November 2020 (ESA-21) Emerging Scholar Award Competition
Listed in alphabetical order

Ahmed, Marya
University of Prince Edward Island

Dual targeting strategy for breast cancer

Breast cancer is one of the leading causes of cancer related deaths in female population in Canada and across the globe. The estimated cases of breast cancer in Canadian women are 26,900 and 5,000 will die from this. This is expected that by 2032 the number of breast cancer patients in Canada will increase from 98.7/100,000 to 116.3/100,000 population. At present, antibodies and chemotherapeutics are commonly used methods to treat breast cancer patients, but strong side-effects of these drugs are a common occurrence. Given the increasing number of breast cancer patients with time and absence of effective and safe treatment to reduce the mortality rate, there is an urgent need to develop safe therapeutics for breast cancer treatment.

The growth of highly aggressive form of breast cancer is supported by the presence of two types of growth promoting proteins. At present, breast cancer patient treatment involves blocking these growth promoting proteins by small molecules (chemotherapeutics, antibodies), however, changes in these growth promoting proteins by mutation or overexpression, leads to failure of these inhibitor therapeutics. One method to provide effective treatment for breast cancer patients is to selectively remove these two growth promoting proteins from cancer cells, using two small nucleic acids (termed as siRNA), specific for each type of protein. siRNAs are small nucleic acids which can delete the expression of growth promoting proteins in cancer cells and can prevent the cancer growth and spread to other organs. siRNA carrying therapeutics are expected to selectively kill breast cancer cells, show reduced side-effects and prevent the spread or relapse of the disease. However, siRNA therapeutics can be easily degraded by proteins in the body and must be protected and delivered inside the cancer cells. This project aims to develop new, degradable and non-toxic materials that can attach to siRNA, prevent their degradation, allow their selective delivery in breast cancer cells and prevent tumor growth, recurrence and metastasis.

The specific aims of this project are to 1) develop non-toxic, degradable peptides for attachment and delivery of two siRNA therapeutics in breast cancer patients 2) study their tumour regression capability in breast cancer cells and in breast tumour models and 3) selective targeting of tumour cell, hence minimizing drug dosage and improving the efficacies.

The development of siRNA loaded peptides will be the first of their kind to deliver precise amount of two siRNA in breast cancer patients. In comparison to existing treatments for breast cancer, that often cause strong side-effects and cannot prevent the metastasis of cancer cells over time, these new therapeutics are expected to improve the toxic side effects of traditional drugs and will improve the patient compliance and efficacy.

Burnier, Julia
The Research Institute of the McGill University Health Centre (RI-MUHC)

Defining the mechanisms of DNA release from cancer cells and their impact on the clinical utility of liquid biopsy

Summary: Tumours are not static; instead, they evolve and adapt, acquiring new genetic and epigenetic characteristics as a result of both the natural evolution of the disease and selective pressures from the immune system, therapy and environment. However, we currently rely on initial tissue biopsy for diagnosis, prognosis, and therapeutic decision-making. This approach provides one static picture, thereby neglecting the dynamics of tumour evolution and spatiotemporal heterogeneity. Differing from traditional biopsy methods, the emerging practice of liquid biopsy provides a minimally invasive way of extracting tumour cells and tumour-derived molecules, such as circulating tumour DNA (ctDNA) from biological fluids (e.g., blood, urine, saliva). As cell-free (cfDNA) and extracellular vesicle bound (EV-DNA) nucleic acids are reflective of systemic disease, such biomarkers represent a powerful and dynamic indicator of disease. However, the clinical utility of such markers remains unclear, with many studies showing conflicting and inconsistent results. We believe that understanding the etiology of ctDNA (cfDNA/EV-DNA), and investigating how they are influenced by treatment could help us to strategically exploit ctDNA for minimally invasive monitoring of cancer patients in a personalized way.

Objectives: To address this unmet need, ***the goal of this project is to investigate DNA emission from cancer cells and to assess the effect of cancer treatment on the kinetics and fragmentation of ctDNA and EV-DNA in cancer patients***. We hypothesize that the release of ctDNA and EV-DNA is emitted as a result of different forms of cell death, and provides real-time information of cellular context*. Moreover, fragment length may reflect cell emission process and could be used alongside quantification as a biomarker. Specifically we aim to) elucidate the mediators leading to DNA release from tumour cells and 2) evaluate the impact of treatment on ctDNA and EV-DNA kinetics and fragmentation in patient blood**.

Methodology: In aim 1, we will assess the etiology of ctDNA in order to understand how cellular context influences DNA emission from tumour cells. Specifically, we propose to uncover the effect of apoptosis, necrosis, and senescence on levels cfDNA and EV-DNA *in vitro* in primary and metastatic human cancer cell lines. To do so we will use a combination of chemotherapy, radiation and cellular stress conditions that induce specific cell death mechanisms, and then quantify ctDNA and EV-DNA emission. Moreover, we will assess fragment length distribution in cfDNA and EV-DNA in cells and determine its relationship with DNA emission. In aim 2, we will evaluate the impact of treatment on ctDNA and EV-DNA kinetics and fragmentation in a clinical study of esophageal and colorectal cancer patients. By monitoring patients through treatment, we will acquire real-time data on changes in cfDNA levels. We will then correlate ctDNA and EV-DNA levels to treatment response. Moreover, using next-generation sequencing, emergence of genomic alterations will be evaluated, with the aim of identifying potential mechanisms of resistance that could be detected by liquid biopsy analysis. Finally, DNA fragmentation will be analysed in clinical samples.

