Canadian Cancer Society

October 2019 (INNOV-20) Competition Awarded Innovation Grants

Listed in alphabetical order by Panel

I1a Biomarkers and Genomics

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Clonal stromapoiesis and BM microenvironment in leukemias and HCT

1. Background and research questions

Hematopoietic stem cells (HSCs) are pluripotent blood cell forming stem cells in bone marrow (BM). They are known to acquire somatic mutation with ageing, resulting in clonal hematopoiesis (CH). However, it is unknown if the same phenomenon can occur in the BM microenvironment (BMM). There is emerging evidence supporting that BMM is playing a pivotal role in the leukemogenesis. A series of 5 studies reported overall 27% of patients with acute myeloid leukemia (AML) or myelodysplastic syndrome (MDS) carries cytogenetic abnormalities in mesenchymal stromal cell (MSC) fraction, which is distinct from hematopoietic cell (HC) fraction. This finding triggers a working hypothesis in Aim 1 that genetic events in MSC are frequent, recurrent in patients with leukemia, and independent from clonal hematopoiesis.

In Aim 2, we will attempt to answer the question if pathological BM niche in the recipient is involved in the development of graft failure after allogeneic hematopoietic stem cell transplantation (HCT). HCT is a curative treatment modality to treat blood cancer but increases mortality by increasing the non-relapse mortality rate. There is still limited knowledge about the mechanism of graft failure. Although bone marrow is replaced with donor-derived HSCs through HCT, the marrow niche is believed to be maintained with recipients' stroma cells. In Aim 2, we will test a working hypothesis that pathological BMM from the recipients is involved in the development of graft failure post-HCT.

2. Objectives

The main goal of the project is to identify recurrent somatic mutations in MSCs, which may be dysfunctional in patients with leukemia or those with graft failure after transplantation.

Aim 1: Clonal stromapoiesis in leukemia

For Aim 1, we will include a total of 100 paired samples from 50 patients with poor-risk or relapsed/refractory acute myeloid leukemia (AML). Whole exome sequencing (WXS) will be applied (100x sequencing depth), and we will evaluate the MSC function according to the presence of mutation in MSCs.

Aim 2: Clonal stromapoiesis in graft failure after HCT

For Aim 2, using the PM leukemia tissue bank, we will test a total of 40 cases including 20 cases developed graft failure and 20 cases with full donor chimerism. MSC cultures, WXS analysis and MSC assays will be performed.

3. Methodology

Ficoll-hypaqued mononuclear cell (MNC) samples collected at initial diagnosis and stored at the PM leukemia tissue bank will be used. MSCs will be cultured according to an established method and separated from the HC fraction. Pairs of HC/MSC fractions will be subjected to WXS (100x). Cultured MSCs at 4th passage will be evaluated using the following assays: 1) flow cytometric analysis, 2) CD34+ HSC proliferation assay, 3) clonogenicity assay, i.e. CFU-F assay, 4) DNA content/cell cycle assay, and 5) response to the TLR ligands, poly IC, and LPS as well as TNFα and IFNγ with respect to release of the anti-inflammatory molecules tumour necrosis facor-inducible gene 6 protein [TSG6], prostaglandin E2 [PGE2] and indoleamine 2,3 dioxygenase [IDO].

4. Significance

Identifying the presence of recurrent mutations in MSCs will open a new avenue of the leukemia genomic research in BMM studies, and will enhance our understanding of the mechanism of leukemia resistance and graft failure. This will

provide a better understanding of the impact of somatic mutations on MSC function. The data generated from our study will be a stepping stone towards the discovery of clonal stromapoiesis as ageing proceeds. The observations may help to facilitate the development of novel therapeutics targeting the BMM.

Saleeb, Rola
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Using nanopore technology to develop rapid, real-time, clinical molecular oncology testing

Background

Pathological examination of tumors remains the mainstay of cancer diagnosis, however, it is often insufficient on its own to stratify patient management. Current practice has evolved to incorporate molecular tests to aid diagnose and predict tumor clinical behaviour. Central nervous system (CNS) tumors represent one such clinical entity where a histo-molecular approach has become standard of care. In 2016, the WHO issued an updated CNS tumour classification scheme that, for the first time, required the use of molecular parameters in conjunction with histological analysis to define the tumor entity. Despite advancements in molecular clinical diagnostics, testing is often piecemeal, involving multiple modalities/workflows to inform a diagnosis. The result is a need for major infrastructural changes and time to results on the order of weeks; the consequences of which can be delays in therapeutic management and, in some instances, an outright inability to access the recommended molecular information owing to limitations in resources. For some CNS tumors such as glioblastomas, where the 2-year survival rate is ~15%, delays and/or inadequate diagnosis can have catastrophic consequences on patient care.

The Proposal

Nanopore-based sequencing is a relatively new technology that offers the potential to overcome many of the current challenges facing tumor molecular diagnostics. . This technology eliminates the need for high capital costs and major infrastructural requirements. It also enables fast turnaround times, owing to the requirement for minimal sample preparation, the ability to run a single case without the need for multiple sample pooling and real-time sequencing analysis which generates long-read sequencing results, and is sensitive to methylation changes in DNA without the need for pre-processing steps.

Hypothesis & Central Objective

Nanopore-based, real-time, sequencing will be a reliable, robust, and timely tool for the diagnosis and characterization of CNS tumors. **The central objective of this proposal is to develop and clinically validate a novel nanopore sequencing-based approach for the molecular characterization of CNS tumors that can be completed in as little as 3 days from the time of specimen receipt, at a cost of <$300 per case.**

Specific Aims

Aim 1. Development and optimization of a streamlined approach to molecular characterization of CNS tumours using nanopore sequencing

WHO classification of CNS tumors and current clinical management guidelines require molecular stratification to complement the pathological diagnosis, including, i) MGMT promoter methylation status, ii) IDH1 and IDH2 mutational status, and iii) co-deletion of the 1p/19q chromosomal arms. In this aim, a targeted approach will be developed that will permit investigation of all three WHO required molecular parameters for CNS tumor characterization into a single nanopore-base sequencing test.

Aim 2: Clinical Validation

Analytical validation is an essential component of any diagnostic used in the clinical laboratory. In this aim, a representative set of brain tumors (n=60) will be selected from the St. Michael's CNS tumor biobank and will undergo testing with the novel sequencing approach. Test performance characteristics such as sensitivity, specificity and reproducibility, among others, will be assessed for validation and quality assurance.

Significance

Present day diagnostics for CNS tumor characterization are challenged by costs and lengthy time requirements. The proposed nanopore sequencing-based solution has the potential to overcome these challenges and, most importantly, to positively impact patient care. To our knowledge, this will be the first application of its kind in a clinical laboratory in Canada. Notably, the proposed approach is significantly scalable, in that it can be applied and expanded to include many current cancer molecular clinical tests. While we are proposing a targeted test which is more suited for clinical molecular needs, the technology has the potential to be used in a comprehensive manner that would be of great value to the clinical and research cancer fields.

Zadeh, Gelareh
The Toronto Hospital (Western Division) - UHN

Proteogenomics of meningiomas to facilitate molecular classification and identify novel therapeutic options

Meningiomas are common brain tumors in adults and are mostly treated with surgery. Whether patients require radiation after surgery is primarily determined by the histopathological evaluation (i.e. the WHO grade). However, meningioma grading based on pathologists' interpretation under the microscope may lead to considerable inter-observer imprecisions. Previous reports have identified specific mutations associated with a subset of meningioma tumors, but these changes have had limited utility in reliably predicted patient outcomes. We have recently investigated changes in the DNA methylation profile and identified a clear methylation signature that can reliably predict the likelihood of early tumor recurrence in meningioma patients. However, this test cannot be performed universally at every hospital. Our recent data have demonstrated that the integration of data from multiple 'omic' platforms is superior compared to individual molecular profiling platforms such as DNA/RNA sequencing or proteomic profiling alone. Therefore, in this application, we attempt to understand how epigenetic or genetic alterations ultimately modify protein structure and/or function and contribute to cancer progression, while also delineating patterns of changes in protein expression that can predict disease aggressiveness. We will leverage extensive genetic data on several hundred meningiomas already characterized by our group, linking them to proteomic alterations to develop novel prognostic biomarkers. Such novel signatures have the potential to reduce over-treatment or under-treatment of meningioma patients harboring specific proteomic biomarkers in their tumors. In addition, these specific protein alterations will be explored as potential therapeutic options in follow-up studies.

I1b Gene Regulation and Cell Biology

Attisano, Liliana
University of Toronto

Modelling brain metastasis

Metastasis is a multistep process in which cancer cells spread from a primary tumour to distant sites to establish secondary tumors, a process that accounts for >90% of cancer related deaths. Clinical observations indicate that most cancers or cancer subtypes metastasize to specific organs, referred to as "organotropism." For example, breast cancers can metastasize to bone, lung, liver, and brain, but the luminal subtype has a higher propensity to colonize the bone, while HER2-positive, and triple-negative breast cancers (TNBC) preferentially colonize the brain. Brain metastases are very difficult to treat and the mechanisms underlying their establishment and progression are poorly understood. Unfortunately, few studies aim to uncover mechanisms underlying brain metastasis, particularly at the early stages. This is due to the inadequacy of in vitro models that lack the 3D spatial organization and cell heterogeneity of tissues, as well as the cumbersome nature of in vivo mouse models that also lack species-specific features. We recently established an entirely human in vitro model of vascularized cerebral organoids (vhCO) using a microfluidics device we developed and call VIVOs. In this system, co-culture of human endothelial cells, fibroblasts and human embryonic stem cell-derived hCOs in a microfluidic device mimicking human vascular pressure leads to robust de novo vascularization of hCOs and the formation of neurovascular units. *Thus, the overall goal of this proposal is to exploit VIVOs to develop a human model of brain metastasis, and map at the single cell level the molecular events underlying TNBC metastatic extravasation and tumour growth.* To achieve these objectives we will:
AIM1. Examine early stage vascularized human CO (vhCO) colonization by triple negative breast cancer (TNBC) cells. In this aim, we will use our VIVOs platform to examine extravasation and the subsequent growth in vhCOs of brain-metastasizing variants of MDA-MB-231 cells, versus parental and lung-metastasizing subclones. Standard analysis of fixed sections by immunofluorescence microscopy will be complemented by live organoid imaging to assess mScarlett-labelled tumour cell dynamics during extravasation and metastatic growth. These studies will provide an unparalleled opportunity to gain insights into tumour cell behavior at the earliest steps of brain colonization.
AIM2. Investigate mechanisms that promote brain metastasis. The metastatic capacity and organotropism of a tumour is critically influenced by interactions with the host organ. Here, we will use single cell sequencing to explore how tumor-vhCO crosstalk alters gene expression patterns in both the tumour and the host tissue to enable extravasation and tumor expansion. Cell trajectory analysis and comparison to our existing hCO single cell profiles will reveal phenotypic transitions during metastasis. These studies will reveal the progressive molecular changes that occur between tumor and host tissues to drive organotropic metastases.
Altogether, we expect that our work will provide key advances in our understanding of the stages of brain colonization by breast cancer cells and may suggest how their disruption could lead to the development of novel treatments for

brain metastases. These advances will not only be relevant for breast cancer, but will likely be applicable to other cancers, such as lung and melanoma that frequently metastasize to the brain.

