonal differences in tumour cells.

This plasticity and heterogeneity compounds challenge of TIC identification. Currently used surface markers are not universal and it is unknown how they change with cancer progression. This raises the need for a robust molecular signature to define TICs and facilitate their clinical evaluation. Identity of every cell including stem and differentiated is determined by its epigenetics. TIC models of glioblastoma (GBM) and leukemia showed that chromatin organisation changes confer TIC like phenotype suggesting epigenetic features can determine tumour initiating capacity. Almost all cancer types exhibit epigenetic changes that are stably inherited, propagating cells with fitness phenotypes. PCAWG consortium demonstrated that pooling samples from different tumour types provides statistical power to identify unifying drivers of cancer. We thus propose a pan-cancer analysis of epigenetic features of TICs can reveal both universal and cancer specific biomarkers of TIC reflective of therapeutic opportunities.

Aims & Methods
Aim 1: Identify epigenetic determinants of TICs
To delineate epigenetic identity of TICs, we will use in-house ATACseq and RNAseq data generated on 100+ TICs isolated from leukemia, GBM, posterior fossa ependymoma (PFA) and pancreatic cancer. ATACseq provides information on chromatin accessibility at regulatory elements such as enhancers and underlying transcription factors (TF). We will cluster accessible sites to identify shared and cancer specific regulatory elements. We will annotate these loci by identifying enriched TF dependencies

Aim 2: Assess epigenetic intra-tumour heterogeneity in TICs
To successfully delineate the epigenetic identity of TICs, it is essential to assess if TICs form a heterogeneous or homogeneous population. We propose to resolve this question using single-cell ATACseq on TIC populations from all four cancer types. We will then measure similarity of subpopulations across tumours via trajectory analysis anchored on signatures identified in Aim1 and identify TF dependency if subpopulations are identified.

Aim 3: Assess efficacy of TIC signature to predict clinical outcome
To assess the clinical relevance of epigenomic TIC signatures identified in Aim1, we will measure the strength of these features in bulk ATACseq data from the TCGA consortium and assess how they correlate to survival using cox regression. Features will be iteratively refined to identify a minimal prognostic signature for potential translation to clinically adaptable assays.

Significance
This project will identify epigenetic signatures to assess TIC burden and disease risk in clinical samples from multiple cancer types. This study will reveal potentially actionable targets to eliminate TICs and lay groundwork for TIC-centric drug development. Furthermore, it will reveal yet unknown TIC subtypes and their unique features. All of the above will facilitate identification, targeting and thereafter response assessment of TICs in bulk tumours in the clinic.

Schaeffer, David
University of British Columbia

Targeting metabolic plasticity in pancreatic cancer

In order to reduce the exceptionally high death rate in pancreatic ductal adenocarcinoma (PDAC), novel therapeutic strategies combining existing and newly developed treatments, aimed at specific tumour molecular signatures are needed. Efforts to combat the disease are plagued by high rates of metastasis at presentation and poor response to therapy, due in part to limited treatment options and lack of knowledge

of clinically validated biomarkers that would help stratify patients into treatments. International efforts to define such biomarkers have intensified including the *Enhanced Pancreatic Cancer Profiling for Individualized Care* (EPPIC) project that we lead in Canada.

PDAC subtypes include 'classical' and 'basal-like' based on a published subtyping methodology, with basal-like tumours being associated with poor outcome. In order to therapeutically target the emerging subtypes of PDAC, their link to specific cellular processes that may confer different vulnerabilities needs to be established. Metabolic reprogramming including glycolysis is a cancer hallmark. PDAC tumours exhibit several factors associated with glycolysis, including oncogenic *KRAS* and *MYC* expression, loss of function in *TP53*, and hypoxia. Another metabolic pathway activated in cancer is cholesterol synthesis. However, the knowledge of how the expression of different metabolic signatures influences PDAC outcome is limited. We postulated that tumour metabolic dependency may be influenced by specific activated pathways, which may in turn affect disease outcome. We recently completed the profiling of glycolytic and cholesterogenic gene expression in 325 PDAC cases and found that: i) tumours with high expression of glycolytic and low expression of cholesterogenic genes have the worst outcome, ii) increased expression of cholesterogenic genes conferred a survival benefit, and iii) there was a correlation of expression of glycolytic and cholesterogenic genes with basal-like and classical subtype genes, respectively.

This has led us to the hypothesis that tumour metabolic profiles confer distinct metabolic vulnerabilities in PDAC which can be exploited for personalized treatment design, and for targeting of the aggressive basal-like subtype. The main objective of the proposal is to investigate whether targeting a specific tumour metabolic signature represents a novel therapeutic approach in PDAC, using patient-derived tumour organoid (PDO) models that recapitulate the donor's molecular signatures.

Goal 1: Analyze the effects of pharmacological metabolic agents targeting the glycolysis-cholesterol synthesis axis on tumour cell survival and chemotherapy response in PDOs derived from genomically and clinically characterized metastatic PDAC tumours.

PDOs are generated from a fresh metastatic tumour biopsy core from patients enrolled in a clinical trial associated with EPPIC at BC Cancer. Patients receive standard chemotherapy treatment or standard treatment with immunotherapy. The pre-treatment biopsies undergo whole genome (WGS) and transcriptome sequencing (WTS), and detailed bioinformatics analysis as part of the personalized oncogenomics program at BC Cancer. This information will be leveraged by the EPPIC project.

PDOs will be grown in matrigel and propagated according to an established protocol. Metabolic activity, cytotoxic and anti-proliferative effects of multiple specific compounds targeting glycolysis and cholesterol synthesis alone, and in combination with standard and non-standard chemotherapeutic compounds, will be analyzed.

Goal 2. Determine if targeting specific metabolic pathways can alter the tumour metabolic gene expression signature and reprogram an aggressive PDAC subtype.

WGS and WTS analysis will be performed in PDOs. Bioinformatics analysis will be conducted to determine the mutational and gene expression signatures of the PDO prior to, and following treatment.

The project will establish whether metabolic tumour subtype-guided therapy represents a new treatment approach in PDAC and will provide proof-of-concept for tumour subtype modification as a strategy to improve outcome.

I1b Gene Regulation and Cell Biology

Audet-Walsh, Etienne
Laval University

Inhibition of the AMPK metabolic program for the treatment of prostate cancer

Most prostate cancer (PCa) cases respond favorably to inhibition of the androgen receptor (AR), the cornerstone therapy in the clinic. Unfortunately, the vast majority will evolve into castration-resistant PCa, the lethal form of the disease, for which the current clinical armament is limited. Our goal is to develop new approaches to reprogram, or actually "de-program" PCa cell metabolism to restore a tumour suppressive metabolic environment.

The two major regulators of cell metabolism, the AMP-activated protein kinase (AMPK) and the mechanistic target of rapamycin (mTOR), are often referred as opposite forces. mTOR is active and consumes energy when nutrients are abundant and growth factors promote proliferation. When nutrients become limited or under energetic stress, AMPK is activated and inhibits mTOR functions and proliferation until a proper energetic state is restored. Accordingly, AMPK activators have gained much interest for PCa treatment and are currently studied in clinical trials. The rationale for using these drugs is that AMPK would inhibit mTOR and block cancer cell proliferation, but most of their beneficial effects, when significant, seem to be attributable to their anti-diabetic effects.

Our results demonstrate that AR simultaneously activates mTOR and AMPK in PCa, by-passing the well-known AMPK-dependent inhibition of mTOR and proliferation. We show that AMPK is activated during induction of PCa cell proliferation and that AMPK inhibition, not activation, is required to block this proliferation and induce cell death. It is thus clear that AMPK does not follow the current paradigm, but that it is rather co-activated with mTOR to promote metabolism and tumour growth. Therefore, we believe that we should not activate but inhibit AMPK for PCa treatment. Our hypothesis is that AMPK acts as a key metabolic regulator and that we can block AMPK or inhibit its functions to kill castration-resistant PCa cells.

First, we will show that AMPK activity is positively linked to PCa aggressiveness, using pharmacological and genetic modulation of this signaling pathway in *in vitro* and *in vivo* PCa models. In addition, immunohistochemistry of the AMPK pathway activity will be performed in two PCa tissue microarrays to further demonstrate this relationship.

Secondly, our results demonstrate that AMPK participate to the regulation of the PCa transcriptome, notably by inducing the AR and the mTOR signalling pathway gene signatures. Accordingly, we will define the transcriptional functions of AMPK in both AR-positive and AR-negative PCa cells using RNA-seq experiments, highlighting a completely new function of AMPK in PCa.

Finally, we will demonstrate using state-of-the-art metabolomics experiments how AMPK controls the two pathways required for ATP synthesis, an essential step to identify metabolic vulnerabilities that we will target to kill PCa cells.

Overall, our work will completely change how we envision AMPK as a therapeutic target for PCa, will

establish a completely new model of AMPK functions in cellular biology, and pave the way for the development of innovative approaches targeting PCa cell metabolism for the treatment of castration-resistant PCa.

Brown, Grant
University of Toronto

Targeting cytosine deaminases in pancreatic cancer cells.

A. Summary of the problem to be investigated
DNA replication stress is widely recognized as an important mechanism of cancer development, progression, and treatment. Surprisingly little is known, however, about the types of replication stress that arise in different cancers and during cancer treatment, or about how DNA replication stress can best be targeted to selectively kill tumour cells. Using genome-scale CRISPR/Cas9 screens we discovered that knockout of a specific cytosine deaminase dramatically increases sensitivity to the DNA replication stress drug gemcitabine in pancreatic cancer cell lines, but not in non-tumour lines. Thus, knockout of this cytosine deaminase is synthetic lethal with gemcitabine, and the synthetic lethality depends on the cancer phenotype. There is no precedent for a protective role for this cytosine deaminase in tumour cells encountering DNA replication stress. We propose detailed molecular analyses to understand the mechanism by which this cytosine deaminase protects tumour cells from DNA replication stress induced by gemcitabine. The resulting knowledge holds promise in improving cancer treatment in that the cytosine deaminase could be leveraged as a target to sensitize tumour cells to killing by gemcitabine and other replication stress inducing therapeutics.

B. Objectives of the proposed investigation
Our hypothesis is that the cytosine deaminase promotes resistance to DNA replication stress caused by gemcitabine and perhaps other DNA replication stress inducing drugs. The key questions that we seek to answer during the 2-year funding term are: 1. How does the activity of the cytosine deaminase reduce the sensitivity of cancer cells to gemcitabine?, and 2. Does the cytosine deaminase respond specifically to gemcitabine, more broadly to cytosine analogues, or even more broadly to DNA replication stress? In parallel, we will validate our discoveries in a distinct cancer model, namely pancreatic tumour organoids in 3D culture. Together, our investigation will define the role of this cytosine deaminase in the cancer cell response to DNA replication stress.