Significance: For liquid biopsy approaches to become clinically valuable, better understanding of the kinetics and etiology of disseminated molecules is critically needed. This project has the potential to highlight many basic concepts of tumour biology, translating into better interpretation of clinical liquid biopsy data. By tracking molecular alterations in the blood and the evolving dynamics of cancer, we might identify circulating molecules that could be used for non-invasive diagnosis, prognosis, and detection of treatment resistance and early metastasis. Such an approach has tremendous impact on cancer discovery as well as on the quality of life of patients on treatment.

del Rincon, Sonia
Jewish General Hospital

Examining the MNK1/2-eIF4E axis in cancer associated fibroblasts as a driver of oncogenic progression

Clinical challenge: Breast cancer is the most frequently diagnosed cancer in women, and despite advances in the diagnosis, treatment, and understanding of breast cancer biology, it remains a deadly disease. Metastasis is the principal cause of breast cancer fatality, so the need for drugs targeting metastatic cancer is critical. Our lab's approach to this clinical challenge is to use inhibitors of MNK1/2 to block invasive and metastatic disease. It is an exciting time to be studying MNK1/2, as these kinases have matured into drug targets that are being used to treat patients with cancer. As part of a Stand Up to Cancer (SU2C) team grant, we are investigating the capacity of MNK1/2 inhibitors to prolong the survival of patients with metastatic breast cancer (NCT04261218). **Rationale:** Aberrant activation of signaling cascades regulating mRNA translation is known to promote breast cancer metastasis. This is best exemplified by the translation initiation factor eIF4E, which acts downstream of key kinases, is overexpressed in breast cancer, and associated with poor prognosis. The oncogenicity of eIF4E depends on its phosphorylation on serine 209 (**S209**) by its only kinases, MNK1/2. We and others have shown that phospho-eIF4E (p-eIF4E) increases the translation of a subset of mRNAs to promote invasion and metastasis. Our approach to inhibit metastasis is to block MNK1/2 with clinically relevant inhibitors. By understanding the role of MNK1/2 in cancer progression, we expect to enhance the clinical utility of these inhibitors. Our team has elucidated mechanisms by which blocking MNK1/2 in tumor cells inhibits their invasion and metastasis. However, new data link MNK1/2-eIF4E axis activation with the function of cells comprising the tumor microenvironment (TME). In support of this concept, our data show that p-eIF4E deficient mice (termed **S209A**) have a TME that *does not* favor metastasis of p-eIF4E competent tumors. A major constituent of the TME are cancer-associated fibroblasts (CAFs), activated fibroblasts which promote tumor progression. There is a gap in knowledge concerning the role of mRNA translation in CAFs. We propose that the lack of p-eIF4E in CAFs plays a contributing role in the protection of **S209A** mice to metastasis. For example, we find that the pro-tumor functions of fibroblasts are regulated by the MNK1/2-eIF4E axis. Breast cancer cells invade poorly when co-cultured with either **S209A** fibroblasts or MNK1/2 inhibitor-treated patient-derived CAFs. *We hypothesize that the MNK1/2-eIF4E axis promotes metastasis beyond its effects on tumor cells, by influencing CAFs to secrete pro-inflammatory cytokines, thus creating a pro-invasive microenvironment.* **Specific Aims:** In this grant, we propose to study how MNK1/2 and p-eIF4E control the activation and function of CAFs as a means to promote metastasis. **Aim 1- Mouse models: 1.1** We will perform in vivo metastasis assays using **S209A** or MNK1/2 double knockout (**DKO**) CAFs comingled with breast tumor cells. **1.2** We will generate a novel mammary fibroblast-specific **S209A** mouse. **Aim 2- Ex vivo work: 2.1** We will define, using MesoScale, whether **S209A** or **DKO** CAFs have a secretome that favours anti-tumor immunity and does not support invasion. Tumor-CAF-immune cell interplay will be modelled using microfluidics. **2.2** We will perform polysome profiling to define CAF specific proteins whose expression are controlled by p-eIF4E-dependent mRNA translation. **Aim 3- Patient samples: 3.1** Patient-derived CAFs treated or not with MNK1/2 inhibitors will be compared using quantitative proteomics. **3.2** Imaging Mass Cytometry will be used to define the immune landscape and fibroblast content of patient-derived breast tumor explants (PDEx) and human breast biopsies, pre- and post-treatment with MNK1/2 inhibitor. **Clinical impact:** If we determine that the MNK1/2-eIF4E axis controls metastasis, at least in part, via its action in CAFs, this will lead to additional biomarkers of response, or resistance, to MNK1/2 inhibition, and reveal novel targets for augmenting the anti-metastatic effects of MNK1/2 inhibitors. We anticipate that execution of the proposed work will directly impact our team's SU2C trial.

Garland, Sheila
Memorial University

Development and evaluation of the iCANSleep app for insomnia in cancer survivors

Problem to be Investigated: Insomnia is one of the most prevalent and enduring cancer-related symptoms, which can exacerbate other side effects and worsen quality of life. The recommended standard of care for insomnia is cognitive behavioral therapy (CBT-I), but access is limited by the lack of trained therapists. Access to qualified face-to-face providers in Canada is further complicated by the fact that a significant proportion of Canadians live outside of metropolitan areas where providers reside. This means that cancer survivors in Canada, many of whom who live in rural or remote areas, do not have adequate access to evidence-based treatment for insomnia. Digital delivery of CBT-I has considerable promise for the treatment of insomnia in the general population, but these programs have not been developed and tested among cancer survivors, who have higher comorbidity and symptom severity.

Objectives of the Proposed Investigation: We propose a 5-year multi-method and sequential study to develop and test a smartphone app called "iCANSleep" for cancer survivors with insomnia.
Specific Aim 1: To co-design user-centered content and program the iCANSleep app (0 to 6 months)
Specific Aim 2: To assess the usability of the iCANSleep smartphone app (6 to 12 months)
Specific Aim 3: To test the feasibility, acceptability, and preliminary efficacy of iCANSleep (12 to 24 months)
Specific Aim 4: To determine the efficacy of iCANSleep compared to a usual care control group (24 to 60 months).
Hypothesis: Participants receiving iCANSleep will exhibit significant and durable improvements on the insomnia severity index (primary outcome), sleep quality, mood, fatigue, and quality of life over a self monitoring control group.