Ferbeyre, Gerardo
Université de Montréal

Targeting a novel pyruvate metabolic cycle to restore the senescent tumour suppression mechanism in tumours

Research on cancer metabolism and tumor suppressors aims to identify key vulnerabilities in cancer cells that can be ultimately translated into novel treatments. The p53 tumor suppressor pathway is disabled in most human cancers. This tumor suppressor inactivate key oncogenic functions hampering tumor progression. To identify p53 target genes critical for tumor suppression our laboratory focuses on oncogene-induced senescence (OIS). OIS engages p53 to prevent tumors by blocking the proliferation of malignant cells and stimulating anti-tumor immune responses. A similar response can be induced by cancer chemotherapy (therapy-induced senescence), which is limited by the toxicity associated to the drugs currently in use. Using senescence as a model system, we have discovered a metabolic cycle involving three enzymes repressed by p53 and highly expressed in multiple human cancers. The enzymes are **pyruvate carboxylase (PC), malic enzyme-1 (ME1) and malate dehydrogenase-1 (MDH1)** and they form a complex that can be immunoprecipitated from cells or detected as distinctive structures using hyperresolution microscopy. We have confirmed that these enzymes are highly expressed in samples from patients with prostate cancer and a mouse model of the disease. This novel metabolic cycle functions by regenerating NAD+ from NADH without consuming any metabolic intermediate. NAD is a coenzyme essential for fuel assimilation such as glucose, lipids and lactate. Recent results in cancer metabolism have recognized that tumors have an enormous plasticity in choosing their sources of energy and carbon. We anticipate that targeting this NAD regenerating complex, we named **CNARC for Cytosolic NAD Regenerating Complex**, has a higher potential to interfere with cancer metabolism than approaches aiming at interfering with single fuel utilisation. In addition, the strategy is potentially safe because the formation of CNARC is specifically induced in tumor cells due to their mutations disabling the p53 tumor suppressor pathway. In this application, we will provide a proof of concept to justify a drug discovery approach to target CNARC. **In the first aim**, we will use prostate cancer cell lines to study their requirement for PC, ME1 and MDH1. We will study the effects of inactivation of these enzymes with shRNAs on tumor growth in cell culture and in mice. We will inactivate each enzyme separately or in combination using a retroviral vector designed to express multiple shRNAs. The metabolic effects of enzyme inactivation will be studied using isotope tracer analysis with 13C6 glucose and biochemical methods to measure the NAD+/NADH ratio. **In the second aim**, we will map the protein-protein interaction motifs required for the interaction of CNARC enzymes and design peptides able to disrupt the complex . We will also use SECS-MAL (size exclusion chromatography and multi-angle light scattering) to obtain biophysical evidence for the proposed new enzymatic complex, its size and stoichiometry. We expect that structural and biochemical data on CNARC enzymes will provide a unique opportunity for drug discovery to reprogram cancer cell metabolism and prevent tumor progression. The validation of a new metabolic cycle carry out by a multimolecular protein complex raises the intriguing possibility of finding drugs that act on the complex rather than on individual components. Since this complex formation is stimulated by loss of p53, it may define a new interesting vulnerability in cancer cells.

Goping, Ing Swie
University of Alberta

Sublethal apoptosis as a driver of breast cancer recurrence

Introduction: Apoptosis is a cellular suicide program that is important for proper development and tissue homeostasis. While apoptosis has long been known to prevent cancer, emerging studies have challenged this paradigm. In fact, at low levels, “failed or sub-lethal apoptosis” induces tumorigenesis and cancer cell aggression in cell- and animal-based models. This paradoxical nature of apoptosis has been the subject of recent reviews (Gama and Deshmukh, Mol Cell; Ichim and Tait, Nat Rev Cancer; Gong and Green, FEBS J). *A major question is whether sublethal apoptosis plays a role in human health and disease.* Our work provides critical evidence that links incomplete apoptosis with breast cancer disease relapse in patients.
An excellent model to investigate the relationship between failed apoptosis and human disease involves the pro-apoptotic protein BIK. BIK is regulated in an Estrogen Receptor (ER)-dependent manner and importantly, is a prognostic marker for poor outcomes in ER-positive breast cancer patients. These results are significant because (i) they identify that ER-positive patient outcomes may be mechanistically tied to BIK-biology, and (ii) they suggest that

sublethal apoptosis contributes to clinical cancer recurrence. We therefore tested whether BIK mediated sublethal apoptosis and whether this induced aggressive cancer phenotypes. We found that BIK triggered caspases and activation of caspase-activated DNase (CAD) generating genomic DNA double-strand breaks. Apoptosis was sublethal and cells survived displaying increased anchorage-independent growth, increased cell motility and enrichment of cancer stem-like cells. *This indicates that BIK mediates failed apoptosis with significant DNA damage, suggesting a mechanism to facilitate tumor evolution and disease relapse. Notably, our results link the emerging paradigm of apoptosis-induced oncogenesis with clinical outcome of ER-positive breast cancers.*

Hypothesis and Objectives: We hypothesize that breast cancer recurrence is mediated in part by BIK-induced sublethal apoptosis that drives the evolution of tumor resistance to therapy.

(1) We will test whether BIK increases mutagenesis through a sublethal apoptotic mechanism. We will assess whether BIK expression increases mutation load through whole genome sequencing. We will additionally use RNA-seq to determine whether phenotypic changes are driven by altered gene transcription. Using a non-apoptotic BIK mutant and CAD-null cells, we will interrogate the molecular mechanism of BIK-induced mutagenesis. Overall this aim will define the causal relationship between BIK --> sublethal apoptosis --> CAD --> mutagenesis --> aggressive cell properties.

(2) We will test whether BIK-induced sublethal apoptosis facilitates cancer cell aggression and therapy resistance in animal models. In vivo tumors are subject to multiple stressors that provide selective pressure and dictate tumor evolution. We will use animal models to establish whether BIK-expressing cells show increased tumor growth and/or metastasis. Using mutant cell lines, we will establish if BIK effects are via sublethal apoptosis and mutagenesis. Finally, since BIK-expressing cells showed increased proportions of stem-like cells, we will determine if BIK facilitates tumor therapy-resistance. Collectively, this aim will establish if BIK drives pre-clinical treatment resistance via sublethal apoptosis and/or mutagenesis.

Significance: BIK is prognostic for poor outcomes of ER-positive breast cancer patients. Our proposal will identify whether the mechanism driving BIK prognostic ability is via sublethal apoptotic pathways leading to the genomic instability required to evolve therapy resistance. Importantly our studies will identify a subset of ER-positive patients that are likely to relapse due to mutation accumulation. This work may provide the rationale to evaluate BIK-high patients for responsiveness to immunotherapy.

Koromilas, Antonis
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2B or not 2B: that is the question about KRAS function.

Background: *H-, N- and K-RAS* genes encode a family of small membrane-bound GTPases, which cycle between GDP-bound inactive and GTP-bound active forms. Around 30% of human cancers contain activating *RAS* mutations at the codon for G12, G13 or Q61, which produce proteins that are preferentially bound to GTP and capable of activating pathways responsible for increased tumor growth and resistance to chemotherapeutic drugs. Although targeting of activated RAS proteins in tumors has proved a daunting task, the recent development of drugs blocking certain forms of mutant RAS (e.g. KRAS G12C) in an inactive GDP-bound state has raised hope that *GTP-GDP exchange is a promising target of anti-mutant RAS therapies.*

Translation of mRNAs is intimately involved in cancer through the selective synthesis of proteins involved in tumor initiation, progression and metastasis. mRNA translation is controlled by key steps at the level of initiation including the formation of a ternary complex between the initiation factor eIF2, GTP and the initiator methionyl-tRNA (tRNAiMet). Recognition of start codon by eIF2.GTP.tRNAiMet requires GTP hydrolysis followed by release of eIF2.GDP. Recycling of eIF2.GDP to eIF2.GTP is essential for subsequent rounds of re-initiation and is facilitated by the translation factor eIF2B, which possesses guanine exchange function (GEF). In cells subjected to different forms of oncogenic stress like genotoxic, oxidative, hypoxic, metabolic or proteotoxic stress, eIF2B GEF is inhibited by the increased phosphorylation of the α subunit of eIF2 at Ser51 resulting in the inhibition of mRNA translation. Despite the translational shut off, select mRNAs are better translated under these conditions leading to production of proteins, like the transcription factor ATF4, with key roles in adaptation to stress, tumor formation and tumor resistance to chemo and radiation therapies.