C. The methodology to be used
We have used an innovative genome-wide CRISPR/Cas9 gene knockout screening technology to understand the DNA replication stress response in tumour cells. Here we focus on a compelling and independently validated hit from our screens, a cytosine deaminase. We will determine whether the in vivo target is free nucleoside or nucleotide, bases in ssDNA, or bases in dsDNA, using a mass spectrometry approach in collaboration with the Caudy lab at U of T. We will use fluorescence microscopy to measure accumulation of ssDNA and micronuclei. DNA fibre analysis will be used to measure DNA replication fork rates and nascent DNA stability. We will define the biochemical properties of deamination of cytosine analogues by the cytosine deaminase in vitro. Our toolbox of molecular biological, biochemical, and microscopy techniques will allow us to develop mechanistic models of the cytosine deaminase's role in the DNA replication stress response in tumour cells. In collaboration with the Tuveson lab at Cold Spring Harbor, we will validate the synthetic lethality of cytosine deaminase gene knockout with gemcitabine in patient-derived organoids grown in 3D cultures, and develop local expertise in this important emerging pancreatic cancer model.

D. The significance of the research to cancer

DNA replication stress is central to cancer biology. Indeed, many cancer treatments act by causing DNA replication stress, and therapies that directly target replication stress response proteins like WEE1 and ATR are advancing toward the clinic. We expect that our study will define a cytosine deaminase as a novel DNA replication stress response pathway that can be targeted to specifically kill cancer cells. Our current focus is on pancreatic cancer, a highly fatal cancer with substantial room for improvement of therapies, and an area where our studies have excellent potential for high impact, influencing treatment and reducing cancer burden. We also seek to broaden the scope of our study to encompass additional cancer types and therapeutic drugs.

Fu, YangXin
University of Alberta

Role of RNA methyltransferase NSUN5 in glioblastoma

Summary: Glioblastoma (GBM) is the most common and aggressive malignant primary brain tumour. There is an urgent need to better understand the molecular mechanisms of GBM to develop novel therapeutic strategies to treat this deadly disease. Using TCGA (The Cancer Genome Atlas), we determined that NSUN5 (a RNA cytosine methyltransferase that methylates ribosomal (r)RNAs) strongly associates with poor survival in GBM patients. Because rRNA methylation modulates structure and activity of the ribosome and thereby regulates protein synthesis, elevated expression of rRNA methyltransferase NSUN5 could alter the translatome in GBM cells to broadly drive tumourigenic phenotypes. In yeast, loss of NSUN5 alters the structural conformation of the ribosome and translational fidelity, resulting in selective translation of a subset of oxidative stress-responsive genes. Our preliminary experiments showed that (a) NSUN5 is highly expressed in 7 out of 9 GBM cell lines and 8 out of 12 patient-derived GBM neurosphere cultures (primary GBM cells); (b) overexpression of NSUN5 promotes sphere formation in U87 and primary GBM cells and tumour formation and/or progression of U87 cells in mice, whereas knockout/knockdown of NSUN5 decreases cell growth and sphere formation in U251 and primary GBM cells; (c) NSUN5 methylates cytosine 3782 (C3782) of 28S rRNA in GBM cells; and (d) NSUN5 overexpression increases, whereas NSUN5 knockout/knockdown decreases global protein synthesis in GBM cells. *These findings led us to hypothesize that elevated expression of NSUN5 promotes progression of GBM by altering the pattern of rRNA methylation, leading to pro-tumourigenic translational reprogramming.*

Objective: The objective of this proposal is to determine if NSUN5 can be a potential therapeutic target for GBM.

Methodology: We will use established GBM cell lines and primary GBM cells and the orthotopic xenograft model, as well as biochemical and molecular biology approaches to determine the role for NSUN5 in GBM.

Aim 1: To determine whether elevated expression of NSUN5 reprograms the translatome in GBM cells by methylating C3782 of 28S rRNA. We posit that elevated expression of NSUN5 alters the pattern of rRNA methylation, leading to translational reprogramming in GBM cells. (i) We will use the conventional RNA bisulfite sequencing to determine whether NSUN5 methylates C3782 of 28S rRNA in primary GBM cells. (ii) We will perform polysome profiling coupled with RNA sequencing experiment using primary GBM 50M cells stably transfected with an empty vector or NSUN5 (50M/Vector vs. 50M/NSUN5) to determine to what extent the elevated NSUN5 reprograms the translatome in GBM cells. Ten candidate genes will be

prioritized based on magnitude of difference in translational efficiency between 50M/Vector vs. 50M/NSUN5 cells as well as documented roles in the maintenance of cancer stem cell (CSC) phenotypes. The translational regulation of these will be validated by conducting polysome profiling followed by qRT-PCR in more primary GBM cells.

Aim 2: To determine whether NSUN5 regulates GBM phenotypes. We will stably overexpress NSUN5 or knock it down in primary GBM cells and examine whether NSUN5 regulates behavior of primary GBM cells using *in vitro* (cell growth, cell cycle, sphere formation and protein expression of the CSC-associated genes) and *in vivo* models*.* For *in vivo* work, we will use the orthotopic xenograft model of primary GBM cells via intracranial injection. Tumour formation and progression will be monitored by *in vivo* imaging. At the end of the experiments, xenografted tumours will be collected for molecular and immunohistochemical analysis, as well as polysome profiling (to determine the extent to which translational alterations discovered in *Aim 1* occur *in vivo*). The function of the differentially translated mRNAs identified in *Aim 1* will be examined as described above to determine the extent to which they mediate the effects of NSUN5 in primary GBM cells.

Significance: This pre-clinical and mechanistic study will determine the role of NSUN5 in GBM, which could lead to discovery of novel therapeutic strategies for this deadly disease.

Hope, Kristin
McMaster University

Identification of novel factors mediating oncogenic splice events

Problem: Alternative gene splicing is one of the major contributors to proteome diversity and is thus tightly controlled throughout normal development. Aberrant splicing is a key driver of cancer, as many cancer-specific splice variants regulate each of the hallmark processes of cancer. These oncogenic splicing events are directed by upstream splicing regulators, which could represent potential drug targets in diverse cancer types. However, existing technologies including spliceosome-wide CLIP-seq and RNAi/CRISPRi screens are highly inefficient in discovering splicing factors governing individual splicing events. In this study, we will apply a novel RNA antisense purification (RAP) proteomic method to identify RNA-binding proteins (RBPs) that interact with the pre-mRNA of a cancer-supporting splice isoform. Specifically, we will use this method to discover factors that control the expression of CD44v6, a leukemogenic splice isoform of CD44 that supports leukemic stem cell (LSC) function.

Background and Rationale: Acute myeloid leukemia (AML) is a malignant hematopoietic disorder with dysregulated clonal expansion of undifferentiated myeloid progenitor cells. Despite significant advances in cancer therapeutics in recent years, AML patients continue to suffer from chemo-resistance, high relapse rates, and low overall survival rate, all of which are attributed to LSCs, a typically rare population of AML cells. LSCs and normal hematopoietic stem cells (HSCs) share common surface markers (CD34hiCD38lo) and similar mechanisms that support self-renewal pathways, which have contributed to the limited success of specifically targeting LSC for AML eradication.

CD44 is a hyaluronate receptor well recognized as a cancer initiating cell marker in AML. As CD44 is also expressed in HSCs, it is not feasible to target CD44 for LSC eradication. On the contrary, CD44v6, a CD44 splice variant that contains the V6 variable exon, is highly selectively expressed on AML cells and is required to support LSC-mediated in vivo growth. Preclinical immunotherapy studies targeting CD44v6 in AML demonstrated efficacy in disrupting primary AML xenotransplantation. However, these experiments

did cause adverse effects related to collateral damage to normal monocytes, which express low level of CD44v6. Together these results provide a compelling impetus to further our understanding of the upstream regulatory molecules that direct heightened LSC-promoting CD44v6 expression.

Hypothesis: We hypothesize that one or more RBPs interacting with the CD44v6 primary mRNA (pre-mRNA) around the V6 exon control the expression of the CD44v6 variants and are crucial for LSC self-renewal. We will test our hypothesis through the following aims:

Aim 1: Identify novel CD44v6 splicing regulators in THP-1 AML cells
We will perform biologically triplicated RAP proteomic experiments to identify the protein interaction partners of the CD44 pre-mRNA in the THP-1 AML cell line, which expresses high levels of CD44v6. We will then confirm the newly-identified RNA-protein interactions and verify that these RBPs mediate CD44v6 splicing in primary AML samples.

Aim 2: Confirm the LSC-regulatory functions of the CD44v6 splicing regulators in primary AML samples
We will perform in vitro and in vivo loss-of-function assays of the identified CD44v6 splicing regulators to confirm their roles in the leukemic progenitors and LSCs of a variety of primary AML samples.

Significance: The proposed studies will identify AS regulators that direct the expression of the LSC-promoting CD44v6 variant in AML. In addition to their ability to enforce expression of an identified LSC regulator, these factors may also drive other aberrant driver splicing events and as such represent important new AML therapeutic targets and diagnostic markers. Ultimately, if validated in the work we propose here, we would demonstrate that the RAP proteomic method has much broader application in identifying upstream splicing factors of oncogenic splice isoforms that represent novel treatment targets in a wide range of cancer types.

Khokha, Rama
Princess Margaret Cancer Centre - UHN

Harnessing therapeutic targets in pancreatic cancer stroma

The problem: Pancreatic ductal adenocarcinomas (PDACs) comprise >90% of all diagnosed pancreatic tumours and have remained untreatable despite decades of intense research. A major factor limiting therapeutic success is the copious PDAC stroma, known to provide extensive support for disease progression and chemoresistance. However, recent efforts towards depletion of PDAC stroma as a therapeutic strategy have revealed unexpected tumour-suppressing functions. This points at convoluted tumour-promoting and tumour-suppressing elements within the heterogenous PDAC stroma, which need to be adequately understood to enable stroma-guided therapy. Notably, human PDAC stroma exhibits histological subtypes (histotypes) that impact disease outcome. We recently isolated morphologically distinct subpopulations of cancer-associated fibroblasts (CAF) from these clinically relevant human stromal histotypes, and found they exhibit intriguing differences in their gene expression and cell behavior. We hypothesize that PDAC stromal subtypes harbor specific pro- and anti-tumour signals, which can be harnessed to mobilize stroma-guided therapy.