Methods: The content of iCANSleep will be adapted from an established CBT-I treatment protocol developed by the PI. We will adhere to a phased sequential approach to the development of complex technology-based interventions. A user centred design will be applied where cancer survivors will be actively engaged in all aspects of the research process, including the app's design, the usability and feasibility testing and the refinement of the prototype. Particular attention will be paid to addressing barriers for use by individuals who are: older aged, lower education, and male.
Phase 1 will conduct virtual focus groups and phone interviews following a semi-structured interview guide with a purposive sample of cancer survivors (n=30) to inform the design of the app and its content.
Phase 2 will use iterative high-fidelity usability testing with 5-7 participants per cycle until no further recommendations for change are suggested.
Phase 3 will test the feasibility, acceptability and preliminary efficacy of the iCANSleep app in a sample of 50 cancer survivors. Participants will complete the Insomnia Severity Index (ISI) and a packet of patient-reported outcomes (PRO) at baseline and 6 weeks.
Phase 4 will test the efficacy of the fully developed iCANSleep intervention using a randomized controlled trial with a heterogenous sample of 146 cancer patients (73 female: 73 male). All participants will complete the Insomnia Severity Index (ISI) and a packet of patient-reported outcomes (PRO) at baseline, 6 weeks and 12 weeks. The follow-up assessment will provide information regarding long-term durability of the effect of iCANSleep.

Significance of the Research to Cancer: This research provides opportunities to build the scientific foundation for improving sleep and quality of life in cancer survivors, extend the reach of insomnia therapy to rural areas, and enhance the translation and dissemination of research findings. It will also allow for the development of current and future leaders in the area of cancer survivorship.

Gujar, Shashi
Dalhousie University

Kynurenine metabolic pathway: implications for oncolytic virus-based cancer immunotherapy

Preamble: Cancer immunotherapies are the most promising anticancer options of the modern era. These therapies exploit the functions of the immune cells (e.g., T cells, macrophages) or mediators (e.g., antibodies, cytokines), and target a multitude of cancers. Immunotherapies can eliminate local as well as metastatic cancers, and are now being approved to be used in clinics. Since the <u>presence of **antitumor T cell responses** almost always correlates with **positive patient outcomes from cancer,**</u> many of these immunotherapies focus on inducing antitumor T cell immunity. Such antitumor T cell responses can destroy existing cancer cells, and further establish continuous protection against possible relapse.

Problem to be investigated: The desired clinical benefits of antitumor T cells are negatively affected by suppressive mechanisms that reside within tumor microenvironment (TME). It is now clear that the TME harbors immune evasion strategies that negatively influence antitumor T cells. In particular, the <u>metabolic milieu within the TME has emerged as a major regulator of antitumor T cell immunity</u>. Latest evidence shows that the TME-associated metabolic perturbations actively resist the development of antitumor immunity, and additionally sustain the process of cancer growth. Thus, the metabolic repercussions accompanying the TME-immune system interaction hold the key to promoting antitumor T cell responses, and will be investigated in the proposed research.

Objectives of the proposed investigation: To achieve these goals, my cancer immunotherapy research program draws upon the advances in genomics, proteomics, and metabolomics. In particular, my laboratory specializes in using cancer killing viruses, and implements oncolytic reovirus as an anticancer immunotherapeutic. Our discoveries thus far have highlighted that reovirus, primarily known for its cancer-killing abilities, can be exploited to overcome the TME-associated immunological barriers, and used to promote antitumor T cell immunity.

In this context, most recently we have made two important discoveries: 1) the targeted metabolic reprogramming of TME enhances sensitivity of otherwise resistant cancers to the 'cancer killing' activity of oncolytic reovirus (*Cancer Research, 2019; Molecular Therapy 2020*); 2) macrophages, the immune cells intimately involved in inducing antitumor T cell response, upregulate the kynurenine metabolic pathway following exposure to reovirus (*Journal of Proteome Research, 2020*).

In particular, our latest discoveries have identified clinical implications for kynurenine metabolic pathway in non-small cell lung cancer (NSCLC) undergoing oncolytic virotherapy. Thus, in line with our latest published reports and convincing preliminary data, <u>the over-arching objective of the proposed research</u> is to dissect the bio-therapeutic implications for the kynurenine metabolism in regulating antitumor T cell immunity during oncolytic reovirus-based therapy of NSCLC.

Methodology: We have developed mass-spectrometry-based platforms that implement the latest concepts in the fields of immuno-metabolomics in my laboratory. In particular, an in-house-developed novel metabolomics platform to map kynurenine pathway in various biological specimens, along with the unique systems, such as knock out (KO) mice (e.g., kynureninase [KYNU] KO, aryl-hydrocarbon receptor [AhR] KO), are established. Finally, widely accepted gold-standard immunological assays, NSCLC mouse models, and highly qualified personnel required to undertake this research are already in place.

Significance: Our research will reveal <u>new scientific paradigms around the metabolic regulation of antitumor T cell immunity</u>. Most importantly, this <u>clinically translatable cancer research of 'bench-to-bedside' potential</u> will inform the development of highly efficacious cancer immunotherapies. We truly believe that this research will ultimately promote long-term cancer-free health for Canadians.

Jones, Courtney
Princess Margaret Cancer Centre - UHN

Amino acid metabolism in relapsed acute myeloid leukemia stem cells

The goal of this project is to eradicate leukemia stem cells (LSCs) in relapsed acute myeloid leukemia (AML) patients. Most AML patients who receive chemotherapy achieve a significant clinical response. However, the majority of AML patients will relapse and succumb to this disease, at least in part due to our inability to eradicate the disease initiating LSC population1. Therefore, there is an urgent need to develop therapeutic strategies to target LSCs. Several preclinical studies have shown that LSCs have unique metabolic requirements that can be inhibited to target LSCs2-12. We have previously demonstrated that LSCs isolated from AML patients who have relapsed post chemotherapy treatment (relapsed LSCs) are differentially sensitive to metabolic targeting therapies compared to LSCs isolated from newly diagnosed AML patients (diagnosis LSCs)3,4,7.

Based on this observation, we hypothesized that relapsed LSCs have distinct metabolic properties compared to diagnosis LSCs and that these metabolic properties can be targeted to eradicate relapsed LSCs. To test this hypothesis, we isolated LSCs from diagnosis and relapsed AML patients, and performed detailed mass spectrometry studies to identify unique metabolic characteristics of relapsed LSCs. Our data shows that relapsed LSCs have increased amino acid levels compared to diagnosis LSCs. Further, our preliminary data suggests that inhibiting arginine metabolism targets relapsed LSCs. Specifically, arginine appears to be essential to support the urea cycle and polyamine synthesis in relapsed LSCs. Based on these data, <u>we hypothesize that elevated levels of amino acids are required for relapsed LSC function by mediating the urea cycle and polyamine synthesis.</u> We will pursue this hypothesis through the following specific aims.