Research relevant to the application: We provide very strong evidence for a novel role of eIF2B in KRAS function. Specifically, we demonstrate a physical interaction between eIF2B and mutant but not normal KRAS in tumor cells. We also show that downregulation of eIF2B by si/shRNAs impairs mitogen activated protein kinase (MAPK) signaling in mouse and human tumor cells with KRAS mutations. Moreover, we demonstrate that treatment with ISRIB, a small compound that increases eIF2B GEF, stimulates MAPK signaling preferentially in tumor cells with mutant KRAS.

Hypothesis and Research Objectives: We hypothesize that the interaction between **eIF2B and mutant KRAS**

exerts reciprocal effects on both proteins. Considering that mutant KRAS proteins contain low levels of intrinsic GTP hydrolysis, eIF2B may act as GEF for mutant KRAS. Also, eIF2B binding to mutant KRAS may facilitate GDP-GTP exchange on normal KRAS in dimers with mutant KRAS. On the other hand, mutant KRAS binding may stimulate eIF2B GEF to promote protein synthesis and tumor growth. Our objectives are to determine the biochemical attributes underlying eIF2B and mutant KRAS interaction and their implication in KRAS signaling and protein synthesis.

Research plans: In aim 1, we perform co-immunoprecipitations and cell imaging techniques to better characterize the interaction between eIF2B and KRAS mutants in lung, colorectal and pancreatic tumors. In aim 2, we determine the effects of purified eIF2B on GDP-GTP exchange of recombinant normal and mutant KRAS proteins by NMR technology. Also, we examine the effects of eIF2B GEF inactivation by genetic means on GTP loading of mutant KRAS and stimulation of the MAPK pathway. In aim 3, we determine the effects of mutant KRAS on the translational effects of eIF2B GEF and expression of tumorigenic genes.

Significance: Our work sheds new light onto mechanisms of mutant KRAS function through its interaction with eIF2B, the only known function of which is the regulation of mRNA translation initiation. Because targeting GEFs is gaining ground in anti-RAS treatments, our work will determine whether eIF2B is a suitable target for the development of anti-tumor treatments of deadly cancers with KRAS mutations.

McCaffrey, Luke
McGill University

Characterizing DCIS heterogeneity and disease progression

Since implementation of widespread screening programs, ductal carcinoma in situ (DCIS) now makes up 20-25% of new breast cancer diagnoses. Rates of progression from DCIS to invasive ductal carcinoma (IDC) are 20-50%, with roughly 8% of patients already having IDC at the time of their surgery. There is currently no way of identifying which DCIS has the potential to progress to IDC and which will remain indolent without any therapy being required. Furthermore, the increase in rates of DCIS diagnoses have not been followed by a decrease in IDC diagnoses or mortality, leading many researchers and clinicians to believe that we are currently overtreating indolent DCIS. This means that every woman with a diagnosis of DCIS must undergo surgery with its associated anxieties, risks and potential complications, though not all have the same likelihood of developing IDC. Recent techniques for studying DCIS have been optimized, including intraductal mammary injections, and it is now feasible to consistently recreate all stages of breast cancer in a laboratory setting from DCIS to IDC to metastatic disease. Accumulating evidence indicates that major mutations and genetic alterations found in invasive cancer clones pre-exist in heterogeneous DCIS. It is also becoming evident that the microenvironment also plays a role in progression, but these are not sufficient.

Our hypothesis is that cell heterogeneity and plasticity are required respond to changing microenvironmental cues to enable progression of DCIS to invasive ductal carcinoma (IDC). To address this, our project has three objectives:

1.Establish patient-matched DCIS-IDC breast PDX models. We will develop a PDX biobank of 10 patient-matched DCIS and invasive breast cancer using a recent intraductal transplant method. We will compare cell heterogeneity from the PDX and primary patient samples.

2.Identify cellular heterogeneity during DCIS-to-Invasive transitions. We will use single cell RNA sequencing to identify unique cell populations during the DCIS-to-invasion transition and multiplexed imaging to map these cell populations within microenvironmental niches.

3.Functionally characterize cell plasticity of DCIS populations. Using PDX-derived organoids, we will evaluate cell plasticity and the ability of different DCIS populations to adopt an invasive phenotype in different microenvironmental conditions.

Siegel, Peter
McGill University

Characterizing molecular mediators of leptomeningeal metastasis

Background: The development of central nervous system (CNS) metastases is a devastating complication of numerous solid cancers. CNS metastases are 10 times more frequent than primary brain tumors and occur in 20-40% of all cancer patients. Of these, 80-90% encompass parenchymal lesions while the remaining 10-20% are leptomeningeal metastases. The development of CNS metastases is associated with a particularly devastating

prognosis, with the median survival of treated patients bearing leptomeningeal metastases being under 3 months, compared to 1 year for patients undergoing treatment for parenchymal metastases.

We have generated a unique patient-derived xenograft (PDX) model of spontaneous leptomeningeal metastasis (called GCRC1971) in which tumors implanted in the mouse mammary fat pad spontaneously metastasize specifically to the leptomeninges in 90% of mice. When comparing the gene expression profiles of matched primary tumors and leptomeningeal metastases from this model with RNASeq, tumors in the leptomeninges express higher levels of CIRBP and RBM3 than the initial lesions located in the mouse mammary fat pad. We have confirmed higher expression of CIRBP and RBM3 expression in leptomeningeal metastases when compared to primary mammary tumors by immunoblotting and immunohistochemistry. However, our preliminary data indicates that CIRBP, but not RBM3, functionally promotes leptomeningeal metastasis formation.

Cold Inducible RNA Binding Protein (CIRBP), is an RNA binding protein that is involved in cellular stress responses. CIRBP is comprised of an RNA recognition motif (RRM) and a glycine rich domain (RGG). The RRM contains two motifs (RNP1 & RNP2) that enable CIRBP to bind its target mRNAs. CIRBP has been proposed to play both tumor promoting and suppressor roles in different contexts but its functional role in metastasis has yet to be studied.

Hypothesis: We hypothesize that CIRBP, through the regulation of specific target mRNAs, enhances the ability of cancer cells to withstand stresses encountered while forming leptomeningeal metastases.

Objectives: In this proposal, we will 1) define and characterize the role of CIRBP in the formation of leptomeningeal metastases using unique PDX models and human clinical material and 2) functionally characterize mRNA targets of CIRBP and define their role in leptomeningeal metastasis formation.

Experimental Approach:

Aim 1: Establish CIRBP as a mediator of spontaneous leptomeningeal metastasis. We will employ unique PDX models that have high levels of CIRBP, which we will diminish using shRNA approaches, and test the impact of CIRBP loss on the formation of spontaneous leptomeningeal metastasis formation. In addition, we will overexpress CIRBP in PDX models that exhibit low endogenous CIRBP levels to determine whether the efficiency of spontaneous leptomeningeal metastases is increased. Finally, we will stain TMAs of brain metastases and primary breast tumors to determine if high levels of CIRBP are associated with metastasis in general and more specifically with the formation of leptomeningeal metastases.

Aim 2: Define CIRBP mRNA targets and their influence on metastatic organotropism to the leptomeninges. We will generate mutants of CIRBP that are impaired in their ability to bind target mRNAs to determine if this function of CIRBP is required for its pro-metastatic functions. We will then identify mRNAs that are bound by CIRBP specifically in leptomeningeal metastases and identify those targets that functionally promote the formation of leptomeningeal metastases.

Significance: We have developed unique PDX models that are capable of forming leptomeningeal metastases, a devastating CNS lesion that is associated with very poor patient outcomes. We have used these models to identify CIRBP, an mRNA binding protein that is functionally involved in the formation of these lesions. The proposed experiments will define the specific mRNAs that are regulated by CIRBP and how the proteins encoded by these CIRBP-regulated mRNAs contribute to the formation of leptomeningeal metastases.

I2 Imaging and Technology Development

Reilly, Raymond
University of Toronto

Radiation nanomedicine combined with checkpoint immunotherapy for treatment of glioblastoma multiiforme (GBM)

RATIONALE: We propose an innovative radiation nanomedicine for treatment of glioblastoma multiforme (GBM) that could be infused at surgery to eradicate residual tumour, prevent recurrence and improve patient outcome. We will study this treatment alone or combined with anti-PD1 checkpoint immunotherapy to eradicate residual tumour cells not killed by the radiation nanomedicine in mice with orthotopic GBM tumours. Anti-PD1 immunotherapy has proven effective for eradicating GBM tumours in mice and multiple clinical trials of immunotherapy of GBM have been launched. In addition, radiation enhances the immunogenicity of tumours, but the immune response is blunted by PD1/PDL-1 immune checkpoints in GBM.**HYPOTHESIS:** Our hypothesis is that local treatment with the radiation nanomedicine will inhibit tumour growth and prolong survival in mice with orthotopic GBM tumours and that survival will be further improved by combining the radiation nanomedicine with anti-PD1 immunotherapy.**EXPERIMENTAL DESIGNAim 1. To construct and characterize the radiation nanomedicine and study its ability to kill GBM cells in vitro by causing lethal DNA double-strand breaks or cell membrane damage, and to estimate the cellular radiation doses.**The radiation nanomedicine will be constructed from 5 nm gold nanoparticles (AuNPs) labeled with 197(m)Hg and modified with anti-EGFR F(ab')2 to bind, internalize and transport the AuNPs to the nucleus of