Objectives & Methodology: To ultimately identify essential regulators underlying CAF subtype-specific impact on PDAC progression and to examine their untapped therapeutic potential, we have 2 major objectives: Aim 1 will define the cellular composition and the global molecular profiles of human PDAC

stroma histotypes. First, we will perform rigorous histopathological assessment and determine major cellular components on a semi-automated IHC platform. Next, we will perform histology-guided OMICs analyses of tissues from well-annotated PDAC patients enrolled in two independent but complementary clinical studies. Using Laser Capture Microdissection, we will extract patient-matched tumour and stroma, with defined stroma histotypes, for analysis using our cutting-edge methods for deep proteomics and transcriptomics. This will define histotype-specific molecular targets associated with clinical outcome; druggable candidates will be shortlisted and validated by IHC. Aim 2 will model the CAF subtype-specific interactions with PDAC cells and therapeutically evaluate the identified molecular regulators. Model systems that recapitulate human PDAC histotypes are lacking yet are essential for dissecting their contribution to PDAC progression. We are excited to have isolated distinct CAF subpopulation from fresh human PDAC tissues, which morphologically reflect their original human stroma histotypes. Our preliminary results have exposed distinct proteome and secretome compositions, as well as major differences in their cell biology and behavior. We now have access to >70 PDAC patient-derived organoids (PDOs) with rich clinical information. We have also optimized functional assays as well as drug testing protocols for PDOs, with and without incorporation of our novel CAF models. Utilizing functional genetics and pharmacological manipulation, our innovative human preclinical platform will facilitate a systematic streamlined interrogation of shortlisted targets from Aim 1. This will unravel i) inherent capacities of human CAF subtypes to promote or restrain aggressiveness of PDAC PDOs, ii) corresponding therapeutic targets, and iii) potential for synergistic effects with current 1st line chemotherapeutics.

Significance: Our compartment-specific and patient-matched expression profiling will illuminate the molecular composition of PDAC stroma subtypes and tumour cells. This knowledge is key to understanding stromal biology in PDAC and molecular mediators underlying its powerful pro- and anti-tumour functions. This work will help instruct molecularly-guided stroma-targeted therapies and improve currently used chemotherapeutic options. We will also generate an unparalleled molecular resource with rich clinical annotation for the scientific community along with new *in vitro* models for recently discovered human stroma histotypes. Overall, our program will propel further mechanistic studies and help reconcile controversies on the disparate role of PDAC stroma.

Muller, William
McGill University

Evaluating the role of integrin signalling in breast cancer dormancy

We have previously demonstrated that mammary-specific disruption of β1 integrin results in a drastic impairment of tumour induction in a GEMM of human luminal B breast cancer. Although this study points to an essential role of β1 integrin in promoting breast cancer, we were not able to study the biological effects of β1 integrin loss because tumours that emerged from this previous GEMM still retain expression of β1 integrin. To this end, we have recently generated a modified version of this GEMM, that results in tumours with effective ablation of any conditional alleles. Furthermore, this model faithfully recapitulates the pathological progression of breast cancer as observed in human. Using this new tool, we have investigated the role of β1 integrin and identified paradoxical effects of its deletion in different stages of mammary tumour progression.

Nepveu, Alain
McGill University

DNA repair properties and vulnerability of triple negative breast cancer cells

Background

It is well established that defects in DNA repair contribute to the development of cancer. Paradoxically, increased DNA repair efficiency, in particular of the base excision repair (BER) pathway, is essential for many cancer cells, not only to resist genotoxic treatments but also to proliferate in the presence of elevated reactive oxygen species (ROS) produced by cancer-associated metabolic changes. We have discovered one strategy that cancer cells use to augment their DNA repair capability. Many cancer cells increase the expression of accessory proteins that stimulate the activity of BER enzymes. In contrast to BER enzymes, these accessory proteins are not essential to normal cells under physiological conditions, but are needed for cancer cells to avoid senescence or apoptosis in response to oxidative DNA damage. This provides a therapeutic window to develop new drugs targeting accessory proteins to which cancer cells have become addicted. We have established the paradigm in this field by showing that CUT domain proteins stimulate OGG1, a DNA glycosylase that initiates repair of <u>oxidized purines</u>. We now have identified another transcription factor as a novel accessory factor for NTHL1, the DNA glycosylase that initiates repair o*f oxidized pyrimidines*. Moreover, this protein also stimulates the polymerase activity of DNA pol β.

The gene that encodes this transcription factor has recently been shown to be highly expressed in triple-negative breast cancers (TNBC) and in the basal-like breast cancer subtype, a subgroup of breast cancers defined by gene-expression profiling. Knockdown of this gene in three TNBC cell lines suppresses tumour growth in xenografts. In the DMBA-induced tumour model, prior deletion of this gene greatly decreases tumour formation, whereas deletion of the gene after tumour appearance causes tumour regression, indicating that it represents a potential therapeutic target. Interestingly, a retroviral mutagenesis screen revealed a strong cooperation between overexpression of this gene and loss of the Nf1 tumour suppressor gene in leukemogenesis, suggesting cooperation between this gene and RAS pathway activation. These results provide a rationale for a targeted treatment in TNBCs.

None of the transcriptional targets of this transcription factor can account for its implication in cancer.

Hypotheses
We hypothesize that increased expression of this gene is needed by TNBC cells that exhibit elevated ROS levels in order to augment their capacity to repair DNA damage and avoid senescence or apoptosis.

Research Plan
1) Role in DNA Repair and Resistance to Genotoxic Stresses
We will investigate the effects of this gene's knockdown and overexpression on the efficiency of DNA repair and the resistance of triple-negative breast cancer cells to genotoxic treatment.

2) Synthetic Lethality of Gene Knockdown
We will measure steady-state ROS levels and investigate the effects of this gene's knockdown on genomic DNA damage, cell proliferation and senescence in a panel of TNBC cell lines of distinct subtypes as well as in normal MCF10A and human mammary epithelial cells (HMEC). We will confirm the synthetic lethality of this gene's knockdown in patient-derived TNBCs established as xenografts in immunocompromised mice.

3) Mechanism of Action

To address mechanism of action, we will identify the protein domains that stimulate the enzymatic activities of NTHL1 and DNA pol β. We will define the DNA binding specificity and kinetics of this protein, determine whether the protein interacts directly with NTHL1 and DNA pol β, stimulates binding of these BER enzymes to their DNA substrates and engages in a ternary complex with the enzyme and DNA.

4) Role in Tumour Development

We will investigate driver mutations that cooperate with this gene's overexpression in breast tumour development, in particular those that lead to RAS-pathway activation.

Significance

These experiments will identify a new base excision repair accessory factor, provide a mechanistic explanation for the role of this gene in triple-negative breast cancers and evaluate the protein encoded by this gene as a potential therapeutic target.

Zhang, Wei
University of Guelph

Engineering HPV oncoprotein E6 for inhibitors of cancer progression

Background: Human papillomavirus (HPV) is directly implicated in the development of cervical cancer, the second leading cause of cancer-related deaths among women worldwide, and a subset of anogenital cancers. While available vaccines potentially prevent infection against a few HPV subtypes, risks of developing cancer for HPV-infected population are still high. Indeed, in recent years HPV-positive oral cancer have drastically increased and the virus is now detected in 60-80% of the cases. Therefore, there remains an urgent need for developing therapeutics to induce HPV-positive cancer cell death by targeting the transforming capabilities of viral oncoproteins.

Objective: The HPV E6 protein is the key determinant for malignant tumour progression through interacting with an E3 ubiquitin ligase E6AP to stimulate p53 ubiquitination and degradation and escape normal cell proliferation control, thereby leading to malignant transformation. Targeting the protein-protein interaction (PPI) between E6 and E6AP thus becomes a promising therapeutic option to induce growth arrest and provoke apoptotic death of HPV-positive cancer derived cells. Unfortunately, despite tremendous efforts the lack of potent and selective protein-protein interaction (PPI) inhibitors has severely hampered attempts to exploit the E6-E6AP-p53 axis for therapeutic benefits. In this proposal, we will leverage our protein engineering platform to generate PPI inhibitors disrupting the E6-E6AP interaction to modulate p53 ubiquitination and validate their potential as pharmacological agents for cancer treatment.

Scientific approach: In the past five years, we have devised and optimized structure-based combinatorial protein design and engineering approaches to develop intracellular PPI modulators to manipulate various aspects of ubiquitin signaling in human cancer cell lines. Here, we plan to leverage our protein engineering and synthetic biology platform to identify, characterize, and optimize protein-based E6-E6AP PPI inhibitors to restore p53 protein level and activity. We will build phage-displayed HPV-E6 variant (E6V) libraries with selected residues diversified based on the co-crystal structure of E6 bound to E6AP leucine (L)-rich LxxLL motif. Synthetic E6V binders with improved binding affinity and specificity to E6AP will then be selected and characterized in biochemical assays for inhibitors hijacking E6-E6AP PPI to prevent p53 ubiquitination. With collaborators structural basis of inhibition will be revealed and this will enable

optimization of next-generation inhibitors with improved potency and selectivity. Finally, we will validate the p53 reactivation function and anti-cancer properties for confirmed inhibitors in cell culture and animal models.

Significance: With support from the Canadian Cancer Society Innovation program, we will provide the first-in-class intracellular inhibitors targeting the E6-E6AP-p53 axis as early-phase pharmacological agents for HPV-mediated cancer therapeutics. In addition, detailed structural analyses of E6AP::E6V complexes will facilitate the design and screen for next-generation of protein-based and chemical inhibitors and shed light on targeting other "druggable" PPIs in host-pathogen interactions. This project will ultimately yield a pipeline for the production, optimization, characterization, and delivery of PPI inhibitors that will be available to our collaborative network and the broader research community to exploit ubiquitin signaling for cancer therapeutics.

I2   Imaging and Technology Development

Li, Shyh-Dar
University of British Columbia

A drug delivery technology for activating the tumour immune microenvironment of peritoneal metastases

Problem: Peritoneal metastases (PM) develop from a spectrum of tumours, mainly colorectal and ovarian cancers. The mainstay of treatment includes cytoreductive surgery and hyperthermic intraperitoneal (IP) chemotherapy. Even with these aggressive treatments, the PM recurrence rate is up to 90%. PM patients present with abdominal distention, pain, and emesis caused by malignant obstruction. The overall survival of patients presenting malignant obstruction is 6.4 months. Tumour cells in the PM recruit and transform the immune cells to create an immunosuppressive environment. The tumour immune microenvironment (TIME) is characterized by decreased numbers of CD8+ T cells and natural killer (NK) cells and increased infiltration of immunosuppressive cells, including tumour-associated macrophages (TAMs), regulatory T cells (Tregs) and myeloid-derived suppressor cells (MDSCs). A few immunotherapeutic agents, such as immune checkpoint blockade (ICB) and CAR (chimeric antigen receptor)-T cells, have been investigated for PM therapy via IP administration. Although the results are encouraging, tumour infiltration of immunosuppressive cells has been shown to reduce the efficacy of these promising immunotherapies. R848, a toll-like receptor 7 and 8 (TLR7/8) agonist, stimulates immune cells to promote the antitumour immunity and switches immunosuppressive cells to the antitumour phenotypes. In a PM model of CT26 murine colon cancer, we found that IP delivered free R848 was only effective in clearing the PM at a high dose, but induced significant bodyweight loss (10-20%), suggesting significant toxicity. We discovered that the IP injected dose was rapidly absorbed into the blood (within 15 min), resulting in poor IP retention and a high blood concentration that induced systemic toxicity.