Aim 1: Interrogate the interplay between amino acid metabolism and chemotherapy in relapsed LSCs. In this aim, we will determine if amino acids influence chemotherapy sensitivity and how chemotherapy treatment results in elevated amino acids levels in relapsed LSCs. To measure the influence of amino acids on chemotherapy sensitivity, we will modulate amino acid levels in culture media and measure LSC viability. To measure amino acids, we will use mass spectrometry-based metabolomics analysis upon chemotherapy treatment. Upon successful completion of these studies we will understand the interplay between amino acid levels and chemotherapy sensitivity.

Aim 2: Determine the mechanism by which relapsed LSCs are dependent on arginine metabolism. In this aim, we will determine the metabolic pathways supported by arginine in relapsed LSCs by performing stable isotope labeled arginine flux analysis and complementary metabolic analysis in primary human relapsed LSCs. Upon successful completion of these studies we will understand how arginine is metabolized in LSCs and identify novel therapeutic targets for relapsed AML.

Aim 3: Determine the consequences of perturbing amino acid metabolism on relapsed LSC function.
In this aim, we will measure the consequences of perturbing arginine metabolism in primary relapsed AML specimens and quantifying the engraftment potential of into immune deficient mice compared to control specimens. These studies will determine if targeting arginine metabolism can reduce LSC function and serve as the basis for further preclinical and preclinical studies.

<u>**Significance:**</u> AML is a devastating and fatal disease in many adult patients. The majority of patients initially respond to conventional therapy; however, most develop disease recurrence and succumb to this disease1. The research objective of this proposal is to eradicate recurrent disease. We propose to accomplish this objective by targeting metabolic vulnerabilities of relapsed LSCs. Our preliminary data suggests that relapsed LSCs have increased levels of amino acid metabolism which we propose is targetable to eradicate relapsed LSCs. Targeting amino acid metabolism, specifically arginine metabolism, may provide novel therapeutic strategies for relapsed AML patients, a patient population that currently has poor outcomes and limited options.

Khademi, April
Ryerson University

Augmenting breast pathology workflows for improved cancer care

One in nine women in Canada are diagnosed with breast cancer and 1 in 29 will eventually die from the disease. It is the second cause of cancer death in Canada where roughly 62 women/day are diagnosed. There are currently two challenges in breast cancer pathological analysis that negatively affect patient care. Firstly, visual examination of tissue slides is subjective with mid-to-high discordance rates. This creates variability in treatments given to patients, and reduces quality of care. A secondary challenge is the pathologist workforce is shrinking rapidly, while cancer cases are on the rise, creating significantly higher workloads for current pathologists. The result is delays in diagnosis and increased time to treatment putting patients at risk. The increased workload also creates pathologist fatigue.

This research program is focused on **overcoming interpretation variability and workload pressures** using innovative **computational pathology tools** for improved quality of care. I am interested in developing a breast panel, with several "apps" that augment pathologists' daily tasks These tasks are chosen because of the inter-rater variability in obtaining measurements, the labourious nature of the tasks, and the potential to improve patient care. Automated tools can reduce diagnostic subjectivity, introduce workflow efficiencies and diagnostic turn-around-time, as well as improve pathologist satisfaction.

There are currently interpretation variability & workload pressures in breast cancer pathological analysis that can negatively affect patient care. The proposed computational pathology tools can reduce diagnostic subjectivity, introduce workflow efficiencies and diagnostic turn-around-time, as well as improve pathologist satisfaction. Through imaging-biomarkers, the reduction in turn-around-times and increase in diagnostic accuracy will improve patient outcomes by helping to choose and deliver the right treatment at the right time

Klein Geltink, Ramon
University of British Columbia

Metabolic conditioning of T cells for immunotherapy of cancer

SUMMARY Metabolic pathway activity is tightly associated with T-cell function and differentiation into various T-cell subsets (reviewed in [1]). Activation of cytotoxic CD8+ T cells, the effector T cell most relevant for the therapeutic eradication of tumors, induces metabolic remodeling. CD8+ effector T (TE) cells are dependent on glucose metabolism not only for proliferation, but also for sustained immune-function [2-6]. When glucose is limiting in the cellular environment, TE cells rewire central carbon metabolism to survive. In the tumor micro environment nutrients are limited, and glucose is competed for by tumor cells [4], therefore TE cells need to be metabolically flexible to sustain anti-tumor function (reviewed in [10]). We have shown that one adaptation is the increase of mitochondrial metabolism, as indicated by elongation of mitochondria associated with increased mitochondrial activity and efficiency [7], which was shown to be a metabolic benefit for T cells in tumors [8-10].
Recently we showed that glucose restricted CD8+ TE cells enhance mitochondrial metabolism, and sustained expression of activated T cell surface markers. This suggested to us that these cells could be metabolically resistant to the tumor micro environment. Strikingly, *in vivo* persistence and anti-tumor function after *in vitro* glucose restriction of TE was significantly enhanced [11]. This suggests that the enhanced mitochondrial metabolism is also accompanied by changes that equip T cells to better compete for nutrients in tumors.

The AIMS of this proposal are to investigate:

    1.*The immediate effects of inhibition of pro-growth and metabolic mediators (PI3K-AKT-mTOR) on glucose metabolism in TE cells compared to glycolytic T-ALL cells.*

    2.*The effects of acute pharmacological or genetic inhibition of nutrient transporters in TE cells on the regulation of glucose metabolism versus that in T-ALL cells.*

    3.*The effects of genetic manipulation of metabolism on anti-tumor immunity via adoptive cellular therapy of cancer.*

The methodology we will use to achieve these objectives utilize established *in vitro* and *in vivo* models of tumor immunology. We can generate and genetically manipulate large numbers of primary TE *in vitro*. By using a TCR transgenic mouse strain we can target CD8+ T cells to tumor cells expressing chicken ovalbumin. T cell-tumor co-cultures will be used for tumor killing in 3D-tumor spheroid cultures (melanoma and colon carcinoma). The 3D-spheroid system allows us to model a nutrient depleted tumor like environment, in which T cells display limited T-cell function. The tumor cell line models we propose to use, (B16 melanoma; MC-26 and MC-38 colon carcinoma) cannot be controlled by the endogenous immune system in C57BL/6j mice, so the *in vivo* persistence and anti-tumor activity of *in vitro* manipulated CD8+ TE cells can readily assessed. The core facilities at BCCHR and UBC provide state-of-the-art flowcytometry and metabolomics. We will measure changes in immuno-phenotype, changes in metabolic engagement by seahorse extracellular flux analysis, metabolomics, and stable isotope tracing, and changes in tumor control *in vivo*.