EGFR-positive GBM cells, where the Auger elecrons (AE) or conversion electrons (CEs) emitted by 197(m)Hg are most lethal. The AuNPs will be radiolabeled with 197(m)Hg by conversion of some gold via the 197Au(p,n)197(m)Hg reaction in a 13 MeV cyclotron, or by complexation of 197(m)Hg by thiol-functionalized polymers conjugated to the AuNPs that present DOTA chelators. Binding to EGFR-positive luciferase-transfected human U87MG-Luc or U-251-Luc GBM cells or murine GL261-Luc GBM cells will be assessed (Kd and Bmax) and stability in human plasma and to challenge with cysteine or glutathione determined. The cytotoxic effect of the radiation nanomedicine on GBM cells will be studied, and damage to DNA or the cell membrane assessed. Cell fractionation studies will be used to estimate the cellular radiation doses using MCNP code.**Aim 2. To determine the local retention of the radiation nanomedicine after stereotactic injection in mice with GBM tumours and determine the normal brain and other normal organ dosimetry.**Orthotopic GBM tumour models will be established by stereotactic inoculation of human U87MG-Luc or U-251-Luc GBM cells into NRG mice or murine GL261-Luc GBM cells into immunocompetent C57BL/6J mice. Tumour growth will be assessed by bioluminescence imaging (BLI). The retention of the radiation nanomedicine at the tumour site following stereotactic infusion in mice and re-distribution to normal brain or nornal organs outside the brain will be assessed by microSPECT/CT imaging up to 5 days post-injection (p.i). Biodistribution studies will be used to estimate radiation doses to the tumour, normal brain and other organs.**Aim 3. To study local treatment of GBM tumours in mice with the radiation nanomedicine alone or combined with anti-PD-1 checkpoint immunotherapy.**NRG mice with orthotopic human GBM tumours will be infused locally with the radiation nanomedicine (2, 5 or 10 MBq) and the effects on tumour growth assessed by BLI, and median survival determined and compared to control mice receiving unlabeled AuNPs or no treatment. Immunocompetent C57BL/6J mice will be treated with the radiation nanomedicine alone or combined with anti-PD1 antibodies administered i.p. every 3 days. Control mice will receive anti-PD1 antibodies or no treatment. Normal tissue toxicity will be assessed by monitoring body weight, complete blood cell counts (CBC) and serum ALT and creatinine.**IMPACT AND INNOVATION:** The proposed research may lead to a new intra-operative treatment for GBM that could improve patient outcome by eradicating residual tumour and preventing recurrence. This will be the first time that a radiation nanomedicine has been studied alone or combined with PD1 immunotherapy for treatment of GBM.

Shuhendler, Adam
University of Ottawa

Aldehydes as an imaging biomarker for lung cancer detection and staging by PET

Problem: Lung cancers are difficult to detect, and often are diagnosed at advanced stages of the disease. As a result of the difficulty of diagnosis, lung cancer is the leading cause of cancer death in Canada, accounting for nearly 26% of all cancer deaths for men and women. In addition to difficulties in diagnosis, the treatment of lung cancer requires that chemotherapy be matched to the various subtypes of lung cancers (e.g. adenocarcinoma, small cell, squamous cell, etc.), each with characteristic drug sensitivities and multidrug resistance phenotypes. Currently, lung cancer screening is performed by low dose computed tomography or with a glucose metabolism targeted PET radiotracer [18F]FDG; however these technique sometimes have poor sensitivity and specificity (false positive and negative results), and fail to distinguish between indolent and aggressive lung tumors. A non-invasive method to characterize lung cancer phenotype and drug sensitivity would significantly improve survivability but is currently lacking.*Approach:* The uniquely oxidative microenvironment of lung tumors contributes to the progression of the disease, and results in enhanced levels of oxidative stress markers in these tumor tissues. This redox imbalance results in high levels of aldehyde production, including acetaldehyde, malondialdehyde, and 4-hydroxynonenal, which have not only been related to tumor subtype, aggressiveness, and multidrug resistance status, but are also prognostic for lung cancer outcomes. While aldehydes have previously been measured from patient breath samples, breath analysis is complicated by a mix of analytes emitted from tumor and normal cell types, its semi-quantitative nature, and a lack of standardization of breath analysis techniques. Our approach is to directly quantify aldehydes in lung tumor tissues using a novel positron emission tomography (PET) radiotracer. This way, lung tumor aldehydes can be non-invasively mapped *in situ* in the tumor.*Objectives:*Our objectives are two-fold, with the overall goal of assessing the diagnostic and predictive power of *in situ* aldehyde imaging in lung cancer. *Objective 1*: Assessment of the ability of our PET radiotracer to distinguish between lung cancer subtypes in orthotopic mouse models of human disease. *Objective 2:* Determine if tumor aldehyde content defined by PET can predict response to conventional lung cancer chemotherapy. With these objectives, the prognostic value of directly detecting the endogenous biomarkers of highly oxidative lung tumors can be evaluated with clinical translation in mind.*Methods:* Unlike the commonly used glucose metabolism targeted radiotracer [18F]FDG, our novel PET radiotracer will selectively provide PET signal in the presence of tumor aldehydes. In Objective 1, our radiotracer will be tested across three orthotopic mouse models of different human lung cancers with varying degrees of aggressiveness, which have been shown to differ significantly in aldehyde content. In Objective 2,

these same three mouse models will be subjected to either carboplatin or cyclophosphamide chemotherapy, and therapeutic efficacy (determined by tumor size change) will be related to aldehyde content measured by our radiotracer. Animal studies will be followed up by histological assessment of oxidative stress (nitrotyrosine staining) and cell death (active caspase-3 staining).*Significance:* This work is a novel application of the first PET radiotracer capable of the selective imaging of aldehydes *in vivo*, and a novel technology that could increase lung cancer survivorship by providing non-invasive, three-dimensional, and quantitative assessment of a prognostic biomarker: tumor aldehydes. This proposal seeks to interrogate the ability of the novel PET radiotracer to stratify lung cancer subtypes based on aldehyde content, and to determine the chemotherapy best-suited to the specific disease of the individual patient. The success of this radiotracer in the non-invasive prognosis of lung cancer would result in a translational mechanism for the personalized treatment of this high fatality disease.

I3 Immunology, Signalling and Stem Cells

Diallo, Jean-Simon
Ottawa Hospital Research Institute

Enhancing oncolytic virus efficacy using effector peptides

BACKGROUND: Oncolytic viruses (OVs) are self-replicating cancer biotherapeutics that selectively infect and kill cancer cells via oncolysis. Many relevant OVs including Imlygic®, the first clinically approved OV for treatment of melanoma, are genetically tailored to encode therapeutic transgenes whose expression is driven by viral replication. Therefore, therapeutic effects induced by transgene expression and the direct oncolytic action of the OV require sufficiently robust tumor-specific virus replication.
We believe that successful and consistent clinical translation of OV therapy requires the alteration of the tumor micro-environment to fully sensitize tumors to OVs and have shown that various small molecule “viral sensitizers” can be effective in this regard. We have also shown that the combination of VSes working by different mechanisms can result in synergistic enhancement of viral growth. One class of VSes are microtubule destabilizing agents (MDA) that increase OV spread and bystander killing in cancer cells. While select MDAs are clinically approved, they suffer from dose-limiting systemic toxicities. Based on proof of concept from our published studies demonstrating therapeutic benefit of an OV encoding an IFN scavenger protein derived from vaccinia virus, we propose to develop OVs encoding biological proteins with VSe functions to take advantage of viral sensitization while mitigating systemic toxicity. Several bacterial effector proteins have been identified that act as MDAs. As preliminary data, we have successfully engineered the OV VSVΔ51 to encode an MDA and observed increased viral spread in human and murine VSV-resistant cancer cell lines compared to VSVΔ51. Building upon this, we hypothesize that VSe-like effector protein domains (VSEPs) synergistic with our MDA, termed VSEP1, could be encoded within VSVΔ51-VSEP1 to further improve oncolysis and anti-cancer efficacy, while retaining a favorable safety profile.
OBJECTIVES: Our data suggests that it is possible to safely enhance the efficacy of VSVΔ51 through the expression of VSe-like viral or bacterial effectors (VSEPs) that mimic the mechanisms of single VSe molecules, like MDAs for example. We therefore propose to design novel and evaluate OVs that express rationally chosen therapeutic payloads (VSEPs) and strategic combinations thereof *in vitro* (**Aim 1**). We further aim to evaluate the safety and efficacy of promising VSVΔ51-VSEPs compared to VSVΔ51 *in vivo* as proof of concept. (**Aim 2**)
METHODOLOGY: We will first examine a panel of bacterial and viral effector proteins for their VSe activity. Promising candidates will be inserted alone or in combination with VSEP1 into VSVΔ51, and rescued viruses characterized. As a first step, oncolytic activity of VSVΔ51-VSEPs will be evaluated in a panel of human and syngeneic murine cancer cell lines as well as normal cells. Candidates able to selectively and robustly increase oncolytic activity in cultured tumour cells will be tested in human samples and a lead selected for further testing in animals, probing for safety, antitumor efficacy, and impact on immune responses. Mechanism will also be evaluated by microarray/ELISA.
SIGNIFICANCE TO CANCER RESEARCH: Our ultimate goal will be to design novel oncolytic viruses with improved therapeutic efficacy without impacting safety. We are well positioned to translate findings to the clinic down the line should this approach continue to show promise as expected.

Li, Shawn (Shun-Cheng)
Western University

Mechanism of resistance to anti-PD-1/L1 immunotherapy and strategies to overcome resistance

RATIONALE AND OVERALL OBJECTIVE

Antibodies blocking the immune checkpoint receptor, programed cell death 1 (PD-1) or its ligand PD-L1, have brought transformative changes in the treatment of certain cancers such as melanoma and classical Hodgkin lymphoma. However, for most cancers, including cancers of the breast and colon, only a minority of patients have shown a favorable response to the PD-1/L1 blockade immunotherapy in the clinic or in clinical trials. Furthermore, with more patients now under continuous treatment with PD-1/L1 antibodies, a significant subset of patients who initially responded to the therapy have eventually stopped responding due to acquired resistance. Indeed, primary and acquired resistance have significantly limited the potential benefits of immunotherapies, especially those targeting the PD-1-PD-L1 immune checkpoint. Elucidating the mechanism of resistance would inform clinicians as to the next steps that need to be taken to potentially overcome resistance to PD-1/L1 blockade therapy. It is remarkable, in this respect, our knowledge on how the immune system adapts to PD-1/L1 blockade and ultimately confer resistance to the treatment is rather limited and fragmented despite that PD-1/L1 blockade antibodies have entered the clinic for cancer treatment since 2014.