Objectives: This project will develop a new technology to reverse the immunosuppression in the TIME of PM for effective treatment. We hypothesize that a drug delivery system that can efficiently retain R848 in the peritoneum will improve the efficacy and reduce the systemic toxicity.

Methods: This project, through 2 aims, will develop a drug delivery technology and examine its mechanism, efficacy, and preliminary safety in a mouse PM model.

Aim 1. Develop a technology to retain R848 in the peritoneum for local activation of the TIME. We discovered that cationic distearoyl(C18:0)-trimethyl ammonium propane (DSTAP)-liposomes were

preferentially retained in the peritoneum after IP delivery, and R848 could be stably loaded into the liposomal aqueous core using our proprietary technology to create a formulation that focused the drug delivery to the IP cells (predominantly immune cells). We will characterize the effects of DSTAP-R848 in the TIME of a PM model. We will examine which immune cells in the PM interact with DSTAP-R848 by fluorescence-activated cell sorter (FACS) and compare the local (IP) and systemic (serum) profiles of cytokines induced by free R848 and DSTAP-R848 using multiplex enzyme-linked immunosorbent assay (ELISA) and real-time polymerase chain reaction (RT-PCR). The immune cell composition in the PM after treatment with free R848 and DSTAP-R848 will also be determined by FACS to examine whether the TIME is converted to an antitumour phenotype.

Aim 2. Compare the efficacy and preliminary safety of free R848 and DSTAP-R848 in mice. We will determine the anti-PM efficacy and preliminary safety profiles of free R848 and DSTAP-R848 by whole body imaging of tumour burden and general health monitoring. Mice will also be examined for the specific antitumour immunity by FACS.

Significance: If DSTAP-R848 is demonstrated effective and safe in the preclinical models, the project will lead to the development of a first-in-class treatment for PM. This DSTAP-liposomal technology will be of use to deliver other agents for PM to advance the research and develop novel therapeutics. Ultimately, the survival and quality of life of PM patients will be improved.

Yasufuku, Kazuhiro
The Toronto Hospital (General Division) - UHN

Enhancing sentinel lymph node mapping in lung cancer surgery using a novel liposome-based dual-modality nanoparticle

Each year >28 000 Canadians are diagnosed with lung cancer. Even with resectable disease, 5-year survival can be as low as 50%. This has driven interest in sentinel lymph node (SLN) mapping for lung cancer surgery, given its success in breast cancer and melanoma in reducing surgical morbidity and guiding postoperative care. Lung SLN mapping identifies the first lymph nodes draining a tumour; SLNs are high-risk for harbouring metastases. Around 20% of 'node-negative' lung cancer patients may have metastases missed by routine pathology. Thorough evaluation of SLNs can therefore improve staging accuracy and patient selection for limited lung resection. This could increase survival and parenchymal sparing via informed treatment planning.

Two lung mapping methods exist: near-infrared fluorescence (NIRF) and computed tomography (CT). The former uses peritumoural NIRF dye injection (e.g. indocyanine green, ICG) for intraoperative visualization, but NIRF light has limited tissue penetration. The latter uses peritumoural CT contrast (e.g. iohexol, IOX) and preoperative CT to define an SLN's location regardless of depth but does not allow intraoperative visualization. These limitations have hindered adoption of lung SLN mapping into standard care; integrating both methods would overcome these barriers. CF800 is a novel polyethylene glycol (PEG)-coated liposome containing ICG <u>and</u> IOX. Its size (100nm vs. 1nm ICG/IOX) may also improve retention in the SLN, reducing spillover into non-SLNs that can confound mapping.

Our goal is to use preclinical models to develop a multimodal approach for lung SLN mapping. We hypothesize dual-modality CF800 will improve SLN detection and pathological accuracy over unimodal ICG/IOX by enhanced visualization and retention.

Aim 1. Compare CF800 with unimodal agents
CF800 performance relative to free ICG/IOX must first be defined. NIRF mapping will be studied by mouse subcutaneous injection. Drainage to local nodes allows measurement of flow, retention, and spillover by overhead NIRF camera. These nodes will then be resected for immunofluorescence microscopy. Co-localized NIRF and cell markers (e.g. F4/80+ macrophages) would implicate cell uptake in SLN retention; possible mechanisms would then be explored *in vitro* by blocking potential pathways (e.g. complement system). For CT mapping, transpleural CF800 will be compared to IOX in pig lungs post-thoracotomy. Lymph flow and nodal retention will be evaluated by serial CT.

Aim 2. Explore non-tracer factors for SLN mapping
Several factors may affect SLN mapping: administration route, ventilation method, and dose timing. First, CF800's PEG coating reduces protein interaction; post-injection leakage may differ by injection route. Transpleural and transbronchial CF800 leakage will be compared in pig lungs by NIRF camera and CT. Second, mechanical ventilation alters lung lymph flow. To assess the effect on SLN mapping, CF800 injected into rabbit lungs will be tracked by CT during mechanical vs. spontaneous ventilation to compare flow and SLN retention. Finally, PEGylated drugs can generate anti-PEG antibodies; sequential dosing of CF800 may alter cell uptake in the SLN. CF800 dosing regimens will be compared in mice for anti-PEG (ELISA) and SLN retention (see methods in Aim 1).

Aim 3. Validate an optimized CF800-SLN protocol
Informed by Aims 1 and 2, we will validate an optimized CF800 protocol in a clinically-relevant model. Rabbit lung tumour models will be created by bronchoscopic injection of VX2 cells (rabbit squamous cell cancer) and survived until early nodal metastases develop. They will then undergo CF800- vs. ICG-/IOX-SLN mapping. SLN pathology will be compared to total nodal dissection to calculate the pathological accuracy of each agent.

This project will have many potential benefits. First, CF800 may outperform current agents, improving the accuracy and ease of lung SLN mapping. This would enable wider adoption and more personalized lung cancer care. Second, we may identify previously unknown factors affecting SLN mapping, such as ventilation method. Finally, the utility of CF800-SLN mapping in lung cancer may encourage study in other cancers.

Zemp, Roger
University of Alberta

Amplification and Enrichment Platforms for Liquid Biopsy-based Early Prognostication of Cancer

In this grant, we aim to develop technologies that (1) amplify vesicle levels in ex-vivo blood samples containing CTCs using ultrasound treatment, along with micro-flow cytometry to detect increased levels of CTC derived vesicles as a powerful new method to quantify sparse CTC levels without missing such rare cells. (2) significantly amplify levels of biomarkers and extracellular vesicles in the blood of animal subjects using ultrasound treatment (3) enrich meaningful vesicle and CTC populations from the whole blood volume using proposed functionalized scaffolds in an IV system (4) detect epigenetic modification in biomarker-amplified and/or enriched samples.

I3   Immunology, Signalling and Stem Cells

Brockman, Mark
Simon Fraser University

Toward improved immunotherapy for ovarian cancer: quantifying the diversity, phenotype and temporal dynamics of tumour-reactive T cells from primary treatment to relapse

High-grade serous carcinoma (HGSC) is the most common and lethal type of ovarian cancer, with only ~30% of patients surviving 5 years or more. The presence of tumour-infiltrating lymphocytes (TIL) is strongly associated with survival in HGSC, suggesting that immune-based strategies should be beneficial. However, immune checkpoint blockade has so far shown disappointing results for HGSC, and adoptive T cell therapy (ACT) has yet to achieve clinical responses better than disease stabilization.

Recent anecdotal reports by us and others reveal the multiple ways in which TIL responses can fail. We contributed to a recent report in *Nature Medicine* showing in two HGSC patients that <5% of TIL were tumour-reactive, with the remainder representing irrelevant "bystander" T cells that may provide no clinical benefit. Moreover, we reported in *Cell* that TIL-positive tumours can lose expression of tumour antigens and/or MHC molecules. Conversely, we previously reported an HGSC case in which tumour-specific T cells emerged during primary treatment but then disappeared from blood and tumour tissue at second relapse. These issues will thwart any immunotherapy that relies on endogenous tumour-reactive T cells, including checkpoint blockade and ACT. Thus, there is a pressing need to move beyond anecdotal reports toward a systematic understanding of the fate and phenotype of tumour-reactive T cells during the clinical course of HGSC.

Hypotheses
1. Tumour-reactive CD8+ T cells express a unique set of cell surface markers.
2. In HGSC patients undergoing standard treatment, the diversity and frequency of tumour-reactive CD8+ T cells in peripheral blood is maximal during first remission and deteriorates during progression to relapsed disease.

Objective
This project will investigate the diversity, phenotype and temporal dynamics of bonafide tumour-reactive CD8+ T cells in HGSC patients during standard treatment and progression to relapsed disease.

Aim 1. To determine the diversity, frequency and surface phenotype of tumour-reactive CD8+ T cells. TIL will be collected from 10 HGSC patients, expanded with IL-2, and exposed to autologous primary tumour. Tumour-reactive (CD137+) CD8+ T cells will be isolated by FACS for single-cell analyses: TCRseq will identify matched TCRɑ and b subunits, and targeted RNAseq will profile 16 genes encoding surface proteins characteristic of activated, tissue-resident memory, and dysfunctional T cells (CXCR6, CD38, CD39, CD49a, CD57, CD101, CD103, CTLA4, GITR, ICOS, LAG3, OX40, PD1, TIGIT, TIM3, 2B4). For the 50 most prevalent TCR, specificity for autologous tumour will be validated using a reporter cell assay. *Deliverable:* An unprecedented compendium of >3500 tumour-reactive TCR clonotypes with linked single-cell data on phenotypic cell surface markers.

Aim 2. To investigate how the diversity, frequency and surface phenotype of tumour-reactive CD8+ T cells changes during standard treatment and disease progression. TCR sequences from Aim 1 will be used to track the temporal dynamics of tumour-reactive T cells in HGSC patients. Serial blood specimens (from

diagnosis, standard treatment, remission, and relapse) will be profiled by FACS and TCRseq to quantify the frequency of each tumour-reactive TCR clone in CD8+ T cell subpopulations defined by candidate surface marker phenotypes from the literature and Aim 1. To investigate the influence of antigen loss, selected TCR clonotypes will be assessed for recognition of matched relapsed tumour. *Deliverable:* The first systematic view of the phenotype and fate of thousands of tumour-reactive CD8+ T cells over the clinical course of HGSC.

Clinical relevance
Our work will reveal the surface phenotype of transient versus persistent tumour-reactive CD8+ T cells over time, informing the targets and timing of future checkpoint blockade strategies. This study may also enable the use of peripheral blood to produce more pure and potent T cell products for ACT. Our innovative methods and data will be applicable to other cancers.