IMPACT ON CANCER Immunotherapy strategies are being studied for many tumour types, but we have a poor understanding of regulation of TE cell function in tumours. Here, our aim is to discover new ways to take advantage of the inherent metabolic flexibility of TE cells. *In vitro* glucose restriction can increase *in vivo* persistence and function of TE cells in mouse models of cancer. This implies that fewer tumour-specific T cells would be needed to achieve clinically-relevant effects. Clearly all patients do not have the same tumour phenotype or immune infiltrate. However, we expect our approach will reveal ways to increase metabolism and give the T cells a fighting

chance against many forms of cancer.

Kridel, Robert
Princess Margaret Cancer Centre - UHN

Rational design of combination therapies for B-cell lymphoma

Background: Forty percent of patients with aggressive B-cell lymphomas fail currently available treatment approaches. These patients have poor outcomes, even when they are candidates for intensive chemotherapy with stem cell support or novel cellular therapies. Given that relapsed lymphoma is frequently resistant to chemotherapy, targeted therapies hold the promise of improving patient outcomes by precisely blocking deregulated oncogenic pathways. Over the last decade, advances in next-generation sequencing technology have led to granular cataloguing of genetic alterations that are recurrent in B-cell lymphomas. A major theme that has emerged is common disruption of genes encoding histone modifiers, illustrating that epigenetic reprogramming plays a pivotal role in lymphoma pathogenesis. Hence, epigenetic modifiers are currently studied in clinical trials, with particular emphasis on inhibition of EZH2 and HDAC3, aiming to counteract key hallmarks of lymphomagenesis. However, as typical for targeted therapies, not all patients respond. The question thus arises how the efficacy of epigenetic therapies can be improved.
Objectives: The major goal of this proposal is the identification of rational combination therapies that allow to overcome treatment resistance in B-cell lymphoma. In Aim 1, we propose to rationally identify drugs that synergize with HDAC3 inhibition and will perform unbiased, HDAC3 inhibitor-sensitizing library screens. In Aim 2, we propose mechanistic studies to elucidate the interaction between a promising gene candidate and response to HDAC3 inhibition. Lastly, in Aim 3, we propose to deploy a novel method for combinatorial screens that we have adopted specifically for the lymphoma context.
Methods: In Aim 1, we will perform genome-scale knockout screens using the CRISPR-Cas9 technology in order to uncover potential targets for co-inhibition with HDAC3. We will rigorously validate individual targets, both *in vitro* and *in vivo*, across a large number of cell lines and *in vivo* models to test the generalizability of our findings. In Aim 2, we will follow up on a first target that was identified in our preliminary work. We will generate isogenic knockout cell lines and test their sensitivity to HDAC3 inhibition. In parallel, we will conduct mechanistic studies of the pathway involved and generate RNAseq and ChIPseq to identify gene expression programs that are modulate by combined inhibition of HDAC3 and gene knockout. In Aim 3, we will perform combinatorial screens that allow us to assess the interaction between random pairs of gene, among a panel of 104 genes that are relevant to B-cell lymphomagenesis and/or can be targeted with small molecule inhibitors. To this effect, we have constructed a novel gRNA library of over 51K unique constructs that we propose to use for screens in at least 3 cell line models. This latter aim will allow to define novel, unexpected gene interactions and highlight combination strategies that can potentially be synergistic or, on the contrary, antagonistic.
Significance: Our proposal aims to identify optimal combination therapies that have high response rates in this pre-clinical study. Our preliminary results illustrate the power of library screens to discover unexpected synergy and gain novel insight into underlying mechanisms. We will harness a team of collaborators with orthogonal expertise in epigenetics, genomics and animal studies, thereby guaranteeing feasibility. Our findings will have clinical implications as our ultimate goal is the translation of the most promising combination therapies into clinical trials.

Mahar, Alyson
University of Manitoba

Measuring Equity and Generating ActioN in CANcer: using research to promote equitable care delivery across Canada (MEGAN-CAN)

Rationale Unjust differences in cancer risk reduction, diagnosis, treatment, survivorship and the provision of end-of-life care persist among Canadians despite a universal healthcare system. The reduction of cancer healthcare disparities is a national and international priority, supported by the World Health Organization, the Canadian Partnership Against Cancer, and CancerCare Manitoba. Organizations dedicated to improving cancer outcomes for all Canadians are challenged by a lack of high quality, Canadian studies to inform the development and implementation of equity-based programming and services. Understanding cancer care inequity is critical to reduce the negative consequences of preventable morbidity and mortality in vulnerable populations.

Aims By taking a social justice lens to improve cancer outcomes for Canadians who may be at risk of experiencing health inequities, I will: A1: Work with patients, survivors, and family members to identify priorities for equity-focused research in cancer care; A2: Investigate associations and intersections among prioritized equity stratifiers and key milestones along the cancer continuum; A3: Engage patients, family members, healthcare providers and policy-makers to understand personal and system-level barriers to receiving and providing equitable cancer care; A4: Create a Canadian resource that can be used to address equity-related gaps in cancer care along the continuum by developing new ways to analyze, interpret and use cancer equity research.