Our overarching goal for this innovation project is to enable the development of more effective and durable cancer treatment that capitalizes on improved understanding of the molecular basis of resistance to anti-PD-1/L1 immunotherapy. *The proposed research is built on our observation that 4T1 mouse breast tumor treated with a kinase inhibitor did not show obvious resistance to continuous treatment whereas those treated with a PD-1 blockade antibody did.* Furthermore, we found that tumor-infiltrated T cells from the two treatment groups had distinct expression profiles for immune checkpoint receptors, including PD-1, CTLA-4, TIM-3, BTLA and TIGIT, and exhibited significantly different cytotoxicity towards the tumor cells ex vivo. We also found that a kinase inhibitor, when applied as a single agent or in combination with PD-1 blockade antibody (αPD-1), could re-sensitize tumors that were pretreated and became resistant to αPD-1. We will build on these exciting findings to define the molecular mechanism of acquired resistance to αPD-1, focusing on alternate immune checkpoint signaling in T cells and the role of tyrosine kinase in mediating resistance. Insights gained from the mechanistic studies will inform the design of strategies to overcome αPD-1 resistance.

HYPOTHESIS

Immune checkpoints that are alternatively expressed or co-expressed with PD-1 mediate resistance to PD-1/L1 blockade immunotherapy. We further hypothesize that tyrosine kinases play a critical role in resistance to PD-1/L1 blockade by facilitating compensatory checkpoint signaling in T cells. *This implies that combination therapies that simultaneously inhibit kinases and block immune checkpoint signaling would afford an effective strategy to overcome resistance to anti-PD-1/L1-based immunotherapy.*

SPECIFIC AIMS

1) *To characterize the adaptive T cell responses mediating acquired resistance to PD-1/L1 blockade therapy*.
2) *To define the signaling mechanism of immune checkpoints that mediate resistance to PD-1/L1 blockade.*
3) *To identify strategies to overcome resistance to anti-PD-1/L1 immunotherapy and evaluate their efficacy in vivo.*

METHODOLOGY

We will employ established cell lines to define the mechanism of resistance to anti-PD-1/L1 immunotherapy. Tumor infiltrated T cells will be employed to identify novel therapies to overcome resistance which will subsequently be evaluated in syngeneic mouse tumor models.

SIGNIFICANCE

This work will generate mechanistic insights into adaptive/acquired resistance to PD-1/L1 blockade immunotherapy and lay the foundation for the development of strategies to overcome this resistance to benefit more cancer patients.

Stein, Lincoln
Ontario Institute for Cancer Research

Oncolytic virus therapy for U1-mutant cancers

Summary: This project brings together experts in genomics and immunotherapy to build on our recent discovery of a new class of somatic driver mutation in the tumour genomes of patients with chronic lymphocytic leukemia (CLL), pediatric medulloblastoma (MB), and multiple other tumour types. These mutations occur at a specific location of the U1 small nuclear RNA (snRNA), a core component of the spliceosome, and act by altering the 5' splice site (5'SS)

recognition sequence to cause a consistent pattern of mis-splicing and expression changes among >1000 genes. We seek to exploit this mutation by engineering a potent cytolytic virus that is dependent on the presence of the mutation for replication and armed with U1-MUT specific neo-epitopes for use as an oncolytic virus vaccine.
Objectives: The project has three major objectives:

1. Engineer candidate U1-MUT oncolytic viruses.
2. Validated the engineered viruses in U1-WT and U1-MUT cell lines.
3. Arm the engineered viruses with U1-MUT specific neo-epitopes.

Methods: U1 snRNA is a core component of the spliceosome and confers 5' splice site's sequence specificity. We have discovered new driver mutation in multiple cancer types that alters a specific base in the binding site for the 5'SS, thereby causing widespread, but highly specific, changes in gene expression and splicing of the cancer cells that carry it. Unlike driver mutations that affect the protein components of the spliceosome, the changes to the transcriptome in U1-MUT cells is highly similar from one patient to another, and are predicted to create multiple cell-surface and intracellular neo-peptides. This raises the possibility of creating a "universal" cancer vaccine that can be applied to any patient carrying the mutation. We are currently performing long-read transcriptome sequencing and proteomics in order to predict and confirm these neo-antigens. In this project, we will apply our extensive experience in viral genome engineering to create strains of HSV and ColoAd1 which depend on the cryptic splicing induced by the U1 mutation to correctly splice genes essential for its replicative and lytic cycles. We will do this by replacing selected canonical 5'SSs in early gene transcripts with the 5'SS sequence preferred by U1-MUT spliceosomes. Using a series of human cell line models for CLL and MB that we have previously engineered to carry the U1 mutation, we will test the engineered HSV for its ability to complete its replicative and lytic cycles in the presence or absence of the U1 mutation. Infected cell lines will be tested by RNA-seq to confirm the expected splicing pattern of engineered early genes, and subjected to standard tests of viral cytolytic competence. Following successful completion of the previous aims, we will arm the engineered viruses with validated U1-MUT specific neo-epitopes discovered in a parallel, non-overlapping project, thereby creating a candidate oncolytic viral vaccine. We will validate this version of the virus for expression of the neo-epitope sequences, in preparation for follow-on in vitro studies of enhanced immunological recognition and killing of infected cells. Significance: The U1-snRNA mutation is the latest entry in a handful of non-coding driver mutations to be described in cancer, and the first mutation in a noncoding RNA for which we can assign a plausible mechanism of action. As such, we are working in a largely unexplored area of cancer biology and have a high potential for making novel and innovative discoveries. Based on our survey of whole genome sequencing data across >2,400 primary tumours from 37 tumour types, U1 mutations commonly occur in four cancer types: MB, CLL, hepatocellular carcinoma and non-Hodgkin's B-cell lymphoma. These tumour types span the gamut from a rapidly-progressing and usually lethal childhood cancer, to a slowly-progressing but incurable disease of older adults. Altogether, we estimate that roughly 500 new cancer cases per year carry the U1 mutation. The current proposal is an innovative, high-risk/high-reward, project that, if successful, opens up a potential new avenue to treat patients with these challenging cancer types.

Watson, Ian
McGill University

Use of oncolytic viruses to overcome immune checkpoint inhibitor resistance in melanoma

Background: Cutaneous melanoma is the most aggressive form of skin cancer. Historically, metastatic melanoma has been one of the most treatment-resistant human malignancies, where no effective treatment options were available to patients. Through advances from basic science research, a number of targeted and immune therapies are now available for patients with the potential for curative outcomes. For targeted therapy, drug resistance remains a major problem. However, immune checkpoint inhibitors (ICIs) have produced durable long-term survival for approximately 20-50% of patients. ICIs include anti-CTLA4 (ipilimumab) and anti-PD-1 (nivolumab and pembrolizumab) that activate the immune system by blocking PD-1 and CTLA4-mediated T-cell inhibition to induce an anti-tumor response. Unfortunately, not all patients respond, and patients do relapse on continuous ICI therapy. Recent sequencing studies have suggested a significant proportion of ICI-resistant melanomas (up to 75%) possess mutations in the IFN-JAK-STAT pathway (Gao *et al.,*2016). Specifically, Loss-Of-Function (LoF) mutations in *Janus kinase 1*and *2 (JAK1*and *JAK2)*have been found in patients with primary and acquired resistance to ICI. These mutations lead to decrease IFN signaling resulting in reduced antigen presentation, immune cell recruitment and PD-L1 expression. We hypothesize these mutations would also lead to a reduced ability for melanomas to mount an antiviral response, which could result in increased vulnerability to oncolytic virus (OV) therapy. Already, Talimogene laherparepvec (T-VEC), a modified OV strain of the herpes simplex virus-1 (HSV-1), is approved for the treatment of melanoma patients and our research performed here has a direct path forward to the clinic.
Preliminary data: We have obtained cell lines from a pre-treatment biopsy (M420) and a pembrolizumab-resistant

progressing lesion (M464) possessing a *JAK2* LoF mutation (Zaretsky *et al.,*2016). We performed a dose-response cytotoxicity assay using a modified prototypical OV, Vesicular Stomatitis Virus (VSV d51) that relies on IFN defects for cancer selectivity. We observed a 22-fold decrease in LD50 in the ICI-resistant *JAK2* LoF mutant line, M464, compared to the patient-matched M420 *JAK2* wild-type line ($p = 0.0197$, $n = 3$). We repeated this experiment with an additional modified OV strain, Herpes Simplex Virus 1 (HSV1-dIPC0) and similarly observed a 7-fold decrease in LD50 ($p = 0.0201$, $n = 3$) in the ICI-resistant M464 line.

Hypothesis: As mutations in the IFNγ response pathway are a bona fide ICI resistance mechanism, which would simultaneously lead to deregulation of the normal cellular antiviral response, we hypothesize that ICI-resistant melanomas are more susceptible to OV treatment.

Research Plan: In aim 1, we will first formally demonstrate using genetic approaches, siRNA knockdown and CRISPR/Cas9 knockout, that targeting JAK1 and JAK2 of the IFN signaling pathway results in increased sensitivity to OV in melanoma. We will perform knockdown and knockout studies in our pre-ICI treatment biopsy (M420) and a panel of ICI-treatment naïve melanoma lines. In aim 2, we will test whether pharmacological inhibition of JAK1/2 in melanomas with intact IFN signaling results in OV sensitization. In aim 3, we will test OV therapy alone and in combination with JAK inhibitors in PDX models from pre-treatment and ICI-resistant melanomas obtained from our live-tissue cell bank.

Implications: Numerous combination immunotherapy trials are ongoing with the goal to improve ICI response, many without biomarkers to select patients that may respond. Currently, OV therapy is being investigated in melanoma in combination with ICIs to promote intratumor infiltration to “turn cold tumors hot”. We believe the results from this proposal may produce a paradigm-shifting clinical change in how OV are used for melanoma patients, offering a potential new precision-medicine strategy for ICI-resistant and *JAK1*-, *JAK2*- and other IFN pathway-mutant cancers. Furthermore, our results will provide pre-clinical data to combine JAK1 inhibitors and OV as a strategy to improve OV response in treatment-naïve melanomas.