Brooks, David
Princess Margaret Cancer Centre - UHN

Visualizing interferon signatures to predict efficacy of cancer immunotherapy

Immunotherapy has revolutionized cancer treatment. However, for unknown reasons, many patients still do not respond to these therapies. One of the most important questions currently facing the use of immunotherapy is how to predict who will respond to therapy in order to direct the best treatment on an individualized basis. Further, these therapies are increasingly becoming associated with adverse autoimmune events that can severely affect the ability to sustain treatment even if it is working. Emerging information indicates that alterations in how a group of proteins called type I interferons stimulate the immune system are critical to the success of immunotherapy. Interestingly, these same proteins are also heavily associated with autoimmunity. Thus, we propose that regulation of type I interferon responses is key to understanding the success of immunotherapy and that measuring the levels and changes in their effects will provide a new way to effectively identify and predict the patients that will respond to immunotherapy to eliminate cancer. Importantly, we propose that by measuring these interferon-induced responses, we will also be able to pre-identify patients that will experience autoimmune events as a result of therapy, and thus prevent these adverse reactions. To achieve this goal, we have developed a new technique to intricately measure type I interferon signaling and its immune system-wide effects in individual patients. This new strategy has the potential to transform the way that therapy is provided and ultimately serve as the basis to guide the use of immunotherapy in the clinic.

Haeryfar, S.M. Mansour
Western University

MAIT cell functions and therapeutic potentials in metastatic colorectal carcinoma

Colorectal cancer (CRC) is a major cause of mortality worldwide. With a 5-year survival rate of only 64%, CRC is also the second highest cause of cancer related death in Canada. A devastating complication of CRC is metastasis to various organs, most frequently to the liver. Surgery for CRC liver metastasis (CRLM) is currently considered the standard of care and is increasingly used in combination with neoadjuvant therapies and chemotherapies. Though preoperative chemotherapy may help reduce the tumour burden to allow for surgery, only a small percentage of CRLM patients have resectable tumours. Therefore, there

is an urgent need for new and efficacious therapies to shrink CRLMs, thus maximizing the number of candidates for hepatic resection. Immunotherapy is typically more tumour-specific and less toxic than chemotherapy and presents renewed hope in the fight against CRC. We recently reported for the first time that mucosa-associated invariant T (MAIT) cells, a type of unconventional innate-like T cells with unique characteristics and remarkable cytotoxic and immunomodulatory properties, infiltrate CRLMs but are rendered dysfunctional within the metastatic masses. MAIT cells are uniquely enriched in the human liver; yet, their roles in anti-cancer immune surveillance have only recently come under scrutiny. We hypothesize that MAIT cells serve a protective role in CRC and CRLM. We also hypothesize that the MAIT cells' functional impairments observed in the CRLMs can be prevented and/or reversed using MAIT cell function-modifying agents.

We will analyze MAIT cells from healthy and tumour bearing liver tissue from CRLM patients via RNA sequencing to determine which genes are exhibiting altered expression patterns. Studies have shown that the presence of certain immune cells can correlate with improved or reduced patient survival. To determine whether MAIT cells are helping or hindering the immune response to CRLM, we will use orthotopic mouse models of CRC. These preclinical models mimic colon tumour invasion, vascular spread, and metastasis to distal organs, including the liver. To test potential immune therapies, mice with CRLM will be given MAIT cell agonists. Morbidity and mortality will be monitored and liver metastasis progression between experimental and control mice will be compared. The timing of ligand administration will be optimized in both preventative and therapeutic settings. If MAIT cell mediated immune responses are detrimental to the fight against CRLM we will need to explore immune therapies that can disable these cells. The findings of this project could lead to the development of novel immunotherapies for CRLM, thus allowing more patients to qualify for surgical resection. Such treatments could greatly improve the overall health and survival of CRC patients suffering from hepatic metastasis. In the future, MAIT cell-based therapies will be combined with chemotherapies in the hope that total CRC/CRLM eradication will eliminate the need for surgical interventions.

Raouf, Afshin
CancerCare Manitoba

Identification and characterization of clonogenic cells in primary ER+ human breast cancer tumours

Majority of breast cancer tumours are Estrogen Receptor positive (ER+) and for these tumours, interfering with estrogen signaling (endocrine therapies) is an effective treatment modality. *Unfortunately, >30% of these tumours develop endocrine therapy resistant* (ETR) *cells* which pose a significant clinical challenge due to increased metastatic disease and poor overall survival as effective therapies against ETR tumours are lacking. In this regard, the existence of breast cancer stem cells (BCSC) has been suggested as a likely explanation for therapeutic resistance and tumour relapse. The identification of BCSCs in ER+ tumours has been limited to serial passaging of cells grown as nonadherent spheres and xenografts in immunodeficient mice. However, since ER+ breast cancer cells (BCCs) have proven difficult to maintain *in vitro*, identification of clonal tumourigenic subsets has not been possible. Such studies therefore, have been limited to breast cancer cell lines which may represent the essential facets of the primary tumours. Recent advances in patient-derived xenografts have shown some promising results for the triple negative and HER2+ tumours. However, these studies have proven challenging for ER+ tumours due to their poor xenograft efficiency (<5%).

To address this issue, we developed a new cell culture system, enabling us to perform colony forming cell (CFC) assays using primary malignant human ER+ breast cancer tumours (ER+BCTs). Based on this

assay we have identified 2 distinct but low frequency (0.1%-0.5%) tumour CFC (T-CFCs) types from the ER+BCTs. Next, we found that these two T-CFC subtypes can be purified based on their differential expression of CD49f and Epithelial Cell Adhesion Molecule (EpCAM). Very interestingly, type A T-CFCs were enriched in the CD49f+EpCAM+ subset while type B T-CFCs were enriched in the CD49f+EpCAM- subset of tumour cells. To demonstrate the clonal origin of these T-CFCs, primary ER+BCCs were transduced with a lenti GFP virus and we found that sorted a single GFP+ T-CFC formed a colony of several 100 cells in 9 days. Next, we developed tissue culture reagents enabling us to expand the primary ER+BCCs *ex vivo*. We expanded cells from 14 primary ER+ human breast tumours at an unprecedented 100% efficiency. These expanded cells contain 75-85% ER+ cells and form ER+ tumours in xenografts within 7 weeks. Also, we found that the *ex vivo* expanded ER+BCCs contained both T-CFC subtypes but at much higher frequencies (5-20%) which can be isolated using CD49f and EpCAM markers. To examine T-CFC sensitivity to endocrine therapies, the ER+ cell lines were treated with tamoxifen (Tam) and Fulvestrant (ICI) in organoid cultures. Whereas Tam reduced T-CFCs by 50%, ICI reduced their numbers by ~90%, suggesting that the remaining T-CFCs are resistant to Tam and ICI. Based on these very exciting and novel data, we hypothesize that T-CFCs share some of the BCSC characteristics. The overarching goals of this proposal are to further characterize the T-CFCs and to explore their role in therapy resistance. Towards these objectives we have 2 Specific Aims;

1. To characterize T-CFCs with respect to their relationship to BCSCs and tumour development. In this Aim, we will examine the tumour forming potential of the different T-CFC subsets of primary ER+ tumours and explore their relationship to the BCSCs using serial in vivo xenografts and in vitro tumoursphere assays.
2. To characterize the tamoxifen-resistant subset of T-CFCs. In this Aim, we will determine which of the two T-CFC subtypes develops resistance to endocrine therapies and ascertain the molecular mechanisms that confers ETR phenotype onto the T-CFCs using the RNA-seq transcriptome profiling platform combined with functional gain and loss of function studies in organoid cultures.

Our novel cell culture system has enabled us to identify and isolate the clonogenic subsets of primary human ER+BCTs, some of which are refractory to endocrine therapies. Characterization of these clonogenic cells and assessing their contribution to ETR development would reveal useful and relevant gene targets towards the development of effective therapies against ETR tumours.

Schramek, Daniel
Mount Sinai Hospital

Mapping the determinants of effective immunotherapy using multiplexed, direct in vivo CRISPR screens

Among the most promising recent advances in oncology is the development of cancer immunotherapies, which are focused on reactivating endogenous antitumour immune responses. Immune checkpoint inhibitors are designed to break the immune tolerance imposed by the tumour particularly against cytotoxic T cells and have shown remarkable efficacy against cancers with high mutation burden. Similarly, adoptive cell transfer (ACT) of T-cells extracted from patients, genetically modified and activated *in vitro* and returned to the same patient has proven powerful therapeutic strategy in experimental studies. While very effective, immunotherapies result "only" in a ~ 50% response rate and mere 25% of patients experience a prolonged survival benefit indicating the existence of resistance mechanisms and other immune escape mechanisms.

This project specifically aims to elucidate novel immune regulators with the goal to (1) deepen our

understanding of cancer immune biology, (2) elucidate potential resistance mechanisms, (3) identify novel immune therapy targets and most important for immediate translation (4) help stratify cancer patients into immunotherapy responders and those who might benefit more from conventional chemotherapy.

Specifically, we choose to focus on lung cancer, as lung cancer is the major cause of cancer-related mortality in Canada and a well-established target for immunotherapy. Non-small cell lung cancer (NSCLC) accounts for ~85% of all lung cancers and standard therapies for NSCLC are often ineffective and remain toxic signifying the need for novel and improved therapies. We will also employ our novel squamous cell carcinoma (SCCs) models, as SCCs have recently been added to the tumours treated with immunotherapy.

Here, we propose direct *in vivo* CRISPR and overexpression screens, where autochthonous KRasG12V-tumours as well as Pik3caR1047R- driven SCCs are transduced with sgRNAs or ORF construct. The effect of an experimental immunotherapy on individual tumours can then be measured using deep-sequencing. While most transfected cells will be efficiently cleared by the immunotherapy, sgRNAs or ORFs that cause resistance to immunotherapy will selectively enrich. Conversely, if sgRNAs or ORFs confer sensitivity to immunotherapy, they will be deplete prematurely or more efficiently from the population.

Given the success of CTLA4 and PD1/PDL1 immune checkpoint blockade in cancer, it is vital to identify additional immune checkpoint inhibitors that may prove safer and show more effective/durable clinical responses. At the same time, it is clear that immune-selective pressure for resistant tumour clones exists, but their identity remains elusive. By direct *in vivo* tissue-specific gene editing using immunocompetent mice, our in vivo CRISPR/ORF technology simultaneously tests hundreds of genes, which are directly associated with the cytolytic activity of TILs in human tumours. Thus, this novel approach allows us to better assess the mechanisms of intrinsic or therapy-induced acquired immune resistance as well as to elucidate novel immune therapy targets.