Methods This is a mixed-methods, inter-provincial study that will occur over four aims using a patient-centered approach. The proposed study will focus on breast, colorectal, lung and hematological cancers. A1: We will conduct a scoping review and a Delphi panel, facilitated by a professional facilitation team, to understand patient and caregiver priorities for equity-focused research in care for Canadians. A2: We will complete a feasibility exercise to understand of those equity-focused priorities identified by patients, which are measurable or actionable in research using administrative data. We will then conduct simultaneous cohort studies in Manitoba and Ontario using routinely-collected provincial administrative databases held at the Manitoba Centre for Health Policy and ICES. Cancer care inequalities in screening, the diagnostic interval, TNM stage at diagnosis, receipt of appropriate treatment within clinically homogenous scenarios, patient reported outcomes, survival, survivorship, and end-of-life care will be investigated using multivariable statistical models. We will examine effect modification and interaction among equity stratifiers. We will apply, modify and develop new approaches to modeling these data, in particular with an emphasis on addressing small sample sizes, intersectionality, and understanding time-based trajectories of inequality. A3: Focus groups and a survey with patients, caregivers, oncology healthcare teams and policy makers will be conducted to better understand personal and health system-level barriers to providing equitable care to all patients. A4: We will create infrastructure, under the principles of Open Science, for Canadian researchers to address equity-related gaps in cancer care. This will include an environmental scan of existing resources, a website aggregating resources and highlighting methods and approaches to patient engagement and statistical analyses, as well development of a workshop for researchers and clinicians, and knowledge products for patients and families. This will create capacity for cancer researchers and their communities to more easily address issues of equity.

Significance This study will provide high quality, patient-driven research on cancer care disparities. We will consult with patients, healthcare professionals and policy-makers to better understand barriers to receiving and providing equitable care and identify interventions and changes to target these barriers in meaningful, concrete ways. By creating a national equity platform for Canadian cancer researchers, we will able to share resources and impact the equitable delivery of cancer care across provinces.

Ramaswamy, Vijay
The Hospital for Sick Children

Personalizing therapy in high-risk childhood ependymoma

Ependymoma, the third most common malignant brain tumor of childhood, can arise anywhere along the neuroaxis, with the cerebellum and cerebral cortex being the most common location in young children. Due to its inherent chemotherapy resistance, current treatments in children over the age of 1 are limited to surgery and radiation3,7. This large field of radiation to the developing cerebral cortex, combined with aggressive surgical resections results in devastating long-term neurocognitive sequelae, and an inability to live an independent life. Recently it has been shown that the majority of supratentorial ependymoma harbor highly recurrent activating fusions of the NFkB co-activator RELA with C11orf95, and in a small minority fusions of YAP1 to MAMLD15. These fusions are highly prognostic, with RELA fusions harboring a very poor outcome. As such, new and novel approaches are urgently required for children with ependymoma, particularly high-risk RELA-fused supratentorial ependymoma to improve both survival and quality of life. My group recently profiled a large international cohort of childhood supratentorial ependymoma and we observed that although the majority of samples harbored c11orf95-RELA fusions, a substantial minority harbor alternate fusions of chromosome 11 with other 3' binding partners known to be involved in other neoplasms. The long-term goal of my research program is to characterize rare fusion driven cancers of childhood, validate them in appropriate model systems and identify fusion specific therapies. *As such, I hypothesize that fusions in supratentorial ependymoma are driver events, which can lead to oncogenesis and be rational targets for therapy.* We will test this hypothesis through the following three aims:

Specific Aim 1: Mapping the molecular landscape of RELA and non-RELA fused ependymoma
Detailed molecular profiling will be conducted across an expanded cohort of supratentorial ependymoma, and actionable pathways identified. Whole genome sequencing, RNA-sequencing and DNA methylation profiling will be employed across both RELA and non-RELA fused models to provide a detailed curation of the somatic landscape of supratentorial ependymoma.

Specific Aim 2: Functional modeling of non-RELA chromosome 11 fusions
We will generate isogenic human and murine neural stem cell models of both RELA and non-RELA fused supratentorial ependymoma. This will allow us to validate the oncogenic potential of each of these fusions, while simultaneously generating models that can be used to identify genetic and therapeutic vulnerabilities, ultimately with the goal developing new treatments. This aim will not only allow us to model the entire spectrum of heterogeneity across supratentorial ependymoma, but also provide my group with the workflow to validate and model rare fusions identified through personalized brain tumor sequencing programs.

Specific Aim 3: Chemical and epigenetic screening of supratentorial ependymoma models
In order to identify therapeutic vulnerabilities across supratentorial ependymoma, my group has been performing high throuput kinome and epigenetic screening across patient derived supratentorial ependymoma models. We found that RELA fused, but not non-RELA fused ependymoma are highly sensitive to FGFR inhibition. We will validate FGFR as a bonafide target in vivo, and extend our screeing to models generated in Aim 2, to identify specific therapies benefiting all supratentorial ependymoma.This has the potential for immediate impact for children with supratentorial ependymoma, with respect to both classification and novel therapeutic targets. My proposed project takes a multiprong approach to develop a robust workflow to efficiently validate fusion driven brain tumors in human and murine model systems, which will provide the isogenic models needed to identify novel therapeutic targets for these children who currently have limited treatment options. This has the potential to be applied to other rare fusion driven childhood brain tumours allowing personalization of care for even the most rare conditions.

Sharif, Tanveer
University of Manitoba

Effect of standard chemoradiotherapy interventions on metabolism and stemness in glioblastoma

PROBLEM TO BE INVESTIGATED: Glioblastoma (GBM) is the most lethal type of primary brain cancer in adults, with an abysmal long-term survival rate of less than 5%. There are minimal treatment options available to GBM patients, and the current standard of care (SOC) consists of extensive surgery followed by aggressive rounds of radiation and/or chemotherapy. Even after receiving treatment, the majority of GBM patients will present with highly aggressive and invasive recurrent tumors that become nearly impossible to treat. Hence, there is a critical need to develop new treatment strategies that can prevent tumor relapse and improve the outcomes for GBM patients.

Targeting the increased metabolic demands of GBM tumor cells seems like a logical therapeutic approach to hamper tumor growth. Despite this, clinical trials utilizing glycolytic inhibitors have not proven successful for treating GBM. Within the heterogeneous tumor mass, there exists different subpopulations of cells, including poorly-differentiated stem-like GBM cells and non-stem-like GBM cells, which may possess distinct metabolic dependencies. However, cellular plasticity allows these populations to transition in response to environmental cues, therefore, it is important to investigate the changes in these populations over time. We hypothesize that metabolic adaptations may regulate cellular transitions that mediate chemoradiotherapy resistance of GBM cells and that targeting distinct metabolic phenotypes at specific stages of disease progression may prevent tumor recurrence and disease relapse. We will attempt to address this through the following **objectives**:

    1.Understanding the metabolic and molecular transitions that occur during GBM disease progression by using a temporal integrated multi-omics analysis.