Watts, Tania
University of Toronto

Investigating the role of signal 4 on inflammatory monocytes in tissue resident memory T cell formation and control of melanoma during STING agonist therapy

Melanoma is mainly caused by UV exposure and is rising in incidence worldwide. Although new therapies, such as checkpoint blockade, are revolutionizing melanoma treatment, not all patients respond. The presence of CD8+ tumor infiltrating lymphocytes (TIL), and especially those with a transcriptome associated with tissue residency, correlates with improved outcome. Accumulation of CD8+ TIL is also associated with a type I interferon (IFN-I) signature. The Stimulator of Interferon Genes (STING) pathway has been implicated in induction of IFN-I in both spontaneous control of melanomas as well as with radiation and oncolytic virus therapy. These findings led to the development of a STING agonist, ADU-S100 that is currently in clinical trials for melanoma. ADU-S100 induces strong and long-lasting anti-tumor immunity in multiple mouse tumor models through a mechanism dependant on IFN-I, CD8+ T cells and cross-presenting Batf3+ conventional dendritic cells (cDC). T cell priming requires MHC and antigen (signal 1), B7 family costimulatory signals (signal 2) and cytokines (signal 3), provided by cDC. Recent work from our laboratory has shown that monocyte derived antigen presenting cells (MoAPC) provide an additional signal, signal 4 for T cell activation. To translate STING therapy to humans and appropriately combine it with other therapies, we need to fully understand its mechanism of action. For example, do responders differ from non-responders in expression of signal 4. This proposal will test the role of signal 4 on MoAPC in control of melanoma in a mouse model of therapy with ADU-S100. Using the mouse B16.ova melanoma model, we will ask if STING agonists improve control of melanoma through effects of signal 4 molecules on MoAPC and whether this correlates with induction of CD8+ TIL with a Trm phenotype. We will also characterize the effect of ADU-S100 on the transcriptome of the tumor infiltrating immune cells and use this insight to determine correlates of effective therapy that could be extended to humans. The following aims will be carried out:

Aim 1. *Assess the role of MoAPC in STING agonist therapy of mouse melanoma.*

Aim 2. *Assess the role of specific molecules on MoAPC in STING agonist therapy.*

Aim 3. *Analyze the transcriptome of immune cells in the tumor after STING agonist therapy*.

Our study will provide definitive evidence as to the importance of signal 4 on MoAPC for STING agonist therapy of melanoma and provide an in-depth characterization of the cells involved. This information will be important for future optimization of IFN-I based therapies.

Yong, V. Wee
University of Calgary

Unexpected expression of PD1 on brain tumour-initiating cells: a novel regulator of glioblastoma growth

Glioblastoma (GBM) is a devastating brain tumor where 80% of patients die within two years of diagnosis despite aggressive treatment. A major reason for the dismal prognosis is the existence of stem-like brain tumor-initiating cells (BTICs). Clearly, improved therapeutic strategies are required, and one approach is to mobilize immune cells against BTICs as described by many groups, including our own (Sarkar et al., Nature Neurosci 17:46, 2014; Mirzaei et al., Trends Immunol 38:104, 2017). This has generated intense interest in immune checkpoint proteins on T lymphocytes such as programmed cell death 1 (PD1) receptor that interacts with its ligand PDL1 on tumor cells; these interactions disarm the capacity of surveilling lymphocytes to kill transformed cells. Inhibitors to PD1 or PDL1 are thus being developed for various types of tumors. During the course of our investigations of PD1 on immune cells, we made the unexpected observation that GBM patient-derived BTICs are immunoreactive for PD1. When we stably reduced PD1 expression in BTICs, their growth was attenuated. Conversely, BTIC lines overexpressing PD1 had a higher growth rate. These surprising and novel results formulate the hypothesis to be tested in this high risk, high reward competition: that BTICs express PD1 for their intrinsic growth regulation, and that neutralizing PD1 on BTICs is an important therapy to directly inhibit BTIC growth independent of immune cell responses. The specific aims are to:**1) Evaluate the commonality of PD1 expression on BTICs and its growth advantages to BTICs:**While we have observed PD1 on 8 of 8 patient-derived BTIC lines and in 5 of 5 GBM tissue specimens, we will now examine our center's over 100 GBM patient-derived BTIC lines and tissue sections from 20 GBM specimens to investigate the commonality of PD1 expression. To corroborate the intrinsic functions of PD1 in BTICs, we will under- or overexpressed PD1 on 10 human BTIC lines. We will focus on clonogenic growth in limiting dilution assays, apoptosis, and cell cycle assay. Finally, to affirm the in vivo promoting properties of PD1 in the absence of adaptive immunity, we will implant BTICs into the striatum of immunocompromised Rag knock-out mice and monitor tumor growth by sequential brain MRIs, survival analysis, and by histology.**2) Investigate the mechanisms by which PD1 promotes BTIC growth**: To test whether PD1 signaling is required to promote BTIC growth, we have generated BTIC lines overexpressing mutant variants of PD1 with defective signaling motifs. We will implant human BTIC lines expressing PD1 with defective motifs into the striatum of Rag knock-out mice to monitor tumor growth. By RNAseq, we will also address the transcriptomes that are over-represented in PD1-overexpressing BTICs. Furthermore, a mutant version of PD1 with defective PDL1 binding was generated to test whether tumor-promoting effects of PD1 depend on PDL1 ligation. We will implant BTICs expressing the PD1 mutant version with defective PDL1 binding into the striatum of Rag knock-out mice and monitor tumor growth by MRI.
3) Investigate a novel CNS-targeted silencing compound to abrogate PD1 within the tumor microenvironment: We will first address whether blocking antibodies for PD1 or PDL1 abrogate the tumor promoting-effects of PD1 in the murine glioblastoma models.Because the blood-brain barrier limits the antibody penetration into the CNS, and also PD1 may promote BTIC growth independent of PDL1 ligation, we will evaluate the small interfering RNA (siRNA) -induced gene knock-down technology to down-regulate PD1 within the GBM microenvironment. To this end, we will use a novel compound composing of PD1 siRNA and a tetrapeptide that homes selectively into the CNS.
This paradigm-shifting application addresses the novel findings of PD1 and its growth modulation of BTICs. Besides new insights into GBM biology, our results emphasize that targeting PD1 with blocking antibodies that may not gain entry into the CNS misses affecting the crucial function of PD1 in BTICs. Moreover, we are excited to test a novel CNS-targeted compound to abrogate PD1 function within the tumor microenvironment.

I4 Novel Therapeutics

Britton, Robert
Simon Fraser University

Small molecule inhibitors of O-GlcNAc transferase: validation of a new anticancer target

During oncogenic transformation, cancerous cells uptake increased amounts of glucose compared to healthy cells. This increase in glucose uptake impacts several biosynthetic pathways, including the hexosamine biosynthetic pathway (HBP), which results in a net increase in the metabolite uridine diphosphate N-acetyl glucosamine (UDP-GlcNAc). UDP-GlcNAc is used by O-GlcNAc transferase (OGT) to transfer GlcNAc to many proteins – a dynamic posttranslational modification that is upregulated in almost all cancer cells. There is a compelling body of evidence that inhibiting GlcNAcylation through inhibition of OGT can impair the growth and proliferation of cancerous cells, while leaving healthy cells largely unaffected.

The overexpression of OGT in cancers is a well described phenomenon, and studies have shown that OGT knockdown in solid many cancers results in impaired growth *in vitro* and in vivo. While reducing hyper O-GlcNAcylation by OGT knockdown inhibits survival of cancer cells, it does not appear to affect counterpart immortalized but non-transformed cells, indicating a therapeutic window. This is relevant for diseases such as AML, whose standard treatment has not changed since 1981 resulting in an average 5-year overall survival of 21% in Canada.
Despite the persuasive *in vitro* evidence that OGT is a viable and promising cancer target, *in vivo* examination of its role in cancer have thus far lagged far behind cell studies. Attempts to discover OGT inhibitors have been carried out, however, most of the inhibitors discovered in these campaigns negatively impact cell viability, likely suffer from off-target effects and display poor water solubility, making them incompatible with *in vivo* studies.
This proposal aims to address the lack of small molecule OGT inhibitors suitable for in *vivo studies*. Using a novel high-throughput screening assay, the Vocadlo group at SFU has identified 4 novel hit structures that display low μM inhibition constants for OGT. We aim to subject these hits to medicinal chemistry efforts in order to optimize potency and water solubility. Potent lead structures (Ki < 400nM) that display adequate water solubility will be advanced to preliminary cell studies in well-established cancer cell lines. Compounds that show cellular inhibition of global GlcNAcylation (EC50 < 5μM) will be advanced into more detailed cell studies. These studies will examine the kinetics of OGT inhibition, as well as several biomarkers of OGT inhibition, including global O-GlcNAc reduction, reduction of GlcNAcylation on specific structures, and the non-canonical proteolytic function of OGT.
Following these studies, we will examine the proliferation of cancer cells and compare their growth to counterpart non-cancerous cell lines. To validate OGT inhibition as a mechanism for cellular growth inhibition, we will profile our compounds in differentially responding cell lines by comparing their growth inhibition profiles to that of known inhibitors. Preliminary data from the Kuchenbauer group shows that 5 out 10 human AML cell lines were equally sensitive to two distinct OGT inhibitors, resulting in significant growth inhibition. To assess this effect in vivo, we will take advantage of a primary murine AML transplantation model based on the combined ectopic expression of homeobox gene Hoxa9 and its cofactor Meis1 (H9M), which are frequently overexpressed in AML patients. We will engraft mice with H9M cells and treat 5 mice/group with OGT inhibitors at 3 increasing doses starting with the *in vitro* cell-based determined IC50. H9M cells overexpress green fluorescent protein (GFP) which allows flow cytometry-based tracking. In addition, we will investigate the combinatorial effect of OGT inhibition with standrad chemotherapy in vivo and eventually test OGT inhibition in patient-derived xenotransplant AML models. Overall, the development of next generation OGT inhibitors will pave the way for a new class of drugs – post-translational modifiers – as novel, selective and effective type of cancer treatment.