I4 Novel Therapeutics

Houry, Walid
University of Toronto

Dysregulation of mitochondrial protein homeostasis for cancer treatment

The mitochondrion is a vital organelle in the human cell that has a plethora of biological functions. Consequently, a dysfunctional mitochondrion gives rise to a wide range of diseases, including various types of cancers. As such, the cell employs a variety of biological processes that are important for the upkeep of a healthy mitochondrion and its proper functions. Molecular chaperones and proteases are key players required to maintain the quality and integrity of the mitochondrial proteome. The human ClpXP complex is an ATP-dependent protease found in the mitochondrial matrix and plays an important role in ensuring protein quality control.

The ClpXP complex is composed of two proteins: the serine protease ClpP and the AAA+ ATPase ClpX. Mitochondrial ClpXP has been found to play an important role in activating the mitochondrial unfolded protein response, in affecting the rate of mitochondrial protein synthesis by regulating the levels of mitoribosome assembly, and in degrading several different subunits of the mitochondrial respiratory chain

complexes. Importantly, ClpP has been found to be universally overexpressed in primary and metastatic human cancers, and its levels correlated with shortened patient survival. For example, it has been found that ClpP levels are significantly increased in AML cells and in patient samples compared to normal hematopoietic cells. Inhibition or downregulation of mitochondrial ClpP induces cell death in human leukemia cells preferentially over normal cells. Furthermore, dysregulated mitochondrial respiration induced by ClpXP targeting caused oxidative stress, which in turn reduced tumour cell proliferation and abolished metastatic dissemination *in vivo*. Therefore, ClpP is a 'drugable' therapeutic target in cancer.

Our group has extensive experience studying the biochemistry, structure and cell biology of ClpP from various species. In the course of these studies, we identified novel classes of compounds that dysregulate ClpP in collaboration with Dr. Robert Batey's group (Department of Chemistry, University of Toronto). These compounds bind at the top of the ClpP cylinder to prevent the formation of the ClpXP complex. However, the compounds also dysregulate the ClpP protease by opening up the entrance pore, hence, causing ClpP to degrade proteins unspecifically causing cell death. About 150 analogs were generated of these compounds, and the co-crystal structures of ClpP with several compounds were obtained.

In the course of the above studies, the analogs were also tested against the human ClpP. To our surprise, several compounds we generated had submicromolar binding to human ClpP. One structural class was found to bind tightly to human mitochondrial ClpP (Kd of tens of nM). The class is based on acyldepsipeptides (ADEPs). These compounds inhibited human ClpXP formation and dysregulated human ClpP activity allowing the proteases to unspecifically degrade model substrates. Importantly, the compounds had IC50 values against wildtype HEK293 cells in the range of 0.5 μM and had no effect on HEK293 cells lacking ClpP. This clearly demonstrated that the compounds are acting on-target in cells. Finally, through biochemical and cell biological assays, we found that the compounds cause apoptosis in cell cultures.

In this project, we are interested in testing these compounds in animal models and in further optimizing these ADEP compounds using structure-based medicinal chemistry approaches. Our goal is to obtain compounds with nM binding to ClpP that dysregulate the protease and show good pharmacokinetic properties. Such compounds can be targeted against several cancers. However, given the interests in our laboratories and given the available published literature, we will likely concentrate on breast cancers for future studies given that ClpP has been reported to be elevated in these cancers. The project will involve structural studies, biochemical, and animal studies of the most promising compounds.

Kafri, Ran
The Hospital for Sick Children

Pharmacologic prevention of malignancy in li-fraumeni syndrome

Li-Fraumeni syndrome (LFS) is a genetic cancer predisposition that associates with high risks of pediatric cancers. Genetically, LFS is typically caused by germline TP53 mutations. Treatment of malignancies in children with LFS is particularly challenging. Children with LFS associate with lifelong risks to develop a wide spectrum of early onset cancers. In this study, instead of seeking treatments for tumours in children with LFS, we propose a daring alternative – to identify drugs that prevent initial tumour onset. Rationale for this study stems from recent findings showing that inhibition of mTORC1 delays or prevents tumour onset in Trp53-deficient mice. While mTORC1 inhibitors are effective in cancer prevention, these drugs are not effective in selectively killing cancer cells. Based on this, we propose that cancer prevention results from altering signaling in the premalignant microenvironment in a manner that disfavors cancer onset.

In this proposal, we rely on two innovative experimental assets: 1) A comprehensive library of patient derived LFS fibroblast lines that vary in TP53 mutations and clinical outcomes. These fibroblasts serve as a proxy of stromal signaling in the premalignant niche. 2) The LFS cancer cell observatory – a computer-vision assisted experimental system dedicated to quantifying morphological and molecular dynamics in millions of single patient-derived LFS cells. The goal of the observatory is to identify features of cell morphology or signaling that correlate with the pro-survival secretome of the LFS pre-malignant stroma. We will then use these signatures as screening assays to identify drugs that match signatures of 'cancer prevention'. Finally, we will test the top compounds hits for their influence on tumour onset in mice models of LFS.

The cancer cell observatory will provide a first of a kind dataset – systematically mapping p53-dependent cellular functions in different LFS lines with genetic and clinical characterizations. Our proposal aims to deliver the following: (i) Validated fingerprints of cancer promoting fibroblasts that can be used as screen assays to identify new cancer preventing drugs. (ii) Cell readouts on cancer promoting functions that can be used to study the mechanisms of cancer promotion. In this proposal, we provide the foundation to that will serve to build a pharmacological toolbox correlated with specific TP53 mutations facilitating a personalized approach to offset the onset of malignancy in LFS. With this approach, we will gain the tumour-delaying benefits of mTORC1 inhibition, while avoiding the consequences of complete shutdown of mTORC1 activity. Ultimately, our goal is to identify tumour preventative pharmacology for children with LFS.

Lorimer, Ian
Ottawa Hospital Research Institute

Tumour suppressor therapy for glioblastoma

Glioblastoma is an aggressive form of brain cancer with a median survival after diagnosis of about one year. Forty percent of glioblastomas have a total absence of expression of the tumour suppressor *PTEN* due to copy loss and/or mutation. Over eighty percent have loss of at least one copy of PTEN. Data from our lab and others suggest that restoration of PTEN function in glioblastoma would have therapeutic benefit.

Here we are proposing to develop a clinically-applicable, cell-mediated delivery strategy to restore PTEN function in glioblastoma. This strategy makes use of PTEN-L, a weakly-expressed endogenous version of PTEN that is generated by the use of an alternate upstream start codon. Published data showed that this longer version of PTEN is secreted by cells and taken up by neighbouring cells. However, this data and our own data show that PTEN-L is actually secreted very inefficiently. To counter this problem, we have developed a novel, engineered version of PTEN-L that has enhanced secretion, which in turn leads to increased uptake by neighbouring cells.

We are proposing to test cell-mediated delivery of this novel protein *in vivo*. Neural stem cells will be used for delivery as they have cancer cell-homing properties and have recently been validated as protein delivery vehicles in a first-in-human study in glioblastoma. Neural stem cells will be derived by direct reprogramming of human fibroblasts and will be genetically engineered to express modified PTEN-L. The therapeutic activity of neural stem cells expressing modified PTEN-L will then be assessed *in vivo*. This will be done in a clinically-relevant model of glioblastoma in which patient-derived glioblastoma cells are injected intracerebrally into immunocompromised mice.

These patient-derived glioblastoma cells show highly invasive growth that is similar to what is seen in patients. Once disease is established in the mice, different doses of neural stem cells expressing engineered PTEN-L will be injected intracerebrally. This mimics the administration of therapeutics into the surgical cavity that has been used in many glioblastoma clinical trials. The primary endpoint for these experiments will be survival. In addition, immunohistochemical analyses will be performed to assess the distribution of neural stem cells within the brain and double immunofluorescence staining will be used to assess *in vivo* transfer of modified PTEN-L from neural stem cells to glioblastoma cells. Effects of neural stem cell-mediated delivery of engineered PTEN-L on glioblastoma cell proliferation and apoptosis will also be assessed by immunohistochemistry.

These studies will determine if we can achieve effective cell-mediated delivery of a tumour suppressor *in vivo* in clinically-valid preclinical models of glioblastoma. The studies will also generate a platform technology for therapeutic protein delivery in glioblastoma. This technology will be readily translatable using existing facilities for clinical biotherapeutics development and clinical trials at the Ottawa Hospital Research Institute.

Penn, Linda
Princess Margaret Cancer Centre - UHN

Exploiting an actionable metabolic vulnerability in high-risk myeloma

According to the Canadian Cancer Society's annual cancer statistics, nearly 3,000 Canadians will be diagnosed with multiple myeloma (MM) this year, and 58% will not survive the next 5 years. These sobering statistics reinforce the urgent need for new, effective treatment options for those diagnosed with this plasma B cell cancer. Within the past 10 years, the introduction of new therapies has significantly prolonged the survival of MM patients; however, the disease remains 100% lethal. MM is a highly heterogeneous disease that is often characterized by one of several disease-associated chromosomal translocations.

Research has shown that as cancers progress, the interplay between genetics, epigenetics and metabolism is important for the survival and continued growth of cancer cells. Recent work from our lab has identified an unexpected metabolic dependency of a particular MM subtype on the mevalonate (MVA) pathway, which is responsible for the production of cholesterol and other lipid metabolites. More importantly, we can demonstrate that inhibiting the mevalonate pathway with the statin family of cholesterol-lowering drugs causes this subtype of MM to undergo tumour-specific cell death and decrease disease burden *in vivo*. Furthermore, as a result of MVA pathway inhibition, statins activate a stress signaling pathway known as the integrated stress response selectively in a particular MM subtype.

Our *hypothesis* is that the within this MM subtype there is an unexpected metabolic dependency on the MVA pathway, which can be exploited using the statin family of drugs to improve treatment options for these MM patients.

Our *objectives* are to delineate the mechanism of this metabolic dependency and provide the necessary preclinical evidence to advance these safe, affordable and immediately-available agents forward to phase I/II trials in a particular subtype of MM patients. Our specific aims are to:

1. Identify the molecular determinant(s) of the MM subtype that confers statin sensitivity
In this MM subtype there is a deregulation of several potent oncogenes. We will use CRISPR/Cas9

technology and isogenic cell models to identify the molecular determinant(s) that confer statin sensitivity and further elucidate how these oncogenes increase the dependency of MM cells on MVA metabolism.

2. Determine the mechanism of statin-induced apoptosis in the sensitive MM subtype
We will evaluate how to best use statins in combination with current standard of care therapies (e.g. bortezomib) for the treatment of this MM subtype. Research has shown that activation of the integrated stress response is necessary for the anti-MM properties of bortezomib, and therefore we hypothesize that combining statins with bortezomib can improve MM patient outcomes through a novel metabolic mechanism.

3. Evaluate statins in combination with bortezomib to treat MM *in vivo*
Using xenograft models, we will evaluate whether combining statins with bortezomib can potentiate cell death, decrease disease burden and prolong survival in gold-standard preclinical models of MM-subtype-positive disease.