    2.Understanding the role of metabolic and cellular plasticity in chemoradiotherapy response of heterogeneous patient-derived primary GBM cells and matched recurrent tumors.

    3.Investigating the efficacy of candidate timed metabolism-targeting combinatorial treatment regimens against primary and recurrent patient-derived xenograft tumors.

METHODOLOGY: This study utilizes heterogeneous GBM cells derived from primary and recurrent patient tumors to investigate the role of metabolic and cellular plasticity in mediating chemoradiotherapy response and tumor recurrence. Using a therapeutically relevant SOC chemoradiotherapy treatment regimen, we will probe the metabolic and molecular phenotypes of GBM cells at various stages of the treatment course. Our preliminary findings demonstrate that stem-like and non-stem-like heterogeneous subpopulations possess distinct metabolic phenotypes and display differential early-stage metabolic adaptations in response to chemoradiotherapy treatment. Our findings suggest that blocking these metabolic transitions may enhance sensitivity to chemoradiotherapy treatment. Moreover, we have found that recurrent patient-derived GBM tumor cells display enhanced proportions of stem-like tumor subpopulations and have distinct metabolic preferences as compared to their primary tumor counterparts. These findings are the first to directly compare the metabolic phenotypes of heterogeneous primary patient-derived tumor cells with the metabolic transitions that occur in primary versus recurrent tumor samples. Using a novel temporal multi-omics approach that combines in-depth metabolomic profiling with molecular transcriptomic, epigenomic, and proteomic analysis, we will investigate the metabolic and cellular transitions that occur over various stages of GBM disease progression. Using the information generated from this integrated temporal analysis, we will design various timed metabolism-based combinatorial treatment regimens for preclinical evaluation in orthotopic patient-derived xenograft models.

SIGNIFICANCE TO CANCER: The findings from this study will significantly improve our understanding regarding the role of metabolic and cellular plasticity in therapy response and GBM tumor progression to design novel timed metabolism-based therapeutic strategies to benefit patients at all disease stages and improve patient outcomes.

Sinnarajah, Aynharan
University of Calgary

Improving cancer survivors' access to timely palliative care: validating and building triggers in the electronic medical record using real world evidence

Problem
Many patients living with advanced cancer have unmet needs around illness comprehension and coping, advance care planning and decision making, symptoms and daily functioning, and coordination of care. Consequently, many advanced cancer patients experience unnecessary distress and suffering which diminishes quality of life. These needs remain unmet because they are challenging to address in the cancer clinic setting where providers have limited time, competing priorities, and a lack of space for longer conversation. One solution is *timely* referral to supportive care services, including palliative care (PC) specialists, who are adeptly skilled and suited to address these needs. The **first problem** is: delivery of PC is inconsistent across Canada, and cancer patients who would benefit from PC services are referred **late, or not at all**. *Timely* or early PC is defined as within eight weeks of advanced cancer diagnosis (the American Society of Clinical Oncology definition). But this introduces a **second problem**: It is **unsustainable** for PC services to see every advanced cancer patient.
From prior work, we know that requiring oncologists to systematically evaluate patients' PC needs and make timely referrals is not feasible. Oncologists value screening cues. Consensus PC referral criteria have been established by an international Delphi study. Research is now needed to **clinically validate** these criteria using real world data (from health care administrative databases and the electronic medical record [EMR]) and clinician input. Then, we will test whether the criteria identify cancer patients with a high burden of unmet PC needs. Finally, we will use these validated criteria to drive EMR flags to alert busy oncology clinicians to patients with high PC needs.
Objectives
   1. Clinically validate performance of the PC referral criteria using health databases (including EMR data).
   2. Build and pilot study EMR-embedded electronic flags (driven by the algorithms developed in Objective 1) to assess the acceptability to oncology clinicians and impact on PC referral.
Methodology
Objective 1. The SaferDx trigger tools development framework will guide Objective 1. Steps in this framework include identifying data sources, constructing the "high PC needs" referral algorithms, testing algorithms with clinician and patient advisors, validating algorithms using real-world (anonymized) patient medical records, and finally assessing algorithm performance. Iterative refinements will lead to the final algorithms that will identify patients with high PC needs.
Objective 2. EMR-embedded flags the cue providers to patients with "high PC needs" will be piloted on 20 oncologists for 4 months. We will gather data on the acceptability of these flags to oncologists. Sekhon's Theoretical Framework of Acceptability for healthcare interventions will be used to ensure that acceptability is comprehensively assessed. We will also gather data on how well the flags worked. For example, did the gap between who *should be* referred, and who *was* referred, narrow for clinicians who participated in the pilot when compared to group of clinicians who did not.
Significance
Even though early PC is **proven** beneficial for people living with advanced cancer, it is not sustainable for all advanced cancer patient to see a specialist PC provider. EMR use is increasing across Canada. Using EMR tools to alert busy oncology clinicians to patients with high PC needs holds promise to improve timely PC referrals. Validating the PC referral criteria using health databases including EMRs (that can be adopted across Canada) is needed to assess feasibility and measure patient volumes generated by each criterion. This knowledge will allow different jurisdictions to selectively pick PC referral criteria that are sustainable for their centre. It will also prioritise PC resources to those with high PC needs while making sure they are not missed. A future randomized controlled trial will provide evidence on whether EMR-embedded flags lead to timely PC access. This approach holds promise to be more systematic and sustainable than relying on oncologists to routinely screen and refer to PC.