Olson, Michael
Ryerson University

MRCK inhibitor optimization for glioblastoma therapy

Glioblastoma is the most common primary brain tumour in adults, and its ability to spread rapidly and aggressively through the brain contributes to its resistance to be treated effectively. Surgery is the first treatment option, but the dissemination of glioblastoma cells throughout the brain makes it virtually impossible to cure this form of cancer. Radiotherapy is used to kill tumour cells that cannot be removed by surgery, but several recent reports have shown that radiotherapy comes at the cost of promoting the ability of glioblastoma cells to migrate and invade further into the brain. This observation that radiotherapy may promote more aggressive glioblastoma spread has profound implications on how best to treat this disease.
The ability of tumour cells to invade their local surroundings is driven by mechanical forces produced by the actin-myosin cytoskeleton within each cell. Our research on regulators of the actin-myosin cytoskeleton resulted in the initiation of a drug discovery program to develop inhibitors for the MRCK protein kinases. Structure-guided medicinal chemistry successfully led to the development of potent and selective compounds that effectively block tumour cell migration and invasion. We determined that the increases in MRCK substrate phosphorylation and glioma migration induced by radiotherapy were blocked by the MRCK inhibitor BDP9066 in a dose-dependent manner. Using an orthotopic glioblastoma mouse model, we determined that systemically administered BDP9066 achieved micromolar concentrations in brain tumours, and blocked radiotherapy induced dissemination of glioblastoma cells. Furthermore, the combination of radiotherapy plus BDP9066 administration significantly prolonged survival relative to no treatment or radiotherapy alone. These results demonstrate that there is clinical benefit derived from inhibition of glioblastoma motility combined with radiotherapy, and that MRCK inhibition is an effective means to block glioblastoma spread.
To advance MRCK inhibitors to a state of their being genuine clinical candidates, there is a need to improve their metabolic stability. In vivo pharmacokinetic analysis revealed rapid clearance indicative of first pass metabolism via the liver. In this proposal, we will use information we obtained from the metabolites produced by hepatocytes, and the

X-ray structural information of drug-protein co-crystals to identify chemical structure sites that could be modified to resist rapid metabolism. In parallel, we will use an artificial intelligence based drug design approach to generate alternative structures derived from the BDP9066 series that will be pre-selected for predicted metabolic stability and blood brain barrier penetration. Having generated modified variants, they will be tested for their potency and selectivity, and top candidates will be profiled for their metabolic stability. Iterative rounds of chemistry and evaluation will lead to the generation of prioritized candidates that will be further tested for their in vivo absorption, distribution (including brain penetration) and metabolism. In addition, their ability to block radiotherapy induced migration and invasion will be evaluated. Ultimately, a clinical candidate will be developed for future pre-clinical efficacy studies.

Park, Morag
McGill University

Developing therapeutic approaches to treat poor outcome metaplastic breast carcinomas

Significance: Invasive breast carcinoma (BC) is a combination of heterogeneous diseases, comprising multiple entities with distinct molecular and clinical features. Among BC subtypes, metaplastic breast cancer (MpBC) is a most aggressive disease that accounts for 1% of all breast malignancies, touching ~300 women in Canada per year, the majority of whom will die from their disease within 3 years. MpBC is characterized by the differentiation of the neoplastic epithelium into squamous and/or mesenchymal cells. In most cases, MpBC is negative for estrogen (ER), progesterone (PR) and HER2 receptors. Although they are classified molecularly within triple-negative breast cancer (TNBC), MpBCs are more aggressive than other TNBC histological subtypes. MpBC presents the highest rates of recurrence, poorer survival outcomes with poor response to current chemotherapies optimized for TNBC. To date, there are no established treatments for MpBCs, due to paucity of clinical trials and near absence of preclinical models for investigation. Hence, the establishment of relevant preclinical models and investigative strategies focused on MpBC is essential to identify suitable therapeutic interventions for MpBC. Patient-derived xenograft (PDX) and 3D tumor organoid cultures (PDXO) are now recognized as gold standard models to study the clinical diversity of BC. They are predictive of clinical outcomes and are used successfully for preclinical drug evaluation, biomarker identification, discovery and validation of precision medicine strategies.
Resource: Among a cohort of 48 TNBCs PDX established by us, we have developed the largest resource of MpBC patient-derived xenograft (PDX) world-wide (n=8), due in part to poor response of these patients to neoadjuvant chemotherapies. Using these we have identified alterations and pathways potentially dysregulated in MpBC, applied machine learning computational approaches for drug prediction and have established conditions that enable consistent production of clinically relevant 3D organoids compatible with High-throughput Screening (HTS) for drug identification and validation.
Hypothesis and Aims: We hypothesize that improved therapeutic options can be identified for MpBC by deep understanding of this disease. Our resources and models provide a unique window of opportunity to identify and develop novel therapeutic strategies. The aims of this project are 1) to establish and expand our preclinical models of MpBC (PDX and PDXO), to perform a deep molecular characterization of PDX and PDXO to identify pathways dysregulated/causal in these tumors and to validate molecular alterations using extensive FRQS reseau breast cancer tissue banks with patient outcome (~ 100 MpBC tissues); 2) using an advanced drug prediction pipeline, identify potential FDA approved drugs for MpBC and evaluate drug response alone and/or in combination using our 3D PDXO organoid HTS platform (allowing rapid screens of FDA approved drugs suitable for drug repurposing); 3) validate *in-vivo*, combinations of compounds that present synergy in 3D organoids using our MpBC PDX cohort (n=8) and others 8 independent MpBC PDXs available in PDX repositories worldwide (n ~20).
Potential outcomes and impact: MpBC remains the worst outcome breast cancer with an estimative of ~150 deaths annually in Canada and more than 3000 deaths worldwide. Our study will build a unique Canadian resource and will permit the evaluation and development of new therapeutic strategies to target MpBC with the aim of clinical translation, providing improved outcomes for women suffering from this disease. Notably, up to 30% of mesenchymal TNBC display metaplastic pathologies and while assays to detect mesenchymal TNBC are under development, MpBC may also serve as a clinically identifiable surrogate to evaluate potential regiments for mesenchymal TNBC.

I5 Prevention and Quality of Life

Ferguson, Sarah
Princess Margaret Cancer Centre - UHN

Bariatric surgery for fertility-sparing treatment of atypical hyperplasia and grade 1 cancer of the endometrium (B-FiERCE): a feasibility study

Overview: The incidence of endometrial cancer (EC) is increasing at an alarming rate (2.6% per year). This trend parallels the rising rate of obesity, the most significant risk factor for EC. Young women with obesity and EC or atypical hyperplasia (pre-cancer, AH) who want to maintain their fertility and thus avoid hysterectomy are treated with progestin therapy, such as the progestin-IUD (pIUD). However, the pIUD achieves cancer regression in only 50-70% of women, and over 50% of initial responders will develop recurrence within two years. The effectiveness of the pIUD may be improved if the driver of EC (obesity) was simultaneously addressed. Recommendations by a physician to lose weight through “diet and exercise” are often unsuccessful; lifestyle modification and pharmacologic treatments result in modest (<10%) weight loss at best. In contrast, bariatric surgery (BS) achieves more significant (>30%), rapid and sustainable weight loss. Multiple epidemiologic studies have demonstrated that BS reduces the risk of developing EC, suggesting biologic plausibility for the use of BS to treat EC; however, this has not been tested directly, and we do not know if BS is a feasible or acceptable therapy in this population. Our research aims to answer the question: “Is BS in addition to the pIUD a ***feasible*** and ***acceptable*** option for young women with EC/AH who wish to maintain their childbearing potential compared to standard treatment (pIUD) alone?” We ***hypothesize*** that many women with EC/AH will decide that BS is an acceptable addition to pIUD for fertility preservation.
Approach: This is a pilot randomized control trial to assess the ***feasibility*** of a full-scale randomized controlled trial (RCT). Eligible women will be consented and participants will be randomized to BS plus standard pIUD (intervention group) or to standard pIUD alone (non-intervention group) in a 1:1 fashion.***Inclusion Criteria.*** Women 18 to ≤ 41 years of age with clinical stage 1a grade one endometrioid EC or AH with a body mass index ≥ 35 kg/m2 who want fertility preservation.***Intervention (Figure 1).*** All patients will have a pIUD placed at baseline (T0A). Women randomized to the intervention group will have BS performed within 3 months (T0B). As per standard of care, all women will undergo imaging, endometrial sampling, and biometric measurements at baseline and every 3 months for 15 months.
Study Objectives:
Primary objective of this study is to assess the feasibility of the addition of BS to progestin therapy in women with obesity who have AH or grade one EC and desire fertility preservation. Our primary outcome will be the proportion of eligible women who agree to participate in the study (recruitment rate). This will assess the acceptability of BS to these women and their willingness to undergo randomization. A full-scale RCT to formally evaluate efficacy will be deemed feasible if a recruitment rate of ≥ 40% is achieved.***Secondary objectives*** will include additional feasibility parameters such as: 1. Proportion of patients who complete BS within 3 months of randomization; 2. Proportion of patients who are lost to follow-up at end of study; 3. Completion rate of patient reported outcome questionnaires; and 4. Proportion of patients with complete histologic response 15 months after randomization.**Sample Size:** To obtain sufficiently precise estimate of our recruitment rate, we aim to approach 60 eligible women. Based on previous recruitment rates (43 to 75%) in clinical trials at UHN for women with EC, we estimate that 33 (55%) of eligible women will consent to this study. This would result in a recruitment rate of 55% with a 95% CI of 41.6 to 67.9 using the exact (Clopper-Pearson) method.**Significance:** By treating both cancer and obesity, our innovative approach may lead to more patients achieving cancer regression and fewer patients experiencing cancer recurrence. These patients may also experience significant improvements in pregnancy, neonatal and long-term health outcomes, with a reduction in the risk of other obesity-related diseases.