Taken together, we aim to provide the necessary preclinical data to warrant the clinical evaluation of the statin and bortezomib combination therapy for the treatment of the statin-sensitive subtype MM. Since statins are well-tolerated and approved for the management of cardiovascular disease, they have the immediate potential to be repurposed to positively impact MM patient care.

The co-PIs, Drs. Penn and Trudel, as well as the team of collaborators, trainees and technicians recruited to this proposal, have the molecular and clinical expertise to ensure these objectives are achieved in a timely manner.

Schimmer, Aaron
Princess Margaret Cancer Centre - UHN

Targeting the mitochondrial protease, NLN, in acute myeloid leukemia

AML (Acute myeloid leukemia) is an aggressive blood cancer. Despite recent new therapies for this disease, most patients ultimately relapse and die for this condition. Therefore, new treatment approaches for AML are urgently needed. This project will identify new biological vulnerabilities in the mitochondria of AML and highlight new treatment strategies for this disease.

Dr. Schimmer will study a new way to treat acute myeloid leukemia (AML) by targeting the mitochondria enzyme neurolysin (NLN). Using drug-like compounds and genetic approaches they will understand the normal function of this complex in the mitochondria. They will also study the effects of blocking it in AML using cells and mouse models. This project will identify new biological vulnerabilities in the mitochondria of AML and highlight new treatment strategies for this disease.

I5   Prevention and Quality of Life

Brotto, Lori
University of British Columbia

Mindfulness-based treatment for sexual difficulties following breast cancer

Prevalence rates of sexual dysfunction after breast cancer (BrCa) treatment are in the order of 45-86%. The most prevalent complaints focus on reduced desire, impaired arousal, and painful sexual experiences (dyspareunia). Women experiencing chemotherapy or endocrine therapy induced menopause are particularly likely to report reduced sexual interest and arousal, and sexual pain. Sexual dysfunction is highly distressing and is unlikely to resolve on its own. It presents challenges to maintaining a satisfying sexual relationship, and ultimately impacts the overall well-being and quality of life of both patients and partners. To date, interventions to treat low sexual interest and arousal within the cancer population are very limited.

Evidence demonstrates that mindfulness-based interventions improve sexual desire, arousal, lubrication, and satisfaction in individuals who do not have cancer. Preliminary data from an 8-session mindfulness-based intervention for otherwise healthy women with Sexual Interest/Arousal Disorder (SIAD) showed significant improvements in participant's sexual desire, overall sexual function, and sexual distress. The intervention employs Mindfulness-Based Cognitive Therapy for sexuality (MBCT-S) which incorporates several empirically supported therapeutic approaches, integrating elements of education, mindfulness meditation skills, and sex therapy.

Two briefer versions of this intervention were conducted in gynecologic cancer survivors with chronic low sexual desire, also with similar reports of success. It is now time to adapt these effective interventions to BrCa survivors. We propose a two-site randomized controlled trial of 8 weekly sessions of group MBCT-S versus 8 weekly sessions of a sex education control to address sexual difficulties. The manualized intervention, created by the PI (Dr. Brotto), will be delivered by clinicians specially trained in group therapy, sexual therapy, and mindfulness. Both treatments will be delivered over eight, 2.25 hour, weekly group sessions. There will be approximately 8 participants per group, and 6 groups per arm, in total. The primary aims are:

1. to test the effects of MBCT-S versus a sex education group on 3 co-primary endpoints: (a) sexual desire, (b) sexual distress, and (c) sexual pain;
2. to test maintenance of effects at 3 and 6 months following treatment;
3. to examine four potential mediators of change following MBCT-S: (a) mindfulness skills, (b) pain acceptance, (c) pain catastrophizing, and (d) depression;
4. to explore the relationship between baseline patient characteristics (e.g., demographics, sexual health characteristics, mood) and treatment outcomes to determine variables that predict the greatest benefits.

Participants are adult women who are sexually active in the past six months, English-fluent, with a history of BrCa, who exceed the clinical cut-off for sexual distress, and who are not currently receiving cancer treatment. Assessments will occur at 4 time-points: pre-treatment, post-treatment, and 3- and 6-months follow-ups. Assessments will include a clinician administered interview, and validated questionnaires assessing sexual distress, sexual function (including interest and arousal), sexual pain, pain acceptance, pain catastrophizing, mindfulness, and mood.

Descriptive statistics and frequencies will be calculated to report participant characteristics. A 2×4 mixed multivariate analysis of variance (MANOVA) will examine changes in primary outcomes (1) within groups over time, (2) between the two treatment groups, and (3) the interaction of the within and between group factors. Based on a power of .9, alpha of .05, a medium effect size for the primary outcomes (according to the pilot), and 20% attrition, it is estimated that 92 patients are needed (n=46 patients per group).

Low sexual desire and arousal, and sexual pain, are profound issues for BrCa patients that are often unaddressed due to patient-provider communication barriers and lack of treatment options. These findings will position our team to develop KT activities designed to reduce existing barriers preventing women from getting effective sexual health treatments.

Cunningham, John
Centre for Addiction and Mental Health

Offering nicotine patches to all households in a municipality with high smoking rates: Test of an innovative idea to promote large increases in tobacco cessation

Smoking is the leading cause of preventable death in Canada. Smoking cessation markedly reduces the risk of cancer and cancer death over time. Some regions of Canada have substantially higher rates of smoking than others. This is true among adults (reports available for people 18 years and older) at both the level of provinces and territories (ranging from 17% in British Columbia to 63% in Nunavut) but also at the municipal level (e.g., in Ontario, ranged from 15.4% to 44.7% between municipalities). Such information may be of key importance to increase the impact of smoking cessation initiatives. However, despite the potential importance, no studies have been conducted to-date to test whether targeting an effective tobacco cessation initiative – the distribution of free-of-charge nicotine patches – to areas with high smoking rates would lead to increased rates of tobacco cessation. Further, we plan a concentrated distribution, with offers of NRT sent to every household in a municipality. We predict that the social network effects of having many smokers attempting to quit in the immediate vicinity will lead to high rates of quitting. Our innovative pilot project will be the first to test this approach, providing much needed evidence regarding the potentially synergistic effects of the concentrated distribution of nicotine patches to a municipality with high smoking rates.

This study proposes to use population-level data from the Canadian Community Health Survey (CCHS) to identify one intervention municipal area with high smoking rates and several comparison municipalities with matched characteristics (e.g., smoking prevalence). We will send a coupon offering five weeks of free nicotine patches to one adult in every household in the selected intervention region. Participants will be able to reimburse their coupon at a mobile lab van, which will be parked at several locations throughout the municipality during the intervention period (there will also be a postal mail option for those unable to access the mobile van). Participants will be asked to complete a baseline survey containing items relevant to predicting smoking cessation. Participants will also provide a saliva sample to identify them as high or low nicotine metabolizers (determined by the CYP2A6 polymorphisms and measured by the 3-HC/cotinine ratio; a factor thought to predict success at tobacco cessation using NRT). Participants will be asked to complete a six-month follow-up survey to assess changes in smoking status. Data from the CCHS surveys conducted before and after the distribution will allow the assessment of changes in smoking status at the municipal level (and to compare these changes to matched municipalities who did not receive the NRT distribution).

This research is feasible: The proposed intervention is feasible, as is demonstrated by research already

conducted by our team showing that nicotine patches sent by postal mail can lead to increased rates of tobacco cessation. Those receiving nicotine patches reported higher rates of 30-day abstinence at 6 months compared to those not receiving free-of-charge nicotine patches (Odds Ratio [OR] = 2.65; *p* = .002). Secondary analyses indicated that mailing nicotine patches to some regions may have led to substantially higher impacts on smoking cessation (as high as an OR = 9.6). While this finding is only tentative because this was a secondary analysis with a small sample size, it does lead to our prediction that the impact of NRT might be large in regions with higher smoking rates and serves as a strong justification for this innovation proposal.

There is the potential for a major advance: This research is important because it will provide a preliminary test of the effectiveness of the concentrated targeting of tobacco cessation interventions to areas where they will have the most impact. The results of the trial will be used to establish whether more research to evaluate the concentrated distribution of NRT is merited. The results will also have strong policy and practice implications because the intervention itself can be scaled-up and implemented quickly and feasibly.

Downar, James
Bruyère Research Institute

Transcranial magnetic stimulation for psychological distress in patients with advanced cancer

The aim of this study is to test the feasibility and preliminary efficacy of accelerated rTMS for the treatment of existential suffering in patients with advanced cancer followed by palliative care (PC) providers. The specific aims are to determine: 1) ease of recruitment; 2) completion of follow-up; 3) effect size and variance estimates of treatment for primary and secondary outcomes; and 4) patient satisfaction with treatment. The results of this feasibility study will assist in the planning of a larger-scale randomized controlled trial of accelerated rTMS for suffering in patients with advanced cancer followed by palliative care providers.

Methods
Study Design: Sham-controlled crossover study. Each patient will be randomized to 5 days of accelerated rTMS in week 1 then 5 days of sham stimulation in week 2, or vice-versa.

Participants: Participants will be patients with advanced cancer with ~1-6 month life expectancy under PC management who are experiencing existential distress and do not have a contraindication to rTMS. Participants will be recruited from the 31-bed inpatient Palliative Care Unit at Bruyere Continuing Care.

Study Intervention. We will administer a low-frequency (1 Hz) rTMS stimulation to the right dorsolateral pre-frontal cortex (DLPFC) for 1 minute, eight times per day for five days, using an rTMS stimulation coil. For the sham intervention period, the rTMS stimulation coil will be replaced by a "sham" coil, which produces similar sound and scalp sensations as the active coils, but delivers no effective cortical stimulation.

Assessment of feasibility and acceptability
We will consider the intervention to be feasible and acceptable if we can enroll 25 participants over a 12-month study period, with a recruitment rate of at least 20%, a treatment completion rate of 80%, and a follow-up completion rate of 75%.

Assessment of efficacy. Patient assessment will take place at baseline, 1 day prior to starting the first

treatment period (sham or active), immediately following the first treatment period, immediately after the second treatment period, then at 2 weeks, 4 weeks, 8 weeks and 6 months after the completion of the second treatment arm (if the participant is alive and able to complete the assessments).

Primary measure of efficacy is depressive symptomatology measured by the Hamilton Rating Scale for Depression, measured immediately after the completion of each treatment session.

Secondary measures of efficacy include:
- Anxiety, measured using the Hamilton Rating Scale for Anxiety.
- Existential distress, evaluated via the Demoralization (DEM) scale.
- Death anxiety, evaluated via the Death Anxiety Scale (DAS).
- Quality of life. evaluated using the World Health Organization Quality of Life Scale, brief version (WHOQOL-Bref).