Struik, Laura
University of British Columbia

Modernizing approaches to tobacco use for the next millennium

Background: Cancer related to tobacco use remains a critical public health concern. While smoking remains the number one preventable cause of cancer-related morbidity and mortality rates, the introduction of e-cigarettes, has significantly shifted how we perceive tobacco use and what the term "tobacco use" entails. While e-cigarette and combustible cigarette use are different, they are also intricately related in several and often opposing ways in that e-cigarettes are touted to assist smokers in quitting, but have been found to be a gateway to smoking among young non-smokers. The rapid uptake of e-cigarettes among young Canadians is a troubling sign for tobacco control advocates in Canada. This is because vaping nicotine alone is increasingly associated with respiratory damage and disease, including cancer, as well as because the increase in vaping among young people is paralleling an increase in smoking in this demographic. In response to this complexity surrounding tobacco use of today, Health Canada has recently called for a review and renewal of the Federal Tobacco Control Strategy to adapt to these changes. In order to navigate this new landscape, it is critical that we bring the voices of youth and young adults to the forefront to inform the development of innovative prevention and cessation support resources that resonate with young Canadians.
Objectives: My program of research aims to **modernize approaches to tobacco use for the next millennium**. An interdisciplinary, pan-Canadian research team and partnerships with key stakeholders ground the activities for my program of research. Objectives for this program are to: 1) understand e-cigarette use among youth; 2) enhance prevention intervention and policy measures around youth vaping; and 3) assess e-health approaches for cessation to include vaping. To achieve this goal, I will conduct a series of research projects nested within two major themes over the next 5 years:
    ·**Theme 1:** Understand and prevent e-cigarette use among diverse populations of youth;
    ·**Theme 2:** Modernize cessation programming to include vaping
The research studies within each theme will follow an iterative process, whereby each strengthens and builds on the other. These projects will include a range of activities, including environmental scans, needs assessment research, intervention design/development, and evaluation research. I will use health behaviour change theories to ground my research, including the Unified Theory of Behaviour and Sociomateriality Theory. My research studies will entail a focus on the use of methodologies and methods that position the target population as central to the research process (e.g., interviews, focus groups, and design charrettes). All of the proposal projects will also include gender-based analysis to ensure that gender-related influences are accommodated, and that any gender-related inequities are addressed. Working with First Nations, I will also ensure that a culturally response approach is incorporated into the work. Finally, I will conduct this research using an integrated knowledge translation approach, whereby key knowledge users, including intervention end-users, are included on the research team.
Significance: Findings from the research conducted over the next five years will address critical gaps in our knowledge as it relates to tobacco use in the context of today. This research will not only contribute to understanding this new landscape of tobacco use since the introduction of e-cigarettes, but also generate a theoretically informed knowledge base for launching efforts to address both combustible cigarette smoking and vaping. With the research partners, this work will represent a concerted effort to navigate the recent changes in tobacco use and position Canada as a leader in cutting-edge efforts to address these changes. This research will lead to optimal solutions to curb tobacco use in the areas of both prevention and cessation and ultimately reduce tobacco-related cancers.

Tai, Lee-Hwa
Université de Sherbrooke

Improving autologous cancer cell vaccines for triple negative breast cancer

THE PROBLEM: Triple negative breast cancer (TNBC) has a poor prognosis due to a high rate of early recurrence and distant metastasis, compared to other breast cancer subtypes. Early stage TNBC is treated with neoadjuvant chemotherapy (NAC) and surgery, where response to NAC is the best indicator of long-term survival. There are no effective treatment options for patients with chemo-resistant, late stage or metastatic TNBC. Compared to other breast cancer types, TNBC has higher rates of genetic mutations and contains more tumor infiltrating lymphocytes, which provides a strong rationale to use immunotherapies. This project proposes to optimize an oncolytic virus-**i**nfected **c**ellular **v**accine (ICV) to treat metastatic TNBC.

RATIONALE: Across the field of oncology, immunotherapeutic agents are poised to transform cancer treatment. Therapeutic vaccines are a form of active immunotherapy that harnesses a patient's own immune system to fight against their cancer. Unlike single tumor antigen-targeted vaccines, treatment with autologous tumor cell vaccines exposes a cancer patient to their complete and individualized tumor associated antigen (TAA) repertoire, therefore reducing the likelihood of tumor escape due to tumor heterogeneity. However, autologous tumor cell vaccines have demonstrated limited success in clinical trials. Prior studies confirm that a measurable anti-tumor immune response is associated with better prognosis, but not all patients are able to generate this immune response. Known barriers include the lack of immunogenicity of the autologous vaccine and immune resistance mechanisms in the tumor microenvironment (TME), which is what we will address in this proposal.

PROJECT OBJECTIVES: This current proposal will develop an optimized ICV strategy using functional immune assays in preclinical and translational models of TNBC. We will investigate what is required for an ICV-generated adaptive T cell response that translates into an effective anti-tumor immune response. Specifically, we will identify the key mediators required to create the highest magnitude effector T cell response against the tumor. Furthermore, we will evaluate the underlying immune mechanisms to ensure generated anti-tumor T cell responses are sustained within the immune suppressive tumor microenvironment, for lasting clinical efficacy. Finally, human autologous immune responses against ICV will be evaluated in reconstituted organoid systems derived from TNBC patient tissues.

METHODOLOGY: We will use immunocompetent mouse models of TNBC, human TNBC cell lines and human TNBC patient tissue to develop and characterize an optimized ICV. 4T1 tumors will be implanted orthotopically into the mammary fat pad. ICV treatments will be carried out in the presence of established tumors. For ICV preparation, viable single cell suspensions of enzymatically dissociated 4T1 primary tumors will be γ-irradiated followed by the addition of oncolytic virus to the cells *ex vivo*. This virus+cell preparation will be injected subcutaneous in BALB/c mice. Immune monitoring of T cells will occur before and after vaccination. Systemic and tumor infiltrating T cells will be used to assess degranulation, cytokine secretion and cytotoxicity by intracellular flow cytometry following re-stimulation with tumor lysates pulsed on APCs or gp70 peptide as well as anti-CD4, CD8 and gp70+ tetramers to enumerate helper, cytotoxic and gp70-specific T cell populations respectively. Immune reconstituted organoids will be generated from human TNBC patient tissues to investigate autologous immune responses against TNBC tumors by multiparameter flow cytometry and IHC.

SIGNIFICANCE OF THE RESARCH TO CANCER: Obtaining preclinical and translational data on the immune and therapeutic outcome of ICV in translational models will accelerate the development of a postoperative cancer vaccination strategy. This novel treatment platform has the potential to induce a patient-specific, multivalent anti-tumor immune response that will improve survival for poor prognosis TNBC patients.