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Identifying pathways for cancer care from family physicians' electronic medical records

Problem to be investigated: Cancer Care Ontario has published pathways of care for several cancers.1 These pathways can help identify potential care inequities, which can help target resources to address these gaps. Canadian family physicians (FPs) see their cancer patients in all stages of their care from the pre-diagnostic, diagnostic, active treatment and post-treatment (survivorship) phase. In fact, FPs in Ontario see their patients with breast cancer a mean of 3.8 (SD. 3.3) visits during their diagnostic stage.2 Most wait time measures in Canada focus on the wait after seeing a specialist physician to having a procedure and they are measured using existing health administrative data .3 Getting information on the pre-diagnostic steps in the care pathway is challenging. In Ontario, algorithms using physician

claims data have been used to estimate the wait time from a FP visit to seeing a specialist physician (wait time 1) for breast cancer.4 This study found the median wait time interval was shorter amongst the screened group than the diagnostic group. While it is important to measure wait times from a primary care perspective and compare them to available benchmarks, it can also identify groups experiencing long wait times. For example, a recent study found women with a high body mass index (BMI) experienced longer wait times than lower BMI women in getting care for their endometrial cancer.5 In Ontario, the adoption of electronic medical records (EMR) in clinical care has markedly increased amongst community-based FPs with just over 80% using an EMR.6 Alongside this, the secondary use of FP EMR data has been used for primary care quality improvement,7,8measure chronic disease prevalence9-11 and also measure access and the coordination of care across the health care sector.12-15 Objectives: The objective of this study is use FP EMR data linked to Ontario health administrative data to identify care pathways and measure the wait times for patients who had colorectal, bladder, endometrial and melanoma cancers. The cancer care pathway would start from the FP EMR description of symptoms, to the initiation of investigations, referral to specialty care and the receipt of specific treatments. Methods: At ICES in Ontario, information from community-based FPs EMR linked to Ontario health administrative data will identify specific points along the cancer care pathway. A trained abstractor will examine the entire FP EMR records for pre-diagnostic symptoms/signs and test results indicating an abnormality. Linkage to health administrative data will identify the receipt of physician consultation visits, receipt of diagnostic tests and cancer treatments. Wait times from the pre-diagnostic dates found in the FP EMR to the receipt of consultation visits and treatments will be determined. Patient (age, sex, socioeconomic status, social deprivation index, comorbidity, continuity of care with their family physician, rurality, health risk factors such as smoking, obesity and alcohol use) and physician factors (age, sex, practice location, primary care funding model) related to these wait time will be examined. Finally machine learning using the pre-diagnostic labels identified during the EMR abstraction will be used to develop algorithms that identify pre-diagnostic cancer care. Significance: A patient's cancer care experience begins before they see their cancer care specialists. Recognizing that a person's health concerns often begin prior to seeing a physician, they most often see their FP at the beginning of this process. This research will provide real-world data measuring cancer care wait times coming from a primary care perspective. The development of machine learning algorithms which use existing FP EMR data, with a linkage to existing health administrative data can more quickly and economically estimate primary care wait times. These primary care wait times can identify people who are experiencing inordinate delays in their care and can inform patients, cancer care providers and policy makers on where to target resources to improve the delivery of cancer care.

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Pilot study of the rectal cancer checklist and a reminder system to improve pre-operative surgeon evaluation of surgical margins among patients with rectal cancer

Problem: Following rectal cancer surgery, a positive (+) surgical margin (i.e., cancer cells at the cut edge of the surgical specimen) is an important predictor of local pelvic tumour recurrence – a devastating outcome for patients. If tumours are very advanced and gross tumour will remain in the pelvis after surgery, then futile surgery should be avoided. Optimal pre-operative surgical assessment can almost always identify patients with surgical margins threatened by tumour, and thus facilitate optimal pre- and intra-operative treatment decisions. We hypothesize that among patients undergoing rectal cancer surgery, important negative outcomes which occur after surgery, such as + surgical margin, local recurrence, or futile surgery, can often be connected to sub-optimal surgeon evaluation of tumour resection margins, which occurs prior to surgery. In various contexts across Ontario we have found evidence of this. Our group has completed major Knowledge Translation (KT) research studies in Ontario and gained insights into optimizing the quality of rectal cancer surgery; though we have not yet achieved our goals. We suggest integrating Patient Safety concepts and interventions into our efforts will help close related quality gaps. Patient Safety efforts accept the inevitability of human error, and attempt to engineer surrounding systems that mitigate the effects of such errors. A main thrust of Patient Safety is to remove latent conditions – or system-level deficiencies that are common, unrecognized, and that pre-dispose systems to errors or adverse events. We suggest that sub-optimal pre-operative surgeon evaluation of rectal cancer resection margins is a latent condition across Ontario, and likely across Canada. We would like to pilot test the feasibility of strategies that may mitigate this latent condition. Objectives: Primary: For patients undergoing rectal cancer surgery, to evaluate at six hospitals in southern Ontario feasibility of a Rectal Cancer Checklist and Reminder System strategy. Secondary: a. To use the Theoretical Domains Framework to identify barriers and facilitators to surgeon use of the strategy. b. To measure if the strategy is associated with improved rates of optimal pre-operative surgeon evaluation of resection margins. Methods: This pilot study will be a stepped wedge non-randomized trial involving 6 high-procedure volume hospitals in southern Ontario. 70% or more of surgeons at a site must consent to participate. Study interventions include a Rectal Cancer Checklist, and, a Reminder System to

encourage surgeon completion of Checklists into the patient chart. The six-item Checklist articulates key evaluation steps for rectal cancer tumour margins. Surgeons will receive reminders to complete the Checklist weeks prior to surgery (through use of a pathology database that allows identification of diagnostic biopsies) and just prior to surgery if Checklists are not completed when patients attend Pre-operative clinic evaluations. For four months Checklist submission will be voluntary, followed by four months where they will be forced – or mandatory to complete surgical bookings. Surgeons at any point can withdraw study consent. We will review charts of all patients treated at study sites in a 6-month pre-study period, and, the 8-month intervention period. Following the intervention phase, we will use interviews and a survey based on the Theoretical Domains Framework to identify barriers to use of the Checklist and reminder system. Main feasibility outcomes will be surgeon participation rates in all aspects of the study, especially completion of Checklists. Other important outcomes will be identification of actionable barriers to intervention use, and, influence of interventions on rates of optimal surgeon evaluation of surgical margins. Significance: Observations and results of this pilot study will inform the design of a much larger stepped wedge cluster randomized trial testing the efficacy of KT/Patient Safety interventions to improve surgeon pre-operative evaluation of rectal cancer surgical margins and related patient outcomes.

Wright, Frances
Cancer Care Ontario

An automated algorithm to identify and characterize skin cancers from population-based pathology reports

The Current Problem:
The incidence of keratinocyte carcinoma (cutaneous basal and squamous cell carcinoma) is increasing and the estimated patient care and economic burden of the disease is significant. Capturing keratinocytes carcinoma is not mandated by most cancer registry associations including the Canadian Association of Cancer Agencies. As a result, keratinocyte carcinomas are not part of most cancer registries in Canada, including the Ontario Cancer Registry. With an estimated 40,000-keratinocyte carcinoma cases in Ontario per year, the standard approach to capturing cancer cases using manual review by cancer registrars is not feasible. Therefore, alternative methods are needed.
Objectives:
1.Develop and implement a robust, sustainable **methodology to identify and characterize** keratinocyte carcinomas from electronic pathology reports obtained from Ontario's public and private laboratories in near real-time.
2.Create a **population-based data repository** of keratinocyte carcinomas for analytics and reporting of incidence, health services utilization, and outcomes in Ontario to enable sustainable disease surveillance, patient care, system planning, quality improvement, and performance management, as well as evaluation and research.
Methods:
The pathology reports we require to identify keratinocyte carcinomas typically consist of unstructured blocks of narrative text. Natural Language Processing (NLP) is a process by which a computer converts a string of text into analytic data. We will use an iterative approach to develop a methodology based on NLP and other data science techniques to create case-finding algorithms to capture cases of suspected keratinocyte carcinomas (develop > test > refine > test). Once cases are ascertained, an analysis-ready data repository will be created and linked to other databases held at CCO to enrich the data with patient demographic information, health service utilization, outcomes, as well as relevant pathology information (e.g. histology and behaviour) obtained using NLP.
Using this cohort, we will address the current information gap on skin cancers by building a comprehensive knowledge base on keratinocyte carcinoma. We will assess whether sociodemographic characteristics, geography, and clinical factors are associated with incidence, health care utilization along the care continuum, and patient outcomes using Poisson regression, logistic regression, and Cox proportional hazards regression, and other statistical methods where appropriate. Anticipated knowledge translation activities will include disseminating methods and analytic results to researchers, healthcare providers, government (e.g. health and labour ministries), clinical and population health programs, and the general public through open-access academic journals, conference presentations, cancer prevention messaging, and other media.
Significance of the research:
The development of a keratinocyte carcinoma data repository will fill a significant knowledge gap in the Ontario and broader Canadian cancer system. The results from our analyses will enable data-informed decision-making and guide the identification and prioritization of future initiatives in health care quality planning, improvement, and performance management. We anticipate health care providers and provincial, national, and international policy makers to be the key audiences for this new knowledge and expect an impact on clinical decision-making and policy development. The data repository will also be available to health services researchers who have an interest in keratinocyte carcinoma. In the future, we anticipate that we and others will be able to extend the approach to capture other diseases requiring a

pathology diagnosis, including but not limited to cancer, to enable broader health monitoring. Furthermore, our NLP methodology could be leveraged to automate the extraction of data from other narrative texts, such as radiology and surgical reports.