Data analysis. Analysis will adopt an intention to treat approach. Main analyses for this feasibility study will include calculation of feasibility outcomes using descriptive statistics with 95% confidence intervals. As our main aim is one of estimation rather than hypothesis testing, variance estimates and effect sizes with 95% confidence intervals will be calculated for primary and secondary efficacy measures in favour of significance testing. Assessment for a significant crossover effect will employ a statistical analysis comparing the reduction in symptoms from before to after treatment in each patient over the sham period versus the active period, following established guidelines for analysis and reporting of crossover trials. Anticipated impact. The burden of psychological and existential distress among people with cancer as they near the end of life is substantial, and our current treatments for this distress have shown poor efficacy and timeliness. Recent improvements in rTMS protocols and equipment have raised the possibility that rTMS could be highly effective, timely and scalable. If we can demonstrate feasibility, acceptability and efficacy for rTMS in this population, we would establish that rTMS has the potential to dramatically improve the experience of many patients with cancer.

Ghert, Michelle
McMaster University

The Surveillance AFter Extremity Tumour surgerY (SAFETY) international pilot randomized controlled trial

RATIONALE AND STUDY OVERVIEW
Following surgical treatment for a localized extremity soft-tissue sarcoma (STS), patients remain at risk for the development of local and systemic disease recurrence. Metastasis (systemic recurrence) to the lung is the most common location of disease recurrence in sarcoma patients, occurring in almost half of all patients. Therefore, careful post-operative surveillance is an integral element of patient care. However, the detection of metastases does not necessarily affect long-term survival and may negatively impact quality of life. Surveillance strategies have been identified as the top research priority in the extremity sarcoma field, and surveys of sarcoma surgeons have determined that surgeons typically follow their patients simply based on the way in which they were trained. We propose the Surveillance AFter Extremity Tumour SurgerY (SAFETY) international pilot trial: a 2X2 factorial pragmatic multi-centre randomized controlled trial (RCT), which aims to identify the most effective surveillance strategy in STS patients with respect to survival and quality of life. Patients from 5 international sites across 4 countries (Brazil, the Netherlands, Spain and the United States) will be randomized to more or less frequent clinic visits (every 3 months vs. every 6 months) and to chest x-rays (CXRs) vs. lung scans (CTs). However, prior to embarking on a large multi-centre RCT, it is necessary to confirm feasibility of international recruitment by conducting a pilot trial.

STUDY OBJECTIVES
The overarching objective of the SAFETY trial is to answer the following question:

Does the frequency and mode of surveillance affect patient survival following extremity soft-tissue sarcoma surgery?

International Pilot Feasibility Research Objectives:
For the purposes of the international pilot phase, the primary objective will be to demonstrate the feasibility of conducting a large international multi-centre RCT that will evaluate the impact of surveillance strategies on patient survival following extremity STS surgery. To do so, we will assess our ability to: A) *recruit patients* across multiple international participating clinical sites; B) *ensure compliance with the study protocol*, including the application of eligibility criteria, timing of intervention phase and post-intervention phase visits and imaging modality; C) maintain *completeness of follow-up and cost analysis* data; and D) maintain *data quality*. We hypothesize that the international SAFETY trial will be feasible due to: A) its pragmatic design; B) our successful international collaborative research network; C) our qualified, multi-disciplinary research team; D) our existing trial infrastructure; and E) the demonstrated importance of the research question.

Definitive Research Objectives:
To determine the effect of surveillance strategy on patient survival following extremity STS surgery, we will answer the following questions: A) In extremity STS patients who undergo surgical excision with curative intent, what is the impact of surveillance frequency (every 3vs. every 6 months) on overall survival at 5 years?; and B) In extremity STS patients who undergo surgical excision with curative intent, what is the impact of surveillance imaging modality (CXR vs. CT scan) on overall survival at 5 years? We hypothesize that: A) more frequent post-operative surveillance, and B) the use of post-operative CT scans in the first 2 years following the surgical excision of STS will improve overall survival at 5 years.

RESOURCES AND RELEVANCE
The international SAFETY trial has been designed and will be executed by an internationally-recognized multi-disciplinary team and with the guidance of STS patients. The trial will be conducted by the Centre for Evidence-Based Orthopaedics at McMaster University, a uniquely specialized group with proven success in running high-impact surgical trials. The trial will provide the necessary evidence to develop evidence-based surveillance guidelines, and is poised to have a significant impact on the post-operative care and outcomes of STS patients worldwide.

Razak, Fahad
St. Michael's Hospital

ONCO-GEMINI: ONcology Continuum GEneral Medicine INpatient Initiative; comparing cancer patients admitted to oncology vs non-oncology services

Background: With advances in cancer therapeutics and improved survival, frequency of hospitalizations has increased but there is limited data characterizing inpatient cancer care. While population-based studies using administrative data have helped characterize hospitalizations, additional clinical information such as use/results from laboratory and radiology investigations and medications are required to understand the delivery of care for hospitalized cancer patients. The GEneral Medicine INpatient Initiative (GEMINI) is a Ministry of Health and Long-Term Care (MOHLTC)-supported platform that electronically

extracts, standardizes and analyses data from clinical records, laboratory/imaging investigations, pharmacy, administrative data for general medicine patients admitted at 7 Toronto hospitals. GEMINI is a unique 'Big-Data' repository used to identify variation in processes and outcomes to support benchmarking, and drive quality improvement (QI) of patient care for general medicine. GEMINI is growing in the next 3 years to include the 30 largest hospitals in Ontario through MOHLTC support.

Objectives: Our overarching objective is to adapt GEMINI to include cancer patients (ONCO-GEMINI) admitted to oncology wards in GEMINI hospitals and combine this information with oncology-related admissions to general medicine already captured in GEMINI to evaluate quality of care and drive ongoing QI. ONCO-GEMINI will contain clinically granular data from multiple hospitals on all admitted cancer patients making it a unique data repository for QI and research.

Our specific aims are: 1) to characterize the continuum of inpatient oncology care; 2) to identify variations in inpatient cancer care processes, outcomes and cost across the continuum and compare these areas between cancer patients admitted to oncology versus general medicine. This proposal is a first step towards leveraging the significant financial investment of the MOHLTC in GEMINI infrastructure to allow detailed study of hospitalized cancer patients across Ontario.

Methods: We will use the existing infrastructure and hospital partnerships to extend GEMINI to evaluate patterns of care of cancer patients admitted in the current GEMINI network where medical, radiation and gynecological oncology are their admitting service and combine this with oncology-related admissions to general medicine already captured by GEMINI. Additional oncology specific fields including clinico-pathological, chemotherapy and radiation therapy details will be captured. In Aim 1, descriptive statistics will characterize patterns of care including prevalence of admitting/discharge diagnoses, frequency of admissions over time, investigations, discharge destinations, outcomes (i.e., length-of-stay, mortality, and 30-day readmission rates) and associated costs. For Specific Aim 2, patterns of care and outcomes between cancer patients admitted to oncology versus general medicine services will be compared. Secondary analyses will compare academic to community cancer centres, clinical trial to standard-of-care patients, and patients admitted at their home cancer centre vs another hospital.

Significance: To our knowledge, detailed study and comparison of hospitalization characteristics in the Canadian cancer patient population have not been conducted. This a crucial gap given the prevalence and burden of hospitalizations among cancer patients which is expected to rise. We anticipate this study will identify potential areas for QI in inpatient cancer care, which is the focus of future work. Furthermore, the infrastructure created will produce a platform for ongoing quality measurement and improvement as part of a learning health system and can help with extracting inpatient data for future research studies. Importantly, these data extraction methods are ideal to develop pragmatic registry-type randomized trials with outcomes and process measures derived from ONCO-GEMINI. As GEMINI expands to include all large Ontario hospitals, this platform will help better characterize province-wide cancer inpatient care and may serve as a proof of principle that can be adapted to other provincial cancer organizations.

Sylvestre, Marie-Pierre
Centre de recherche du CHUM

A prognostic tool for regular cannabis use among youth: A precursor to lowering cannabis-related cancer risk

Problem – Cannabis use typically begins in adolescence. 15% of youth become chronic or escalating

users, raising concerns about the risk of cancer in organs exposed long-term (lung, upper aero-digestive tract, bladder). Risks could be elevated in two primary ways. First, cannabis smoke contains carcinogens and mutagens, many of which are present in tobacco smoke, and have been shown to be carcinogenic in laboratory assays. Second, cannabis is frequently mixed with tobacco, and weekly or more cannabis use is associated with both cigarette smoking initiation and nicotine dependence. With recent cannabis legislation, public health strategies must anticipate and prepare for possible long-term sequelae of increasing cannabis use. A first step in preventing cannabis-related cancer is identifying youth at risk of long-term or escalating use. While numerous tools screen for identifying cannabis abuse or disorders, there is no prognostic tool to identify adolescents at risk of becoming regular users.

Objectives -To develop a prognostic tool for use by health professionals and others to identify adolescents at risk of becoming regular cannabis users. Specific objectives are to:
(i) construct a prognostic tool to assess the 1-year risk of regular cannabis use in adolescents;
(ii) conduct an internal validation to assess the discriminatory and calibration potential of the tool;
(iii) conduct an external validation to investigate the predictive ability of the tool in an independent sample;
(iv) evaluate the added value of incorporating individual perception of the risk of using cannabis on the predictive ability of the tool.

Methods - Machine learning, which is new to cancer prevention, will be used to construct the tool using data from COMPASS, a large prospective study including more than 50 000 high school students in Ontario (ON) and Québec (QC). Participants will be categorized as regular cannabis users (yes, no) if they used cannabis at least weekly.

Variables eligible as prognostic variables (i.e., measured prior to the measure of regular cannabis use) include sociodemographics, personality traits, mental health, school connectedness, bullying/victimization, academic achievement, lifestyle behaviors (tobacco use, physical activity, alcohol, other substance use, peer cigarette smoking, age at first cannabis use). The prognostic tool will be constructed in the ON sample using the bolasso algorithm, which combines the variable selection algorithm of lasso (**l**east **a**bsolute **s**hrinkage and **s**election **o**perator) with bootstrap aggregating (bagging), to improve the stability and accuracy of the prediction. We will benchmark and improve the prognostic model by comparing its predictive ability with that of deep learning feedforward (neural) networks. Internal validation will be implemented using bootstrapping and calibration curves and c-statistics will be used to evaluate the discriminating predictive ability of the tool. We will use bootstrapped copies of the dataset used to construct the prognostic tool. External validation will be conducted in the QC sample. The added-value of participants'' perceptions of the risk of using cannabis in the prognostic tool will be assessed using coefficient model updating strategies.

Significance to cancer – A validated prognostic tool will permit early intervention targeted specifically to youth at-risk of long-term or escalating cannabis use (which in turn relates to an increased risk of cancer). Identification of risk can promote discussion of harm reduction strategies for cannabis use and cannabis-tobacco co-use, or trigger referral to interventions that prevent escalation and encourage cessation. Our prognostic tool is the first step to the later design of a web-based intervention to provide personalized recommendations and resources based on responses to the prognostic tool.